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Original Article

Overexpression of sineoculis homeobox homolog 1 predicts poor prognosis of hepatocellular carcinoma

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Abstract: High expression levels of the human sineoculis homeobox homolog 1 (*SIX1*) gene have been correlated with numerous human malignancies. The SIX1 protein is involved in chromatin reconstruction and gene transcription, and plays an important role in cell apoptosis. This study explores the role of SIX1 in tumor progression and in the prognostic evaluation of hepatocellular carcinoma (HCC). Real-time PCR, Western blotting analysis, immunofluorescence (IF) staining, and immunohistochemistry (IHC) were performed to examine SIX1 expression in HCC cell line/tissues compared with adjacent non-tumor and normal liver tissues. Statistical analysis was applied to evaluate the correlation between SIX1 overexpression and the clinicopathological features of HCC. Survival rates were calculated using the Kaplan-Meier method, and the relationship between prognostic factors and patient survival was analyzed using the Cox proportional hazard models. The SIX1 protein was detected in 80.9% of HCCs, which was significantly higher than that in either adjacent non tumor liver or normal liver tissues ($P < 0.01$). SIX1 overexpression was positively correlated with tumor size, pTNM stage and venous infiltration. Moreover, the 5-year survival rate of patients with high expression of SIX1 was significantly lower than that of patients with low SIX1 expression. Multivariate analysis suggested that pTNM stage and SIX1 protein expression were independent risk factors for survival in HCC. In conclusion, SIX1 plays an important role in the progression of HCC. High level expression of SIX1 is an independent poor prognostic factor of HCC.

Keywords: SIX1, immunohistochemistry, prognosis, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the most common malignant tumor of the liver and its incidence is particularly high in China relative to other countries [1, 2]. The treatment options are greatly limited for patients with advanced HCC due to the presence of large size tumors and potential metastasis [3].

Tumor recurrence and metastases are the major causes of death in HCC patients after surgical treatment. Available modalities for HCC screening include serologic markers and radiographic tests. Alpha-fetoprotein (AFP) and Des-gamma-carboxy prothrombin (DCP) are the most commonly used serologic screening test for HCC. In some studies DCP was more sensitive than AFP while in other studies AFP was more sensitive [4, 5]. A recent Japanese study of 1377 HCC patients and 355 non-HCC controls with chronic hepatitis or cirrhosis showed that the accuracy of DCP was inferior to AFP particularly for small tumors [6]. In a word, both DCP and AFP have a limited sensitivity when standard cutoff recommendations are used. As an increasing number of early-stage, small HCC nodules (< 3 cm) in patients with normal serum level of AFP and DCP, prediction of prognosis of these patients represents a major challenge in clinic [7]. Hence, the discovery of dependable biomarkers is important for early diagnosis, prognostic evaluation, prediction of recurrence, and as potential therapeutic targets of HCC.

The sineoculis homeobox homolog 1 (*SIX1*) gene encodes a homeodomain-containing transcription factor that belongs to the 6th family of

homeoproteins and is highly expressed during embryogenesis. The gene, located on chromosome 14q23, is involved in the early development of diverse organs such as the brain, and kidney [8, 9]. Initial studies have shown that *SIX1* is related to the development of tissues and organs, which could promote proliferation and survival of precursor cells before cell differentiation; the loss of *SIX1* can cause abnormal development of various organs [10]. Recently, evidence suggests that *SIX1* plays a pivotal role in tumor invasion and metastasis. Behbakht *et al* [11] found that *SIX1* mRNA was elevated in early stage ovarian tumors compared with normal ovary, and it was significantly elevated in late-stage compared with early-stage tumors or normal ovary. Overexpression of *SIX1* occurs in a large percentage of primary breast cancers and strongly correlates with metastatic breast lesions [12].

However, up until now, *SIX1* protein expression and its correlation with the clinicopathological characteristics of HCC have not been adequately addressed. Here we investigated the clinicopathological significance of SIX1 protein overexpression in HCC, and demonstrate its prognostic value for patients with HCC.

Materials and methods

Clinical samples

This study complied with the provisions of the Declaration of Helsinki and was approved by the Human Ethics Committee and the Research Ethics Committee of the Yanbian University Medical College. Through the surgery consent form, patients were informed that the resected specimens were kept by hospital and might be used for scientific research, and that their privacy would be maintained. Follow-up survival data were collected retrospectively from the medical records.

A total of 284 cases of human tissue samples, including 162 HCCs, 87 adjacent non tumor liver tissues and 35 normal liver tissues, were collected from Shanghai Outdo Biotech Co. Ltd. and the Department of Pathology and Tumor Tissue Bank, Yanbian University Medical College for immunohistochemical staining of SIX1. Eight cases of HCC fresh tissues with matched adjacent non tumor liver tissues were selected for qRT-PCR and Western blot, and HepG2 HCC cell line was cultured for immunofluorescence staining of SIX1.

The pathological parameters, including gender, age, tumor size, pathological tumor-node-metastasis (pTNM) stage, venous infiltration, AFP level and HBVs antigen-positivity were carefully reviewed for all 162 HCCs cases. There were 121 males and 41 females. There were 87 patients aged ≤ 50 years and 75 patients aged > 50 years. Of the 162 HCC cases, 119 patients had tumors ≥ 5 cm in size, and 43 had tumors < 5 cm in size. Of the 162 HCC cases, 69 were early pTNM stage (I-II), and 93 were advanced pTNM stage (III-IV) according to Union for International Cancer Control (UICC) criteria (7th Edition) and WHO classification (Pathology and Genetics of Tumors of the Digestive System). There were 136 HCC patients who tested positive for hepatitis B surface antigen, whereas only 26 were tested negative for hepatitis B surface antigen. In 49 patients, the AFP level was ≤ 20 ng/ml and in 113 patients the AFP level was > 20 ng/ml. Of the 162 HCC patients, 107 of them had died from HCC, while 55 remained alive.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total tissue RNA was extracted from eight pairs of HCC and corresponding adjacent non tumor liver tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was synthesized using PrimeScript reverse transcriptase (Takara Bio, China) and oligo (dT), according to the manufacturer's instructions. To examine expression, qPCR was performed using a Bio-Rad sequence detection system according to the manufacturer's instructions, and a double-stranded DNA-specific SYBR Premix Ex TaqTM II Kit (Takara Bio, China). Double-stranded DNA specific expression was assessed using the comparative Ct method ($2^{-\Delta\Delta Ct}$). The following forward and reverse primers were used for SIX1: 5'-AAGGAGAAGTCGAGGGGTGT-3'; 5'-TGCTTGTTGGAGGAGGAGTT-3', and for GAPDH: 5'-CATCACCATCTTCCAGGAGCG-3'; 5'-TGACCTTG CCCACAGCCTTG-3'. All assays were performed at least three times and each sample was done in triplicate.

Western blotting

Total of 10 mg of tissue samples preserved in liquid nitrogen was taken, ground, and lysed in

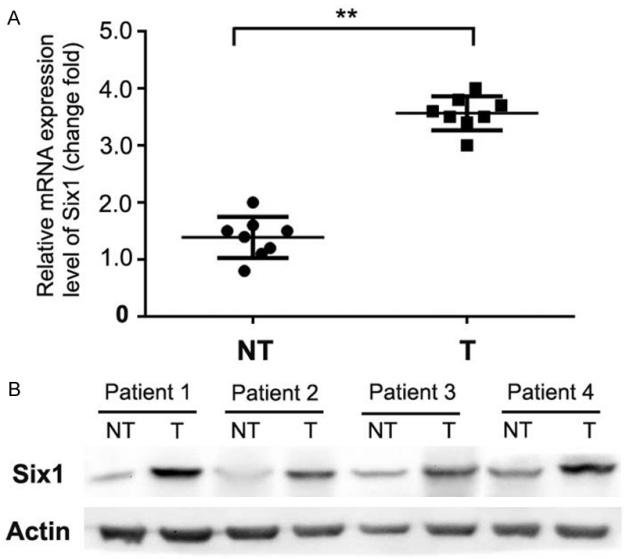


Figure 1. The mRNA and protein expression of SIX1 in HCC and adjacent non-tumor liver tissues. SIX1 expression using (A) qRT-PCR and (B) Western blotting analysis in fresh tissues of HCC matched with adjacent non-tumor tissues. Experiments were performed in triplicate for each case. SIX1 expression level was significantly higher in HCCs (T) compared with adjacent non-tumor tissues (NT). (**$P < 0.01$).

RIPA lysis buffer containing complete protease inhibitor cocktail (Roche). Equal protein samples (20 μg) were separated on 10% SDS polyacrylamide gels and transferred to PVDF membranes (Millipore). Membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1.5 h at room temperature. Membranes were incubated with anti-SIX1 antibody (1:1000, Sigma) and b-Actin (1:1000, CWBIO) overnight at 4°C, and then with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1000, CWBIO). SIX1 expression was detected using ECL Western blotting detection reagent (CWBIO) according to the manufacturer's instructions.

Immunofluorescence staining for SIX1 protein in HepG2 cells

HepG2 HCC cell line was grown on coverslips to 70% confluence; the cells were then fixed with 4% paraformaldehyde for 10 minutes and permeabilized with 0.5% TritonX-100 for 10 minutes. Blocking was performed with 3% Albumin Bovine V (Solarbio) for one hour at the room temperature. After washing with PBS, cells were incubated with anti-rabbit SIX1 (1:100, Sigma, HPA001893) at 4°C overnight, and followed the incubation by Alexa Fluor®488 Goat Anti-Rabbit IgG (H+C) (1:1000, Invitrogen) for one hour at room temperature. After washing with PBS, the cells were counterstained with 49-6-diamidino-2-phenylindole (DAPI) (Beyotime, China) and the coverslips were mounted with Antifade Mounting Medium (Beyotime, China). Finally, the immunofluorescence signals were visualized and recorded by Leica SP5II confocal microscope [13].

Immunohistochemical (IHC) for SIX1 in paraffin-embedded tissues

An IHC study was performed using DAKO LSAB kit (DAKO A/S, Denmark) as described previously [14]. Briefly, 4-μm thick tissue sections were deparaffinized, rehydrated and incubated with 3% H_2O_2 in methanol for 15 minutes at room temperature to eliminate endogenous peroxidase activity. Antigen retrieval was carried out at 95°C for 20 min by placing the slides in 0.01 M sodium citrate buffer (pH 6.0). The slides were then incubated with a primary anti-SIX1 antibody (1:100, Sigma, HPA001893) at 4°C overnight. After incubation at room temperature for 30 min with a biotinylated secondary antibody, the slides were incubated with the streptavidin-peroxidase complex at room temperature for 30 min. Immunostaining was developed by using the 3,3'-diaminobenzidine chromogen and counterstained with Mayer's hematoxylin. We used a rabbit IgG isotype controls, which showed negative staining. Also, positive tissue sections were processed omitting the primary antibody (rabbit anti-SIX1) as a negative control.

IHC evaluation

All specimens were examined by two investigators (Lin Z & Kong J) who had no knowledge of the clinical data. In case of discrepancies, a final score was mutually agreed upon by reassessment using a double-headed microscope. Briefly, the immunostaining for SIX1 was semiquantitatively scored as '-' (negative, no or less than 5% positive cells), '+' (5-25% positive cells), '++' (26-50% positive cells) and '+++' (more than 50% positive cells) [13]. The cytoplasmic, nuclear and membranous expression patterns were all considered as positive staining, and '++' and '+++' was considered as strongly positive staining. For the survival data

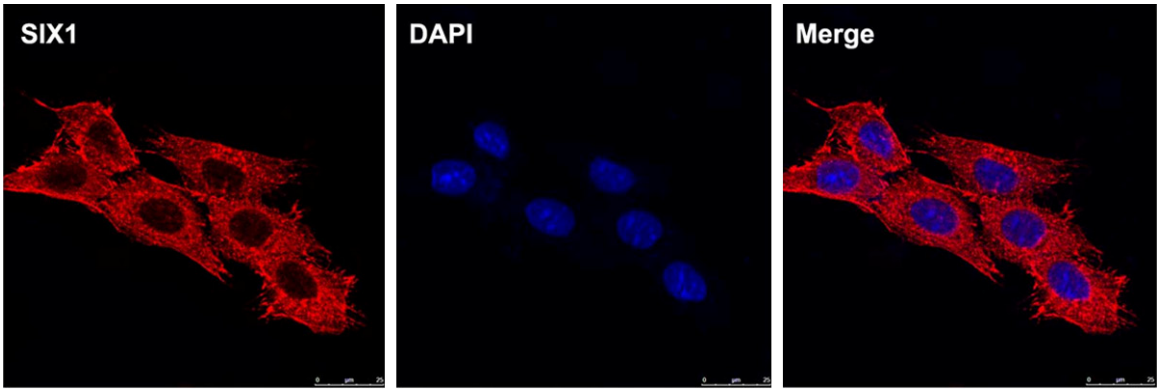


Figure 2. Immunofluorescence staining for SIX1 proteins in HepG2 cells. SIX1 protein mainly located at the cytoplasm of the HepG2 cells, and the weak positive signals was also observed in the nucleus (Red indicates SIX1, Blue indicates DAPI).

analysis, SIX1 expression scored as '++' and '+++' was considered as high expression and '-' and '+' as low expression.

Statistical analysis

Statistical analysis was carried out using the SPSS 17.0 statistical package (SPSS Inc., Chicago, IL, USA). The association of SIX1 protein expression and clinicopathological parameters were analyzed using the Chi-square test. Survival rates after tumor removal were calculated using the Kaplan-Meier method, and differences in survival curves were analyzed by the Log-rank test. Multivariate survival analysis was performed on all significant characteristics measured using univariate survival analysis through the Cox proportional hazard regression model. A *P* value of less than 0.05 was considered to be statistically significant.

Results

SIX1 expression and its protein localization in HCC

The SIX1 mRNA from eight pairs of HCC and corresponding adjacent non tumor liver tissues was examined using qRT-PCR. The results showed that the relative mRNA expression level of SIX1 was up-regulated in HCCs compared with adjacent non tumor liver tissues (**Figure 1A**). Western blot data also demonstrated that SIX1 protein was highly expressed in HCC tissues and weakly expressed in the adjacent non-tumor tissues (**Figure 1B**).

For confirmation the subcellular localization of SIX1 protein, the immunofluorescence staining for SIX1 protein was performed, and it revealed that SIX1 protein is mainly located at the cytoplasm in HepG2 HCC cells. Meanwhile, the positive signals for SIX1 protein is also observed at the nucleus (**Figure 2**).

The immunohistochemical staining also showed that SIX1 protein was mainly positive in cytoplasm/perinucleus in HCC, only small number of cells is nuclear staining pattern. Of note, scattered adjacent non tumor liver cells were strongly positive in perinucleus and nucleus for the SIX1 protein (**Figure 3C**), and cytoplasmic/perinuclear high expression of the SIX1 protein was frequently seen in blood and lymph vessels in both adjacent non tumor liver tissues (**Figure 3D**) and HCC (**Figure 3F**).

The SIX1 protein was expressed in a significantly greater number of HCCs (80.9%, 131/162) than in either adjacent non tumor liver tissues (36.8%, 32/87) or normal liver tissues (22.9%, 8/35) ($p < 0.01$). Similarly, SIX1 protein expression was strongly positive in 61.1% (99/162) of HCCs, which was significantly higher than in adjacent non tumor liver tissues (12.6%, 11/87) or normal liver tissues (5.7%, 2/35) ($P < 0.01$). (**Table 1**; **Figure 3**).

Clinicopathological significance of SIX1 protein overexpression in HCC

To evaluate the role of the SIX1 protein in HCC progression, we analyzed the correlation between SIX1 protein overexpression and the clinicopathological features of HCC. Clinicopathologic profiles and their relationships with SIX1 expression are summarized in **Table 1**. SIX1 protein overexpression is significantly cor-

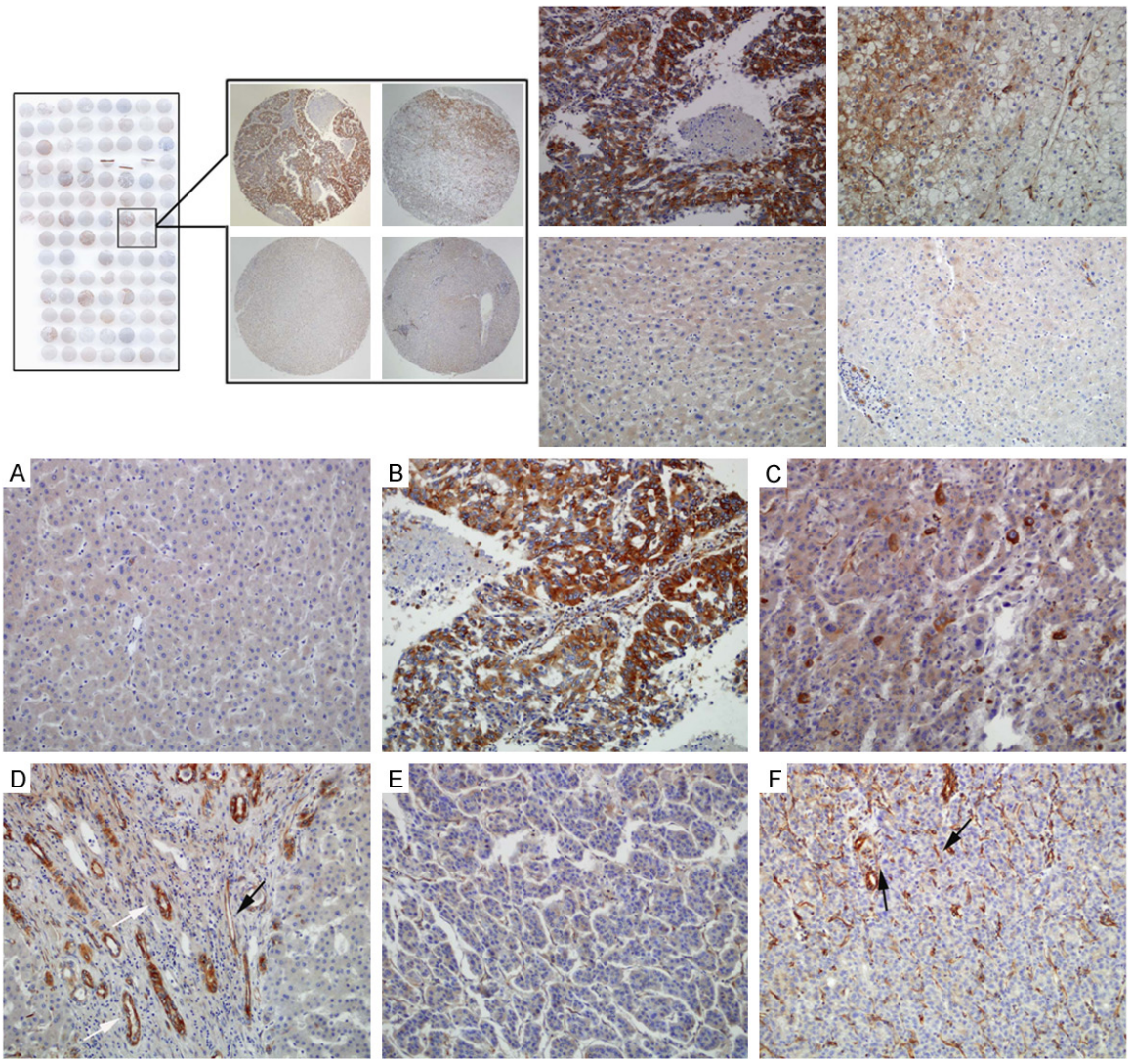


Figure 3. Immunohistochemical staining of the SIX1 protein in HCC tissuearray and normal liver tissues. A: SIX1 protein staining is negative in normal liver tissue. B: SIX1 protein shows strongly positive cytoplasmic signals in HCC tissue with venous infiltration. C: Scattered positive cells are observed in adjacent non tumor liver tissues of HCC. D: SIX1 protein is moderately positive in the blood vessels (black arrows) and bile ducts (white arrows) in the stroma of adjacent cirrhosis tissue of HCC. E: SIX1 protein staining is negative in HCC with no venous infiltration. F: Six1 protein is moderately positive in blood vessels in the stroma of HCC (black arrows). (Original magnification, 200 × in A-F).

related with tumor size, pTNM Stage and venous infiltration of the HCCs. Strongly positive SIX1 protein expression was significantly higher in HCCs that were ≥ 5 cm in size (60.5%) than in cases that were < 5 cm in size (39.5%) ($P < 0.05$). Similarly, we found that the strongly positive expression of the SIX1 protein in HCCs was significantly higher in patients with advanced stage (III-IV) tumors was (77.4%, 72/93) than in patients with early stage (I-II) tumors (24.6%, 17/69) ($P < 0.01$). For venous infiltration, we also found that strongly positive expression of the SIX1 protein was significantly higher in HCC cases with venous infiltration (89.5%, 51/57) than in cases without venous infiltration (36.2%, 38/105) ($P < 0.01$). However, it was found that SIX1 overexpression was not related to sex, age, AFP level or HBV infection status (**Table 2**).

SIX1 expression and pTNM stage are independent prognostic factors in HCC using the cox proportional hazard regression model

A total of 162 HCC patients with complete survival data were identified for prognostic evalua-

Table 1. SIX1 protein expression in HCC and normal liver tissues

Diagnosis	No. of cases	SIX1 protein expression				Positive rate (%)	Strongly positive rate (%)
		-	+	++	+++		
Tumor in HCC	162	31	32	53	46	80.9%**	61.1%**
Adjacent non-tumor	87	55	21	11	0	36.8%	12.6%
Normal liver tissues	5	27	6	2	0	22.9%	5.7%

Strongly positive: ++ and +++. **$P < 0.01$ compared with normal tissues.

Table 2. Correlation of SIX1 protein overexpression and clinicopathological features of HCC patients

Clinical features	No. of cases (*n*)	SIX1 strongly positive cases (%)	*P* value
Gender			
Male	121	68 (56.2)	0.600
Female	41	21 (51.2)	
Age			
≤ 50 years	87	47 (54.0)	0.800
> 50 years	75	42 (56.0)	
Tumor size			
< 5 cm	43	17 (39.5)	0.018*
≥ 5 cm	119	72 (60.5)	
pTNM Stage			
Early stage (I-II)	69	17 (24.6)	0.000**
Advanced stage (III-IV)	93	72 (77.4)	
Venous infiltration			
Absent	105	38 (36.2)	0.000**
Present	57	51 (89.5)	
AFP level			
≤ 20 ng/ml	49	29 (59.2)	0.475
> 20 ng/ml	113	60 (53.1)	
HBsAg status			
Negative	26	18 (69.2)	0.110
Positive	136	71 (52.2)	

AFP = alpha fetoprotein; HCC = hepatocellular carcinoma; pTNM = pathologic tumor node metastasis. *P < 0.05; **P < 0.01.

tion. Of the 162 patients, 107 (66.0%) had died from HCC, while 55 (34.0%) remained alive. HCC patients with strongly positive SIX1 expression had lower disease-free and 5-year survival rate compared with those that had low SIX1 expression using the Kaplan-Meier method (**Figure 4A** and **4B**). Moreover, we analyzed the prognostic value of SIX1 expression in selective patient subgroups stratified according to pTNM. The expression of SIX1 was strongly associated with the 5-year survival rate duration of early stage (I-II) patients with HCC, but not related with advanced stage (III-IV) patients (**Figure 4C** and **4D**; $P = 0.865$), indicating that SIX1 protein expression might be a useful biomarker for prognostic evaluation of HCC.

Additionally, on univariate analysis, tumor size, pTNM stage, venous infiltration and SIX1 expression were also associated with 5-year survival rate of the HCC patients with high SIX1 expression (**Table 3**). These data suggest that SIX1 could also be a valuable prognostic factor of HCC. Therefore, the further multivariate analysis was also performed using the Cox proportional hazards model for all of the significant variables in the univariate analysis. We found that pTNM stage (95% CI: 1.101-2.511; $P = 0.016$) proved to be an independent prognostic factor for survival in HCC. Importantly, SIX1 overexpression also emerged as a significant independent prognostic factor in HCC (95% CI: 1.059-2.521; $P = 0.026$) (**Table 3**).

Discussion

Homeoprotein transcription factor SIX1 is located at chromosome 14q23 of the chromosome, is involved in the early developments of diverse organs such as the brain, and kidney [8, 9]. Iwanaga *et al* [15] and Wan *et al* [16] reported that SIX1 protein was mainly localized in the nucleus in both breast cancer and cervical cancer; however, Fougerousse *et al* [17] found that the Six homeoproteins are mostly observed in the cytoplasm at 4 weeks of embryogenesis, and Wu *et al* [18] also demonstrated that distinct regions of Six1 protein and different servers predicted different localizations in cells. Our previous study has shown that SIX1 protein was mainly positive in cytoplasm/perinucleus in PDAC, only small number of cells is nuclear staining pattern. Here we

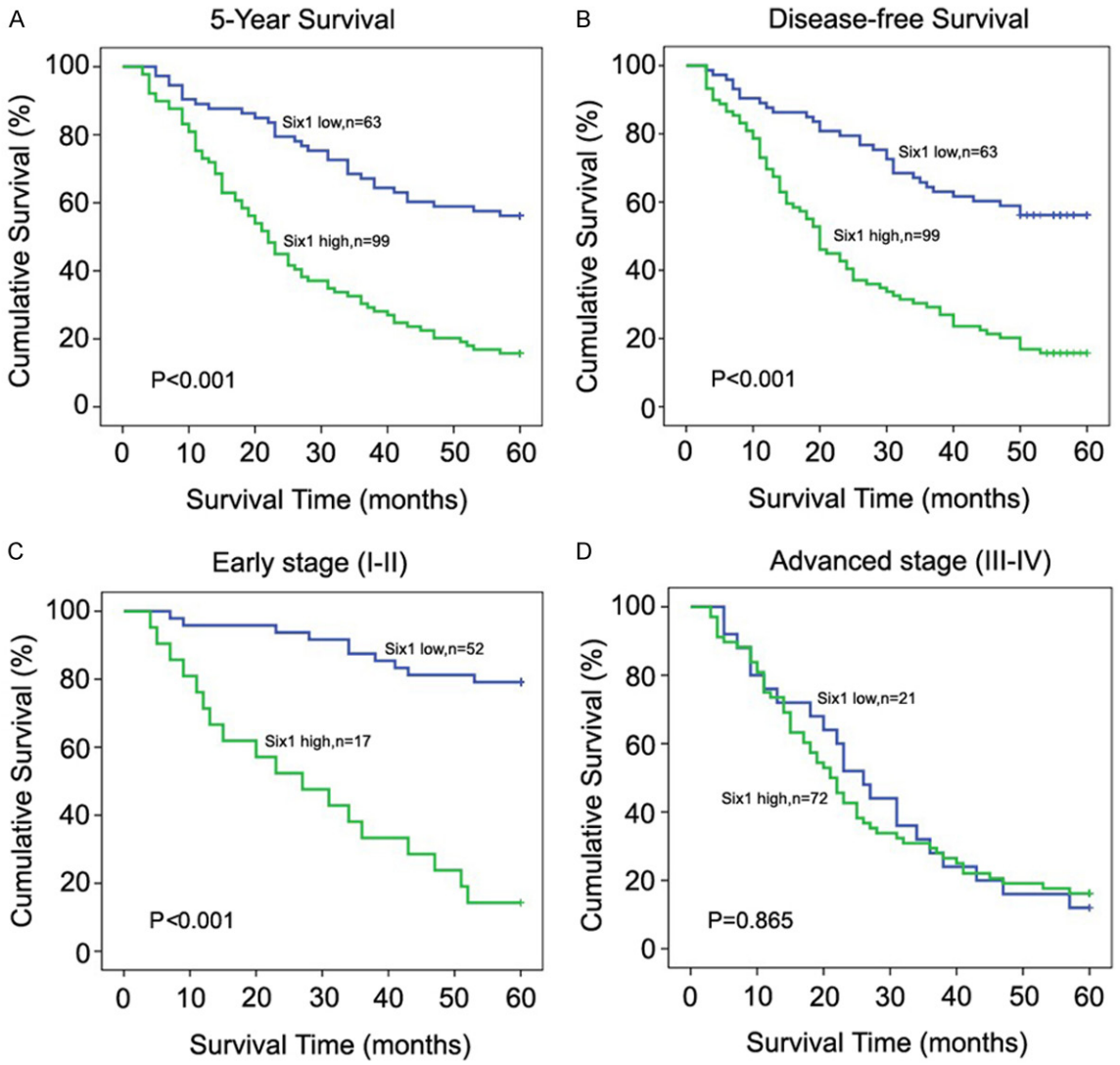


Figure 4. Kaplan-Meier survival curves illustrating the significance of SIX1 expression in HCC. (A, B) HCC patients with high SIX1 expression had a lower 5-year and disease-free survival rate compared to those with low SIX1 expression ($P < 0.001$). (C) The expression status of SIX1 was strongly associated with the 5-year survival rate duration of patients with early stage (I-II) of HCC, (D) But not related with the 5-year survival rate of patients with advanced stage (III-IV) of HCC ($P = 0.865$).

found that SIX1 protein was mainly localized at the cytoplasm of HCC cells using immunofluorescence staining in HepG2 HCC cells and immunohistochemical analysis with paraffin-embedded HCC tissues, indicating that the molecular mechanisms controlling Six homeoproteins localization are still unclear.

Accumulating evidence suggests that SIX1 is deregulated in various mammalian cancers and overexpression of SIX1 leads to an increase in the malignancy of tumors, which results in higher mortality rates for cancer patients [12, 19]. Reichenberger *et al* [20] reported that SIX1 was not expressed in normal breast epithelial cell lines, but was highly expressed in breast cancer cell lines; additionally, SIX1 expression was highly in metastatic cell lines than in primary breast cancer cell lines. Ng *et al* [21] found that ~85% and 60% of HCCs overexpressed SIX1 mRNA and protein, respectively, compared with non tumor liver tissues. They also found that SIX1 protein was not detected in HCC non tumor liver tissues or normal liver tissues, and increased SIX1 protein expression in HCC patients is significantly correlated with pTNM stage, venous infiltration and poor overall survival.

Table 3. Univariate and multivariate survival analyses (Cox regression model) of various factors in patients with HCC

Factors	B	SE	Wald	Exp (B)	95% CI for Exp (B)		*P* value
					Lower	Upper	
Univariate analysis							
Gender	0.305	0.184	2.765	1.357	0.947	1.945	0.096
Age	0.063	0.158	0.161	1.065	0.782	1.451	0.688
Size	0.389	0.179	4.719	1.475	1.039	2.095	0.030*
pTNM stage	0.816	0.164	24.733	2.261	1.639	3.119	0.000**
Venous infiltration	0.332	0.166	3.977	1.394	1.006	1.931	0.046*
AFP level	0.191	0.171	1.243	1.211	0.856	1.694	0.265
HBVs antigen	0.389	0.216	3.240	1.476	0.966	2.256	0.072
SIX1	0.756	0.162	21.710	2.129	1.549	2.925	0.000**
Multivariate analysis							
Size	0.174	0.188	0.858	1.191	0.823	1.722	0.354
pTNM stage	0.508	0.210	5.842	1.663	1.101	2.511	0.016*
Venous infiltration	-0.114	0.191	0.355	0.892	0.614	1.298	0.552
SIX1 expression	0.491	0.221	4.931	1.634	1.059	2.521	0.026*

*P < 0.05; **P < 0.01.

The present study investigated the clinicopathological significance of SIX1 overexpression in HCC patients using IHC. We found that the SIX1 protein was expressed in a significantly greater number of HCCs than in either adjacent non tumor liver tissues or normal liver tissues. Similarly, SIX1 protein expression was strongly positive in 61.1% of HCCs, which was significantly higher than in non tumor liver tissues or in normal liver tissues. qRT-PCR and Western blot showed that SIX1 expression was up-regulated in HCCs compared with non tumor liver tissues. These data indicate that SIX1 protein is upregulated in HCC and is implicated in the progression of HCC. Additionally, recent reports showed that SIX1 could induce lymphangiogenesis [22, 23]. In the present study, we also noticed that high expression of the SIX1 protein was frequently seen in blood and lymph vessels in adjacent non tumor liver tissues and in HCCs. Further study is needed to demonstrate the correlation between SIX1 expression and lymphangiogenesis in HCC.

Overexpression of SIX1 was correlated with advanced tumor stage in pancreatic cancer [24] and cervical cancer [25]. Ng *et al* [21] showed that the SIX1 protein was expressed only in metastatic HCC cell lines (MHCC97L and MHCC97H) but not in non-metastatic HCC cell lines (Hep3B, Huh7 and PLC), suggesting that SIX1 may be a metastasis-associated oncogene that participates in the metastatic process in HCC. Here we found that overexpression of the SIX1 protein significantly correlated with pTNM stage, venous infiltration and tumor size, suggesting that SIX1 may play an important role in HCC progression and invasion. However, SIX1 expression level did not correlate with sex, age, AFP level or HBV infection in HCC. Of note, Ng *et al* [21] found that high level expression of SIX1 in HCC did not correlate with tumor size at the mRNA or protein levels by RT-PCR and western blot, respectively. However, in the present study, strongly positive SIX1 protein expression was significantly higher in HCCs ≥ 5 cm in size than in cases < 5 cm in size ($p < 0.05$). Accordingly, further study is needed to explore the mechanisms involved in SIX1 overexpression in HCC progression.

Tumor recurrence and metastases of HCC after hepatectomy are the major obstacles to long-term survival. In the present study, survival analysis showed a strong correlation between SIX1 protein expression level and prognosis of HCC patients. HCC patients with higher SIX1 protein levels had a significantly lower disease-free and 5-year survival rate when compared with patients with lower SIX1 protein expression levels using the Kaplan-Meier analysis. Interestingly, the expression level of SIX1 protein was strongly associated with the 5-year survival rate duration of patients with early stage HCC, but no correlation was found in patients with advanced stage HCC. Similar

results were reported by Ng *et al* [21] in that SIX1 played an important role in the progression of HCC and might be a new molecular target for HCC therapy. Li *et al* [26] provided evidence that knockdown of Six1 may inhibit colorectal cancer progression. Additionally, Patrick *et al* [27] demonstrated that mice injected with MCF7 breast cancer cells transfected with SIX1 had an overall shortened survival compared to mice injected with MCF7-Control cells. Here we found that pTNM stage ($P = 0.016$) proved to be an independent prognostic factor for survival in HCC. Importantly, SIX1 overexpression also emerged as a significant independent prognostic factor in HCC, indicating that SIX1 might be a biomarker for prognostic evaluation of HCC.

In conclusion, SIX1 protein could be a novel biomarker for predicting short-term overall survival of HCC patients. It has also been suggested that SIX1 could be a novel therapeutic marker for selectively targeting cancer cells. Further studies are therefore worthwhile to explore the mechanism of action of SIX1 and to investigate its potential as a therapeutic target in HCC progression.

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Disclosure of conflict of interest

None.

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