Int J Clin Exp Pathol 2019;12(7):2682-2690
www.ijcep.com /ISSN:1936-2625/IJCEP0094480

Original Article

The prognostic value of homeobox B7 expression in patients with hepatocellular carcinoma

Yarong Guo[1], Xiaojiang Qin[2], Bao Chai[3], Junmei Jia[1], Jiahong Yi[1], Kang Wang[1], Xiaomin Hou[4]

[1]Department of Oncology, The First Clinical Hospital of Shanxi Medical University, Taiyuan, Shanxi Province, China; [2]School of Public Health, [3]Department of Pharmacology, Shanxi Medical University, Taiyuan, Shanxi Province, China; [4]Department of Gastroenterology and Hepatology, Shanxi Academy of Medical Sciences Shanxi DAYI Hospital, Taiyuan, Shanxi Province, China

Received March 27, 2019; Accepted May 23, 2019; Epub July 1, 2019; Published July 15, 2019

Abstract: The prognostic role of homeobox B7 (HOXB7) in hepatocellular carcinoma (HCC) is not clearly established. The present study aimed to evaluate the associations among the clinicopathological characteristics, HOXB7 expression, and the overall survival (OS) of patients with HCC. An immunohistochemical analysis was used to detect the expression level of HOXB7. In addition, the association between the expression of HOXB7 and the clinicopathological characteristics of HCC was analyzed. The Kaplan-Meier method was used to calculate the survival rates, and the COX proportional hazards model was used to investigate univariate and multivariate analyses. A total of 80 patients were enrolled in this study. Of the 80 HCC samples, HOXB7 was up-regulated in 28 samples (35.0%). The high HOXB7 expression was significantly associated with OS by univariate Cox regression analysis (HR = 2.0; 95% CI = 1.1-3.4, P = 0.016). The median survival with high HOXB7 expression and low HOXB7 expression was 12.5 months ± 3.7 months versus 32.5 months ± 4.7 months, respectively, as visualized on Kaplan-Meier curves (P = 0.014). After adjusting for possible factors related to survival time after HCC resection, the results suggested that survival time was negatively correlated with high HOXB7 expression (HR = 2.592, 95% CI = 1.283-5.239, P = 0.008). The present data indicate that the HOXB7 expression was negatively associated with survival time after HCC resection. As HOXB7 was a common and readily available measurement in the clinical setting, it was a convenient and feasible way to identify those patients who were at high risk and who had a poor prognosis.

Keywords: Hepatocellular carcinoma, homeobox B7, postoperative survival

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and a leading cause of cancer-related death worldwide [1, 2]. HCC represents the major histological subtype, accounting for 70%-85% of the total liver cancer burden worldwide [3]. Traditionally, the prediction of HCC outcome is based on tumor-cell-based risk stratification systems, such as the TNM staging system, tumor invasion depth, lymph node metastasis, and distant metastasis [4]. However, these clinicopathological factors cannot provide complete prognostic information because they do not incorporate tumor-microenvironment information. Therefore, the identification of novel clinical biomarkers to predict prognosis and recurrence, as well as further investigation of therapeutic targets, are critically required to improve outcomes in HCC patients.

Hepatectomy is the only curative treatment available in most patients with HCC [5]. However, its long-term prognosis is not satisfactory, and the probability of survival decreases further, even at more than 5 years after the first operation [6]. Therefore, there is a great need to identify biomarkers that can be used to predict long-term clinical outcome in patients with HCC. To the best of our knowledge, there is no study investigating the clinical significance of homeobox B7 (HOXB7) in HCC. In this study, we showed that the overall survival (OS) of patients with high HOXB7 expression was significantly lower than it was in patients with low HOXB7 expression. Moreover, HOXB7 expression was an independent prognostic factor for

OS, suggesting its usefulness as a promising predictor of long-term prognosis.

HOXB7, an important member of the HOX family, has been found to be involved in the process of many cancers, including oral squamous cell carcinoma, breast cancer, and colorectal cancer [7, 8]. In addition, the overexpression of HOXB7 in these patients is associated with poor prognosis [7]. Additional studies revealed that enforced HOXB7 expression promoted cell proliferation [9], angiogenesis [10], invasion, and metastasis [11]. The results suggest that HOXB7 may be a valuable prognostic biomarker for HCC patients. However, little is known about the association between the expression level of HOXB7 and the survival time after the resection of HCC.

In the present study, immunohistochemistry was performed to analyze the correlation between HOXB7 expression and the clinicopathological factors of patients. Additionally, we evaluated the HOXB7 protein as a biomarker for predicting survival in patients with HCC.

Materials and methods

Tissue samples from HCC patients and follow-up

From January 2010 to September 2015, all patients with HCC were identified retrospectively through a review of hospital admitting notes at the Institution of Hepatobiliary Surgery, First Hospital, Shanxi Medical University (Taiyuan, China). The preexisting paraffin-embedded tissue samples of HCC were collected from patients who underwent curative resection. The final survival rate was determined via the review of hospital admitting notes or telephone follow-up. Patients were observed until January 2018, and the median follow-up time was 31 months while the longest was 80 months. Follow-up data were obtained for all the 80 patients. The follow-up period was defined as the interval between the date of surgery and the date of patient death or the last follow up. All patients were followed up one month after hepatectomy, then every three months during the first year after surgery, and every six months thereafter. OS was defined as the interval between the dates of surgery and death or the last visit. The baseline clinicopathological features of these patients, including age, gender, the degree of differentiation, pathological type, the location of the tumor, size of the tumor, number of tumors, Child-Pugh, liver function parameters, serum alpha-fetoprotein (AFP) level, and other tumor-related parameters, were all recorded.

All patients submitted an informed consent according to protocols approved by the Institutional Review Board of the First Hospital, Shanxi Medical University (approval no. 20100109), and this study complied with the ethical guidelines of the Helsinki Declaration. The inclusion criteria of this study were: (1) Age ≥ 18 years; (2) Confirmed hepatocellular carcinoma by Pathology after the operation; (3) The HCC patient underwent surgical resection for the first time. Patients were excluded if: (1) They had been previously diagnosed with hepatobiliary cell carcinoma, mixed cell carcinoma, or fibrous lamellar cell carcinoma; (2) They were preparing to undergo a liver transplantation (except those patients who had undergone a liver transplantation in the past); (3) They had ascites with clinical symptoms, requiring therapeutic paracentesis or drainage, or a Child-Pugh score > 2. For all eligible patients, we reviewed all of their computed tomography angiography scans. The anatomic characteristics were evaluated and documented in the chart at the time of assessment.

Immunohistochemistry

The surgical tissues were fixed with 10% formalin, then the paraffin-embedded tissue samples from the HCC patients were cut into 4 μm thick sections, deparaffinized with xylene, and rehydrated through graded alcohol washes. Tumor tissues were also sectioned at 4 μm thickness using a freezing microtome (Leica, Barnack, Germany). Antigen retrieval was performed for all sections by heating in a microwave oven, and endogenous peroxidase activity was blocked with a 3% H_2O_2 solution. The sections were then incubated with the anti-HOXB7 antibody (1:200; Abcam, Cambridge, MA, USA) at 4°C overnight. Immunohistochemical assays were carried out using the DAKO EnVision Detection System.

The immunohistochemical staining scores of HOXB7 in liver tissues were assessed using a semi-quantitative method by two experienced pathologists who were blinded to the clinico-

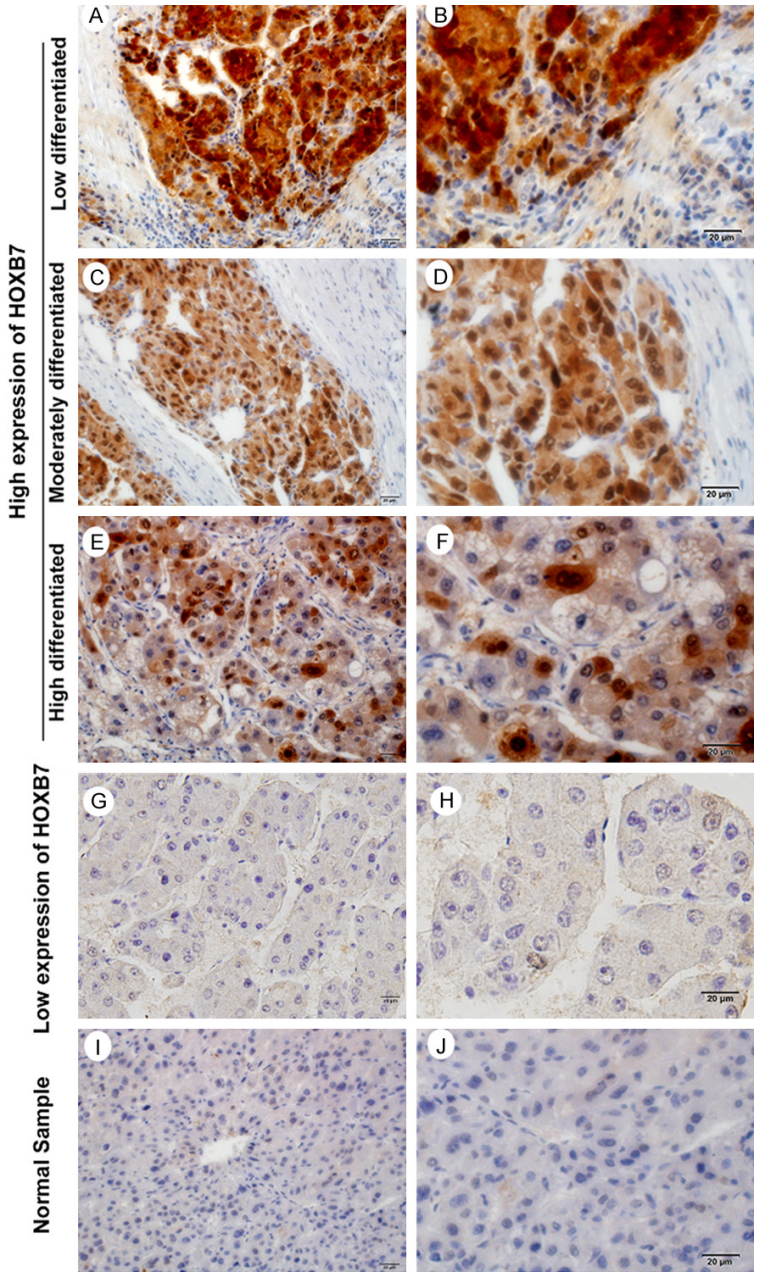

Figure 1. Immunohistochemical staining of HOXB7 expression in HCC tissue and normal tissue. The high expression of HOXB7 in low differentiated tissues (A, B), moderately differentiated tissues (C, D), high differentiated tissues (E, F) and low expression of HOXB7 in HCC tissue (G, H) and normal samples (I, J). Scar bar = 20 μm.

pathological data of the patients as described previously [9, 12]. The staining score was assessed as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). A high expression level was defined as staining score ≥ 2 with at least 50% of malignant cells showing positive HOXB7 staining, and a low expression level was defined as < 50% of malignant cells showing nuclear staining or a staining score < 2 [13].

Statistical analysis

All data were double entered and then exported to tab-delimited text files. All analyses were performed with R (http://www.R-project.org), and SPSS 19.0 statistical software (IBM, Armonk, NY, USA) was used for the statistical analysis. The association between HOXB7 expression and the clinicopathological parameters of HCC was tested using the χ^2 test or Fisher's exact test. The Kaplan-Meier method was used to estimate the survival rate of patients with high or low HOXB7 expression, and the differences between the survival curves were estimated by the Kaplan-Meier method and compared using a log-rank test. The Cox proportional hazards model was used to adjust the potentially confounding variables and to determine the independent prognostic factors. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The selection of patients in HCC

During the study period, 403 HCC patients received treatment at our hospital, and 116 (28.8%) were excluded because they had been treated initially for HCC at other centers. Among the remaining 287 patients, 127 (44.3%) had solitary nodular tumors without extrahepatic metastases or portal tumor thromboses. Thirty-five of these patients (27.6%) were excluded because they had received only local ablation therapy, ethanol injection or transarterial chemoembolization, and another 12

Table 1. The association between HOXB7 expression and the clinicopathological features in patients with hepatocellular carcinoma (n = 80)

Characteristic	HOXB7 expression		*P*-value
	Low, n (%)	High, n (%)	
Gender			0.528
Male	47 (90.4%)	24 (85.7%)	
Female	5 (9.6%)	4 (14.3%)	
Age			0.527
< 60	24 (30%)	15 (18.75%)	
≥ 60	28 (35%)	13 (16.25%)	
Differentiation			0.832
Well-differentiated	18 (34.6%)	8 (28.6%)	
Moderately differentiated	28 (53.8%)	17 (60.7%)	
Poorly differentiated	6 (11.5%)	3 (10.7%)	
Pathological type			0.761
Hepatocellular carcinoma	15 (28.8%)	6 (21.4%)	
Cholangiocarcinoma	21 (40.4%)	12 (42.9%)	
Mixed cell carcinoma	16 (30.8%)	10 (35.7%)	
Hepatic cirrhosis			0.472
No	19 (36.5%)	8 (28.6%)	
Yes	33 (63.5%)	20 (71.4%)	
HBV			0.205
No	30 (57.7%)	12 (42.9%)	
Yes	22 (42.3%)	16 (57.1%)	
HbsAg			0.160
Negative	29 (55.8%)	11 (39.3%)	
Positive	23 (44.2%)	17 (60.7%)	
Vessel invasion			0.039
No	38 (73.1%)	14 (50.0%)	
Yes	14 (26.9%)	14 (50.0%)	
Lymph node invasion			0.082
No	31 (59.6%)	11 (39.3%)	
Yes	21 (40.4%)	17 (60.7%)	
Diameter of tumor			0.121
≤ 5 cm	28 (53.8%)	10 (35.7%)	
> 5 cm	24 (46.2%)	18 (64.3%)	
Number of tumor			0.091
Single	25 (48.1%)	8 (28.6%)	
Multiple	27 (51.9%)	20 (71.4%)	
Location of tumor			0.961
Left lobe of liver	17 (32.7%)	10 (35.7%)	
Right lobe of liver	23 (44.2%)	12 (42.9%)	
Border around the liver	12 (23.1%)	6 (21.4%)	
Pre-operation AFP			0.087
≤ 400 μg/L	21 (40.4%)	6 (21.4%)	
> 400 μg/L	31 (59.6%)	22 (78.6%)	
Child-Pugh			0.027
5-6 Score	23 (44.2%)	5 (17.9%)	

(9.4%) were also excluded because they did not participate in the follow-up. The remaining 80 (63.0%) patients satisfied our inclusion criteria and were included in our study.

The expression of HOXB7 protein in HCC tissues

Immunohistochemistry was used to confirm the expression of the HOXB7 protein in HCC. The HOXB7 protein in the HCC samples was mainly localized in the nuclei and cytoplasms. 28 patients had a high HOXB7 expression (score ≥ 2), and 52 patients had a low HOXB7 expression (score < 2), and the representative images of patients with various differentiation and control are shown in **Figure 1**.

The association between HOXB7 expression and the clinicopathological characteristics of patients with HCC

A total of 80 patients were enrolled in this study. The correlation between HOXB7 expression and the clinicopathologic characteristics of patients with HCC are shown in **Table 1**. Of the 80 HCC samples, HOXB7 was highly expressed in 28 samples (35.0%). The χ^2 test showed that the high expression of HOXB7 was significantly associated with the vessel invasion (P = 0.039) and Child-Pugh (P = 0.027), but was not correlated with gender, age, differentiation, pathological type, hepatic cirrhosis, HBV, HbsAg, lymph node invasion, diameter of tumor, number of tumor, location of tumor, pre-operation AFP, or death events (P > 0.05).

Prognostic significance of HOXB7 expression in HCC

To identify independent factors for predicting patient survival, we put all the clinical and pathological parameters that exhibited significant correlation with HCC patient survival (including age, HBsAg status, anti-HCV status, liver cirrhosis, serum AFP levels,

7-9 Score	20 (38.5%)	12 (42.9%)	
10-15 Score	9 (17.3%)	11 (39.3%)	
Death events			0.062
No	22 (42.3%)	16 (57.1%)	
Yes	30 (57.7%)	22 (78.6%)	

HOXB7, homeobox B7; HBV, hepatitis B virus; HbsAg, hepatitis B surface antigen; AFP, alpha fetoprotein.

vessel invasion, lymph node invasion, tumor size, tumor number, tumor location and Child-Pugh) in a univariate analysis. As presented in **Table 2**, the results indicated that high HOXB7 expression was significantly associated with OS by a univariate Cox regression analysis (HR = 2.0; 95% CI = 1.1-3.4, *P* = 0.016). Specifically, HCC patients with low expressions of HOXB7 had a relatively better survival prognosis. In addition, hepatic cirrhosis, vessel invasion, lymph node invasion, the diameter of the tumor, the number of tumors, and pre-operation AFP were independent prognostic factors for OS (all $P < 0.05$).

As shown in **Figure 2**, Kaplan-Meier survival curves were used to evaluate the correlation between the OS of patients with HCC and HOXB7 expression. The log-rank test showed that OS was significantly different between low HOXB7 expression and high HOXB7 expression (*P* = 0.014). The median survival times of high HOXB7 expression and low HOXB7 expression were 12.5 months ± 3.7 months versus 32.5 months ± 4.7 months, respectively, as visualized on Kaplan-Meier curves.

Multivariable linear regression models suggested that the survival time after the resection of HCC was obviously negatively correlated with a high HOXB7 expression level in the non-adjusted model (HR = 1.978, 95% CI = 1.136-3.444, *P* = 0.016, **Table 3**). In the adjust I model, after adjusting for possible factors related to survival time after the resection of HCC, including hepatic cirrhosis, preoperative AFP, the diameter of the tumor, surgical procedures, and Child-Pugh, the results suggested that the survival time after the resection of HCC was obviously negatively correlated with high HOXB7 expression (HR = 2.487, 95% CI = 1.242-4.981, *P* = 0.010, **Table 3**). In the adjust II model, after adjusting for possible factors related to the survival time after the resection of HCC, including degree of differentiation, pathological type, hepatic cirrhosis, vessel invasion, surgical procedures, Child-Pugh, preoperative AFP, the number of tumors, and the diameter of the tumor, the results suggested that the survival time after resection of HCC was obviously negatively correlated with high HOXB7 expression (HR = 2.592, 95% CI = 1.283-5.239, *P* = 0.008, **Table 3**). Thus, HOXB7 expression is a significant independent prognostic factor in patients with HCC.

Discussion

HCC is one of the most common malignant tumors in the world, with almost 600,000 to 700,000 deaths occurring as a result of the disease every year, and with more than 50% of all such deaths occurring in China alone [14, 15]. Currently, hepatectomy is the only curative treatment available in most patients with HCC [14]. However, the high recurrence rate affects the long-term prognosis of HCC. Previous studies have shown that the probability of recurrence of HCC within 5 years after treatment is also very high (up to 70%) [16]. Thus, there is a great need to identify reliable biomarkers that can be used to predict long-term clinical outcomes in patients with HCC.

To the best of our knowledge, this is the first study to investigate the preoperative HOXB7 expression level as a prognostic marker in HCC patients initially treated with curative hepatectomy. HOXB7 is a member of the homeobox gene family and has been shown to play an important role in the regulation of tumorigenesis and in the metastases of many cancers, including proliferation [17, 18], metastasis [9], and angiogenesis [18, 19]. HOXB7 is reported to function as a transcription factor for various oncogenes by binding chromatin in the nucleus [17]. Our results suggest that HOXB7 protein expression is enhanced in both the cytoplasms and nuclei in HCC patients. Moreover, several epidemiological studies have demonstrated that high HOXB7 expression was also found to be associated with poor prognosis in renal cell carcinoma [20], gastric cancer [21], and breast cancer [22]. In agreement with these studies [23], we confirmed that HCC patients with lower HOXB7 expressions showed longer survival times after curative resection.

Table 2. Univariate analysis of HOXB7 protein expression with various clinical and pathological features in HCC patients (n = 80)

Variable	No. (%)	HR (95% CI)	*p*-Value
Gender			
Male	71 (88.8%)	1.0	
Female	9 (11.2%)	1.2 (0.5, 2.9)	0.643
Age			
< 60	39 (48.75%)	1.0	0.564
≥ 60	41 (51.25%)	1.1 (0.6, 1.9)	
HOXB7			
Low	52 (65.0%)	1.0	
High	28 (35.0%)	2.0 (1.1, 3.4)	0.016
Degrees of differentiation			
Poorly differentiated	9 (11.2%)	1.0	
Moderately differentiated	45 (56.2%)	1.2 (0.5, 3.2)	0.663
Well-differentiated	26 (32.5%)	1.8 (0.7, 5.0)	0.222
Pathological type			
Hepatocellular carcinoma	21 (26.2%)	1.0	
Cholangiocarcinoma	33 (41.2%)	1.7 (0.8, 3.7)	0.140
Mixed cell carcinoma	26 (32.5%)	2.2 (1.0, 4.7)	0.049
Hepatic cirrhosis			
No	27 (33.8%)	1.0	
Yes	53 (66.2%)	11.5 (4.5, 29.4)	< 0.001
HBV			
No	42 (52.5%)	1.0	
Yes	38 (47.5%)	1.4 (0.8, 2.4)	0.219
HbsAg			
Negative	40 (50.0%)	1.0	
Positive	40 (50.0%)	1.5 (0.8, 2.5)	0.172
Vessel invasion			
No	52 (65.0%)	1.0	
Yes	28 (35.0%)	7.4 (4.1, 13.4)	< 0.001
Lymph node invasion			
No	42 (52.5%)	1.0	
Yes	38 (47.5%)	12.7 (6.5, 25.0)	< 0.001
Diameter of tumor			
≤ 5 cm	38 (47.5%)	1.0	
> 5 cm	42 (52.5%)	14.3 (6.9, 29.8)	< 0.001
Number of tumor			
Single	33 (41.2%)	1.0	
Multiple	47 (58.8%)	19..1 (7.8, 46.7)	< 0.001
Location of tumor			
Left lobe of liver	27 (33.8%)	1.0	
Right lobe of liver	35 (43.8%)	0.8 (0.4, 1.4)	0.384
Border around the liver	18 (22.5%)	1.1 (0.5, 2.2)	0.816
Child-Pugh			
5-6 Score	28 (35.0%)	1.0	
7-9 Score	32 (40.0%)	3.5 (1.7, 7.0)	< 0.001
10-15 Score	20 (25.0%)	2.4 (1.1, 5.2)	0.034

Pre-operation AFP			
≤ 400 μg/L	34 (42.5%)	1.0	
> 400 μg/L	46 (57.5%)	7.3 (3.7, 14.5)	< 0.001
Surgical procedures			
The left and right half of liver resection	27 (33.8%)	1.0	
Liver segment resection	35 (43.8%)	3.5 (1.7, 7.0)	0.001
Lobe or local excision	20 (25.0%)	2.4 (1.1, 5.2)	0.034
Survival time	30.5 + 23.9	0.0 (0.0, inf.)	0.963

HOXB7, homeobox B7; HBV, hepatitis B virus; HbsAg, hepatitis B surface antigen; AFP, alpha fetoprotein.

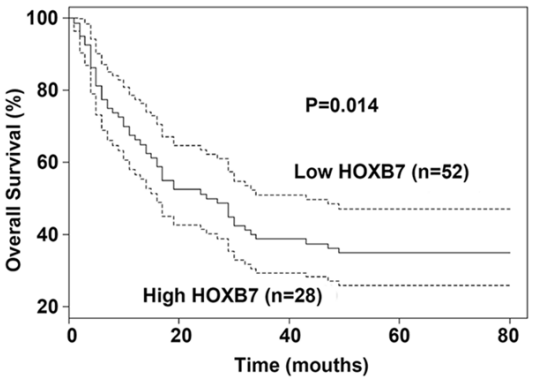

Figure 2. Kaplan-Meier overall survival curves for the 80 hepatocellular carcinoma patients eligible for survival analysis with regard to HOXB7 expression.

In the present study, we found that HOXB7 was highly expressed in 35.0% of HCCs, and its expression significantly correlated with hepatic cirrhosis, vessel invasion, lymph node invasion, the diameter of the tumor, the number of tumors, pre-operation AFP, and patient survival. Currently accepted survival indicators are still based on tumor staging and histopathological observation [24, 25]. To further characterize the clinical presentation and implication of HOXB7 in HCC, our results revealed that high HOXB7 expression was significantly associated with OS by univariate Cox regression analysis (HR = 2.0; 95% CI = 1.1-3.4, *P* = 0.016). Meanwhile, the subgroup analysis did not reveal any significant differences between the OS and the HOXB7 expression in HCC patients (Table S1). A Kaplan-Meier survival analysis revealed that HOXB7 expression was significantly correlated with poor prognosis after surgical resection in HCC patients. Moreover, multivariable linear regression models suggest that the survival time after resection of HCC is clearly negatively correlated with high HOXB7 expression levels in the non-adjusted model (HR = 1.978, 95% CI = 1.136-3.444, *P* = 0.016). In the adjust II model, after adjusting to possible factors related to survival time after the resection of HCC, including the degree of differentiation, pathological type, hepatic cirrhosis, vessel invasion, surgical procedures, Child-Pugh, preoperative AFP, the number of tumors, and the diameter of the tumor, the results suggest that the survival time after resection of HCC is obviously negatively correlated with high HOXB7 expression (HR = 2.592, 95% CI = 1.283-5.239, P = 0.008). Thus, these results suggest that HCC patients with a high expression level of HOXB7 probably have a poor prognosis, and the HOXB7 protein may become a useful prognostic indicator for HCC.

Although we have proved the prognostic significance of HOXB7 expression in HCC patients, there are some limitations in our study. First of all, due to the retrospective design of the study, selection bias was inevitable, which might influence the survival analysis. Secondly, we detected the expression of HOXB7 by means of immunohistochemistry, which was somewhat subjective. Recurrence-free survival was not analyzed owing to lack of RFS data. Last but not least, as the study cohort was comprised of a small single-center sample, we were unable to divide the data set into a training set and a testing set for statistical validation. The study was retrospectively designed in nature, so a large, multi-center, prospective studies with larger cohort data is needed to validate the results.

In summary, our findings showed that HOXB7 expression in HCC tissues might help clinicians predict the survival time of patients after resection. As a simple and cost-effective biomarker, prospective studies with larger cohorts are awaited to validate the clinical significance of HOXB7 as a prognostic marker for HCC patients. These findings have important clinical and public health implications.

Table 3. Multivariate logistic model for HOXB7 associated with postoperative survival of HCC (N = 80)

Exposure	Non-adjusted model		Adjust I model		Adjust II model	
	Hazard ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value
HOXB7						
Low	1.0		1.0		1.0	
High	1.978 (1.136, 3.444)	0.016	2.487 (1.242, 4.981)	0.010	2.592 (1.283, 5.239)	0.008

Non-adjusted model adjust for: None. Adjust I model adjust for: hepatic cirrhosis; preoperative AFP; diameter of tumor; surgical procedures; Child Pugh. Adjust II model adjust for: degrees of differentiation; pathological type; hepatic cirrhosis; vessel invasion; surgical procedures; Child Pugh; preoperative AFP; number of tumors; diameter of tumor.

Acknowledgements

This study was funded by the Shanxi Natural Science Foundation (nos. 201601D011131 and 201701D221259) and the Health and Family Planning Commission of Shanxi Province Scientific Research Project (no. 2015034).

Disclosure of conflict of interest

None.

Address correspondence to: Junmei Jia, Department of Oncology, The First Hospital of Shanxi Medical University, 85 South Jiefang Road, Taiyuan 030001, Shanxi Province, China. Tel: +86-351-4639461; Fax: +86-351-4639452; E-mail: jiajunmeishanxiedu@163.com

References

[1] Erridge S, Pucher P, Markar S, Malietzis G, Athanasiou T, Darzi A, Sodergren M and Jiao L. Meta-analysis of determinants of survival following treatment of recurrent hepatocellular carcinoma. Br J Surg 2017; 104: 1433-1442.

[2] Balogh J, Victor D 3rd, Asham EH, Burroughs SG, Boktour M, Saharia A, Li X, Ghobrial RM and Monsour HP Jr. Hepatocellular carcinoma: a review. J Hepatocell Carcinoma 2016; 3: 41-53.

[3] Li S and Mao M. Next generation sequencing reveals genetic landscape of hepatocellular carcinomas. Cancer Lett 2013; 340: 247-253.

[4] Zhang H, Wang X, Shen Z, Xu J, Qin J and Sun Y. Infiltration of diametrically polarized macrophages predicts overall survival of patients with gastric cancer after surgical resection. Gastric Cancer 2015; 18: 740-750.

[5] Tunissiolli N, Castanhole-Nunes M, Biselli-Chicote P, Pavarino E, da Silva R, da Silva R and Goloni-Bertollo E. Hepatocellular carcinoma: a comprehensive review of biomarkers, clinical aspects, and therapy. Asian Pac J Cancer Prev 2017; 18: 863-872.

[6] Gluer AM, Cocco N, Laurence JM, Johnston ES, Hollands MJ, Pleass HC, Richardson AJ and Lam VW. Systematic review of actual 10-year survival following resection for hepatocellular carcinoma. HPB 2012; 14: 285-290.

[7] Liao WT, Jiang D, Yuan J, Cui YM, Shi XW, Chen CM, Bian XW, Deng YJ and Ding YQ. HOXB7 as a prognostic factor and mediator of colorectal cancer progression. Clin Cancer Research 2011; 17: 3569-3578.

[8] De Souza Setubal Destro MF, Bitu CC, Zecchin KG, Graner E, Lopes MA, Kowalski LP, Coletta RD. Overexpression of HOXB7 homeobox gene in oral cancer induces cellular proliferation and is associated with poor prognosis. Int J Oncol 2010; 36: 141-149.

[9] Nguyen Kovochich A, Arensman M, Lay A, Rao N, Donahue T, Li X, French S and Dawson D. HOXB7 promotes invasion and predicts survival in pancreatic adenocarcinoma. Cancer 2013; 119: 529-539.

[10] Heinonen H, Lepikhova T, Sahu B, Pehkonen H, Pihlajamaa P, Louhimo R, Gao P, Wei G, Hautaniemi S, Jänne O and Monni O. Identification of several potential chromatin binding sites of HOXB7 and its downstream target genes in breast cancer. Int J Cancer 2015; 137: 2374-2383.

[11] Chen H, Lee JS, Liang X, Zhang H, Zhu T, Zhang Z, Taylor ME, Zahnow C, Feigenbaum L and Rein A. Hoxb7 inhibits transgenic HER-2/neu-induced mouse mammary tumor onset but promotes progression and lung metastasis. Cancer Res 2008; 68: 3637-3644.

[12] Du YB, Dong B, Shen LY, Yan WP, Dai L, Xiong HC, Liang Z, Kang XZ, Qin B and Chen KN. The survival predictive significance of HOXC6 and HOXC8 in esophageal squamous cell carcinoma. J Surg Res 2014; 188: 442-450.

[13] Wang JX, Yi Y, Li YW, Cai XY, He HW, Ni XC, Zhou J, Cheng YF, Jin JJ, Fan J and Qiu SJ. Down-regulation of sirtuin 3 is associated with poor prognosis in hepatocellular carcinoma after resection. BMC Cancer 2014; 14: 297.

[14] Chen F, Wang H, Zhu J, Zhao R, Xue P, Zhang Q, Bud Nelson M, Qu W, Feng B and Pi J. Camptothecin suppresses NRF2-ARE activity and sensitises hepatocellular carcinoma cells to anticancer drugs. Br J Cancer 2017; 117: 1495-1506.

[15] Zhang J, Zhang Q, Lou Y, Fu Q, Chen Q, Wei T, Yang J, Tang J, Wang J, Chen Y, Zhang X, Zhang J, Bai X and Liang T. Hypoxia-inducible factor-1α/interleukin-1β signaling enhances hepatoma epithelial-mesenchymal transition through macrophages in a hypoxic-inflammatory microenvironment. Hepatology 2018; 67: 1872-1889.

[16] Choi S, Kim S, Park C, Min J, Lee J, Choi Y and Lee B. Imaging features of gadoxetic acid-enhanced and diffusion-weighted MR imaging for identifying cytokeratin 19-positive hepatocellular carcinoma: a retrospective observational study. Radiology 2018; 286: 897-908.

[17] Tsuboi M, Taniuchi K, Shimizu T, Saito M and Saibara T. The transcription factor HOXB7 regulates ERK kinase activity and thereby stimulates the motility and invasiveness of pancreatic cancer cells. J Biol Chem 2017; 292: 17681-17702.

[18] Stiegelbauer V, Vychytilova-Faltejskova P, Karbiener M, Pehserl A, Reicher A, Resel M, Heitzer E, Ivan C, Bullock M, Ling H, Deutsch A, Wulf-Goldenberg A, Adiprasito J, Stoeger H, Haybaeck J, Svoboda M, Stotz M, Hoefler G, Slaby O, Calin G, Gerger A and Pichler M. miR-196b-5p regulates colorectal cancer cell migration and metastases through interaction with HOXB7 and GALNT5. Clin Cancer Res 2017; 23: 5255-5266.

[19] Yuan W, Zhang X, Xu Y, Li S, Hu Y and Wu S. Role of HOXB7 in regulation of progression and metastasis of human lung adenocarcinoma. Mol Carcinog 2014; 53: 49-57.

[20] Pritchett T, Bader H, Henderson J and Hsu T. Conditional inactivation of the mouse von Hippel-Lindau tumor suppressor gene results in wide-spread hyperplastic, inflammatory and fibrotic lesions in the kidney. Oncogene 2015; 34: 2631-2639.

[21] Joo M, Park J, Yoo H, Lee B, Chun H, Lee S and Bak Y. The roles of HOXB7 in promoting migration, invasion, and anti-apoptosis in gastric cancer. J Gastroenterol Hepatol 2016; 31: 1717-1726.

[22] Errico M, Jin K, Sukumar S and Carè A. The widening sphere of influence of HOXB7 in solid tumors. Cancer Res 2016; 76: 2857-2862.

[23] Komatsu H, Iguchi T, Masuda T, Ueda M, Kidogami S, Ogawa Y, Nambara S, Sato K, Hu Q, Saito T, Hirata H, Sakimura S, Uchi R, Hayashi N, Ito S, Eguchi H, Sugimachi K, Eguchi H, Doki Y, Mori M and Mimori K. HOXB7 expression is a novel biomarker for long-term prognosis after resection of hepatocellular carcinoma. Anticancer Res 2016; 36: 2767-2773.

[24] Feng L, Dong H, Lau W, Yu H, Zhu Y, Zhao Y, Lin Y, Chen J, Wu M and Cong W. Novel microvascular invasion-based prognostic nomograms to predict survival outcomes in patients after R0 resection for hepatocellular carcinoma. J Cancer Res Clin Oncol 2017; 143: 293-303.

[25] Huang Y, Liu Z, He L, Chen X, Pan D, Ma Z, Liang C, Tian J and Liang C. Radiomics signature: a potential biomarker for the prediction of disease-free survival in early-stage (I or II) non-small cell lung cancer. Radiology 2016; 281: 947-957.

Table S1. Subgroup analysis for the effect of the expression of HOXB7 on death events

Subgroup	N	Death events	
		HR (95% CI)	*p*-value
Gender			
Male	71	1.7 (1.0, 3.2)	0.067
Female	9	3.8 (0.7, 22.0)	0.130
Age Tertile group			
Low	27	2.8 (1.0, 7.4)	0.046
Middle	26	1.3 (0.5, 3.3)	0.547
High	27	2.1 (0.8, 5.5)	0.155
Degrees of differentiation			
Poorly differentiated	9	2.5 (0.4, 15.2)	0.334
Moderately differentiated	45	1.8 (0.9, 3.8)	0.123
Well-differentiated	26	2.5 (1.0, 6.6)	0.056
Pathological type			
Hepatocellular carcinoma	21	2.1 (0.6, 7.6)	0.264
Cholangiocarcinoma	33	1.5 (0.6, 3.4)	0.360
Mixed cell carcinoma	26	2.3 (0.9, 5.9)	0.070
Hepatic cirrhosis			
No	27	4.6 (0.8, 28.0)	0.096
Yes	53	2.1 (1.1, 3.8)	0.018
HbsAg			
Negative	40	2.6 (1.1, 5.9)	0.026
Positive	40	1.5 (0.7, 3.2)	0.285
HBV			
No	42	2.3 (1.0, 5.0)	0.039
Yes	38	1.6 (0.7, 3.5)	0.251
Vessel invasion			
No	52	1.5 (0.6, 3.4)	0.374
Yes	28	2.1 (0.9, 4.7)	0.083
Lymph node invasion			
No	42	1.6 (0.6, 4.7)	0.380
Yes	38	2.0 (1.0, 4.2)	0.054
Pre-operation AFP			
≤ 400 μg/L	34	1.3 (0.4, 5.0)	0.676
> 400 μg/L	46	1.7 (0.9, 3.2)	0.094
Location of tumor			
Left lobe of liver	27	2.6 (1.0, 6.7)	0.050
Right lobe of liver	35	1.5 (0.6, 3.5)	0.401
Border around the liver	18	2.5 (0.8, 7.6)	0.111
Diameter of tumor			
≤ 5 cm	38	3.2 (1.0, 10.6)	0.054
> 5 cm	42	1.5 (0.8, 2.8)	0.227
Number of tumor			
Single	33	1.6 (0.3, 8.6)	0.601
Multiple	47	2.1 (1.1, 4.0)	0.020
Surgical procedures			
The left and right half of liver resection	27	2.6 (1.0, 6.7)	0.050
Liver segment resection	35	1.5 (0.6, 3.5)	0.401
Lobe or local excision	18	2.5 (0.8, 7.6)	0.111
Child Pugh			
5-6 Score	28	0.4 (0.1, 3.2)	0.401
7-9 Score	32	2.7 (1.2, 6.2)	0.016
10-15 Score	20	2.3 (0.8, 7.0)	0.134