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Original Article

Latent transforming growth factor-beta binding protein-1 in circulating plasma as a novel biomarker for early detection of hepatocellular carcinoma

Bangrong Cao[1], Lei Yang[1], Weiqi Rong[2], Lin Feng[1], Naijun Han[1], Kaitai Zhang[1], Shujun Cheng[1], Jianxiong Wu[2], Ting Xiao[1], Yanning Gao[1]

[1]*State Key Laboratory of Molecular Oncology, Department of Etiology and Carcinogenesis, Cancer Institute & Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China;* [2]*Department of Abdominal Surgical Oncology, Cancer Institute & Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China*

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Abstract: This study aimed to assess the diagnostic value of the latent transforming growth factor-beta binding protein-1 (LTBP-1) in distinguishing hepatocellular carcinoma (HCC) from patients with hepatitis or liver cirrhosis. The protein levels of LTBP-1 or AFP in circulating plasma were measured by enzyme-linked immunosorbent assay (ELISA) or chemiluminescence in four cohorts: HCC (n = 167), liver cirrhosis (n = 50), chronic hepatitis B (CHB, n = 50), and healthy individuals (n = 104). Receiver operating characteristics (ROC) curves and area under the curves (AUC) of the proteins were calculated. Results showed that plasma levels of LTBP-1 were significantly higher in HCC patients than those in other three groups. LTBP-1 showed a better diagnostic performance (AUC = 0.74, 95% CI: 0.67-0.80) in distinguishing HCC from the CHB or cirrhosis patients, compared to AFP (AUC = 0.59, 95% CI: 0.52-0.65). In the early-stage HCCs investigated, diagnostic performance of LTBP-1 (AUC = 0.77, 95% CI: 0.70-0.84) remained better than that of AFP (AUC = 0.61, 95% CI: 0.52-0.69). Combination of LTBP-1 and AFP showed increased diagnostic efficiency than any of the two proteins performed alone, for both all HCC (AUC = 0.78, 95% CI: 0.72-0.83) and early-stage HCC (AUC = 0.80, 95% CI: 0.74-0.87). These findings proposed that LTBP-1 may be a promising biomarker for distinguishing HCC from the CHB or liver cirrhosis patients, especially for the early-stage HCC.

Keywords: LTBP-1, biomarker, hepatocellular carcinoma, early detection, AFP

Introduction

Hepatocellular carcinoma (HCC) accounts for 70-80% of liver malignancies, is the third most common cause of cancer-related death worldwide [1, 2]. In China, chronic infection with hepatitis B virus (HBV) is one of the most important risk factor for HCC, and nearly 10% of the country's population is HBV carrier (accounting for two-thirds of all carriers worldwide) [3]. It is estimated that about 263,000 people died from HBV-related liver cancer or cirrhosis each year in China [3]. The poor survival rate of HCC is largely due to diagnosis at an advanced, non-resectable stage, resulting in inestimable indirect economic losses [4]. Hence more effective surveillance strategies should be developed to screen for early-stage HCC among the population at risk.

Alpha-fetoprotein (AFP) is a routinely used diagnostic biomarker for HCC, but its sensitivity is only 39-65% at the frequently-used cut-off 20 ng/ml [4, 5]. The unsatisfactory sensitivity limited the utilization of AFP as a screening biomarker for distinguishing HCC from patients at high risk [6]. Other biomarkers, such as lectin-bound AFP (AFP-L3), α-l-fucosidase, des-γ-carboxyprothrombin (DCP), γ-glutamyl transferase, glypican-3, etc., seemed to improve the diagnosis, but were dissatisfactory for early-stage HCC. Therefore, there is certainly a requirement of identifying novel biomarkers for early detection of HCC [7, 8].

Transforming growth factor-beta 1 (TGF-β1) controls various important pathophysiological processes including tissue homeostasis, fibrosis, and carcinogenesis. The latent transform-

ing growth factor-beta binding protein 1 (LTBP-1), a secreted protein, is a part of the extracellular matrix (ECM). LTBP-1 targets latent TGF-β1 and localizes it to ECM by interacting with integrin, fibrillin and fibronectin [9-11]. It has been reported that expression of LTBP-1 increased gradually, from grade II to grade IV, in human malignant gliomas [12]. Immunohistochemistry (IHC) analysis showed that the IHC staining of LTBP-1 was exceptionally strong in the tumor stroma of malignant mesothelioma [13], pancreatic ductal adenocarcinoma [14] and ovarian carcinoma [15]. However, the clinical significance of the LTBP-1 level of peripheral plasma in cancer patients is unknown. In the present study, we measured the plasma levels of LTBP-1 and AFP to investigate whether LTBP-1 could improve the diagnostic performance for HCC, along with AFP.

Materials and methods

Patients and specimens

We recruited peripheral blood plasma samples from 167 HCC patients (33 females and 134 males with the mean age of 54.2 years) before hepatectomy, at the Cancer Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences (PUMC & CAMS). The plasma samples from 50 chronic hepatitis B (CHB) patients (17 females and 33 males with the mean age of 49.0 years) and 50 liver cirrhosis patients (17 females and 33 males with the mean age of 53.8 years, all of these patients were CHB positive) were collected at Beijing You'an Hospital, Capital Medical University. The plasma samples from 104 healthy individuals without viral hepatitis or malignant disease (39 females and 65 males with the mean age of 47.6 years) were taken from a health screening program at the Cancer Hospital, CAMS. Written informed consent was obtained from all patients.

Diagnosis of the HCC cases was based on abdominal ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI), biochemical profiles (AFP serology and liver function enzymes), and was confirmed after surgery by histopathology examination, according to the American Association for the Study of Liver Disease guidelines (AASLD) [16]. The TNM stage of the HCC cases was defined according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (the seventh edition). In this study, stage I (solitary tumor, no vascular invasion) HCC was classified as the early stage [17]. Diagnoses of CHB and liver cirrhosis were according to those previously described [18].

Prior to surgery (for the HCC patients), peripheral blood was collected by venipuncture and kept in an EDTA-coated tube. Blood samples were centrifuged at 4°C for 10 min at 1000 *g* to separate the plasma and blood cells. The supernatants were collected, divided into aliquots and stored at -80°C until analysis.

Assessment of plasma LTBP-1 and AFP concentrations

The concentrations of LTBP-1 protein in the plasma samples were measured using an ELISA kit (cat.# E01L0245, BlueGene, Shanghai, China) according to the manufacturer's instructions. In brief, 100 μl of plasma sample (1:1 diluted) was added to each well in the antibody pre-coated microtiter plate, then 50 μl of conjugate was added to each well and mixed properly. After incubating the plate at 37°C for one hour, excess plasma sample was then washed off, and color development was achieved. The absorbance was measured at wavelengths of 450 nm using a microplate reader (Bio-Rad Laboratory, Hercules, CA, USA) immediately. The coefficient of variation (CV) of intra-assay and inter-assay given by the manufacturer is less than 10%.

AFP levels were tested using a commercial electrochemiluminescent immunoassay kit (Roche Diagnostics, Mannheim, Germany) at the Clinical Laboratory of the Cancer Hospital, CAMS.

Statistical analysis

Differences of LTBP-1 levels in the plasma samples among various groups were tested by Mann-Whitney test (two groups) or Kruskal-Wallis rank sum test (more than two groups) using the R statistic software (http://www.r-project.org/). Receiver operating characteristics (ROC) curves, respective areas under the curves (AUCs) with 95% confidence interval (CI), and statistic comparison of two ROC curves were assessed by the R package "pROC". The optimal cut-off value for diagnosis was calcu-

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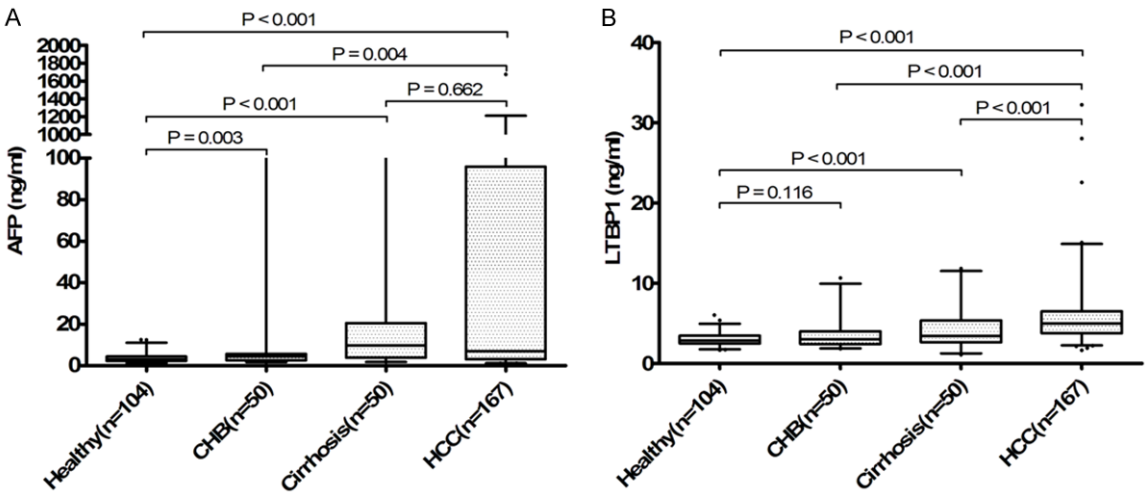

Figure 1. Distribution of plasma levels of AFP and LTBP-1 in HCC and other groups. Box plot shows the distribution of plasma concentrations of AFP (A) and LTBP-1 (B) in each group. Box refers to the 25th and 75th percentile values, with a line indicating median levels, whereas the 95% range is presented by short lines outside the box, points outside the 95% range are outliers. Statistic comparison between HCC and each of other three groups was performed by two-sided Mann-Whitney test, and P < 0.05 was considered to be significant.

Table 1. Plasma concentrations of AFP and LTBP-1 in HCC (positive of CHB and cirrhosis) and three other cohorts

†Cohorts	N	AFP (ng/ml)		LTBP-1 (ng/ml)	
		Median (range)	‡*P*	Median (range)	‡*P*
Healthy	104	3.0 (1.1-12.5)	7.09E-12	2.9 (1.7-6.0)	2.14E-24
CHB	50	4.5 (1.6-638.0)	4.34E-4	3.0 (1.8-10.7)	2.7E-10
Cirrhosis	50	9.8 (1.8-282.0)	0.199	3.4 (1.1-11.8)	1.74E-4
HCC	120	9.5 (1.2-1674.0)	-	5.0 (1.6-14.3)	-

†CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; ‡*P* values were assessed by comparison between HCC (positive of CHB and cirrhosis) and each of the other cohorts, two-sided Mann-Whitney test.

lated by minimum distance to the top-left corner of the ROC curve. In combination analysis of AFP and LTBP-1, logistic regression model was employed to estimate the probability of the two combined variables. The sensitivity, specificity and overall accuracy with 95% CI for the optimum cut-off were calculated using the R package "DiagnosisMed". Box plot and scatter plot of AFP and LTBP-1 plasma levels were performed by GraphPad Prism 5. All statistical tests were two-sided, and $P < 0.05$ was considered as statistical significance.

Results

The plasma levels of AFP and LTBP-1 in HCC patients and other groups

As Shown in **Figure 1A**, the protein levels of AFP in HCC patients (median = 7.0 ng/ml, range: 0.9-1674.0 ng/ml) were significantly elevated than that in CHB patients (median = 4.5 ng/ml, range: 1.6-368.0 ng/ml, $P = 0.0037$) and healthy controls (median = 3.0 ng/ml, range: 1.1-12.5 ng/ml, $P < 0.0001$); but had not statistical difference with that of liver cirrhosis patients (median = 9.8 ng/ml, range: 1.8-282 ng/ml, $P = 0.662$); and the AFP levels were higher in both CHB and cirrhosis patients, when compared with healthy. The LTBP-1 levels were significantly higher in HCC group (median = 5.0 ng/ml, range: 1.6-32.2 ng/ml) than that in the cirrhosis patients (median = 3.4 ng/ml, range: 1.1-11.8 ng/ml, $P = 0.00015$), CHB patients (median = 3.0 ng/ml, range: 1.8-10.7 ng/ml, $P < 0.0001$), and healthy controls (median = 2.9 ng/ml, range: 1.7-6.0 ng/ml, $P < 0.0001$); there was no statistic significance between healthy and CHB patients, and significant difference between healthy and cirrhosis patients (**Figure 1B**).

Furthermore, we also investigated the HCC patients who were CHB positive and suffering cirrhosis ($n = 120$, **Table 1**). Similarly, the protein levels of AFP were significantly elevated in HCC patients, compared with CHB patients ($P = 0.0004$) and healthy controls ($P < 0.0001$); but had not statistical difference with that of liver

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Table 2. Clinical association of the plasma levels of AFP and LTBP-1 in HCC patients

†Variables	‡Phenotypes	N	AFP (ng/ml)		LTBP-1 (ng/ml)	
			Median (range)	§*P*	Median (range)	§*P*
Age				0.523		0.003
	< 55 yrs	88	5.8 (1.1-1674.0)		4.5 (1.6-10.0)	
	≥ 55 yrs	79	7.1 (0.9-1210.0)		5.6 (2.1-32.2)	
Gender				0.042		0.058
	Female	33	33.1 (1.3-1210.0)		5.6 (2.8-28.1)	
	Male	134	5.7 (0.9-1674.0)		4.8 (1.6-32.2)	
Ves.Inv.				0.265		0.322
	No	106	9.3 (0.9-1210.0)		5.1 (1.9-32.2)	
	Yes	56	5.7 (1.2-1674.0)		4.7 (1.6-15.1)	
pTNM				0.737		0.207
	I	80	9.3 (0.9-1210.0)		5.2 (1.9-28.1)	
	II/III/IV	79	6.3 (1.2-1674.0)		4.7 (1.6-32.2)	
Tumor Size				0.264††		0.086††
	≤ 2 cm	17	4.4 (1.2-359.8)		4.8 (1.6-7.5)	
	2~5 cm	103	9.4 (0.9-1674)		4.8 (2.2-22.6)	
	> 5 cm	45	5.9 (1.3-1210.0)		5.7 (1.9-32.2)	
Tumor Dif.				0.042††		0.853††
	High	20	4.3 (1.3-1210.0)		4.4 (2.4-14.3)	
	Moderate	109	8.3 (0.9-1674.0)		5.0 (1.6-28.1)	
	Low	32	36.5 (1.3-1210.0)		5.1 (1.9-32.2)	
CHB				0.326		0.647
	No	7	7.1 (0.9-1210.0)		4.1 (2.4-32.2)	
	Yes	156	6.7 (1.1-1674.0)		5.0 (1.6-28.1)	
Cirrhosis				0.017		0.716
	No	40	4.5 (0.9-1210.0)		4.9 (1.9-32.2)	
	Yes	121	10.7 (1.2-1674.0)		5.0 (1.6-14.3)	

†CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; Ves.Inv., vessel invasion; Tumor Dif., tumor differentiation. ‡yrs, years. §*P* values were assessed by two-sided Mann-Whitney test. ††*P* values were assessed by Kruskal-Wallis rank sum test. The patients with missing value of clinical variables were not listed in this table.

cirrhosis patients ($P = 0.199$). The LTBP-1 levels were higher in HCC patients compared with any of the other groups ($P < 0.01$).

Correlation between the plasma levels of AFP and LTBP-1 and clinical variables in HCC patients

The relationship between clinical characteristics and the plasma protein levels of AFP and LTBP-1 in the HCC were summarized in the **Table 2**. Interestingly, the plasma levels of LTBP-1 were significantly elevated in the older patients (≥ 55 years old, $P = 0.003$), while the AFP levels showed no correlation with age. The two proteins were associated with gender, both raising in female patients (marginally significant for LTBP-1, $P = 0.058$). Plasma levels of AFP were significantly higher in the HCC patients with low-differentiated tumor ($n = 32$, median = 36.5 ng/ml, range: 1.3-1210 ng/ml, $P = 0.042$), and patients with cirrhosis ($n = 121$, median = 10.7 ng/ml, range: 1.2-1674 ng/ml, $P = 0.017$). In contrast, the LTBP-1 levels showed increased trend along with tumor size (marginal significance, $P = 0.086$), but no significant correlation with the TNM stage, vessel invasion, tumor differentiation, CHB or cirrhosis status.

Performance of LTBP-1 in discriminating HCC from CHB or liver cirrhosis patients

We further evaluated the diagnostic performance of LTBP-1 in distinguishing HCC ($n = 167$) from the patients at high-risk (with CHB or cirrhosis, $n = 100$). ROC analysis revealed that

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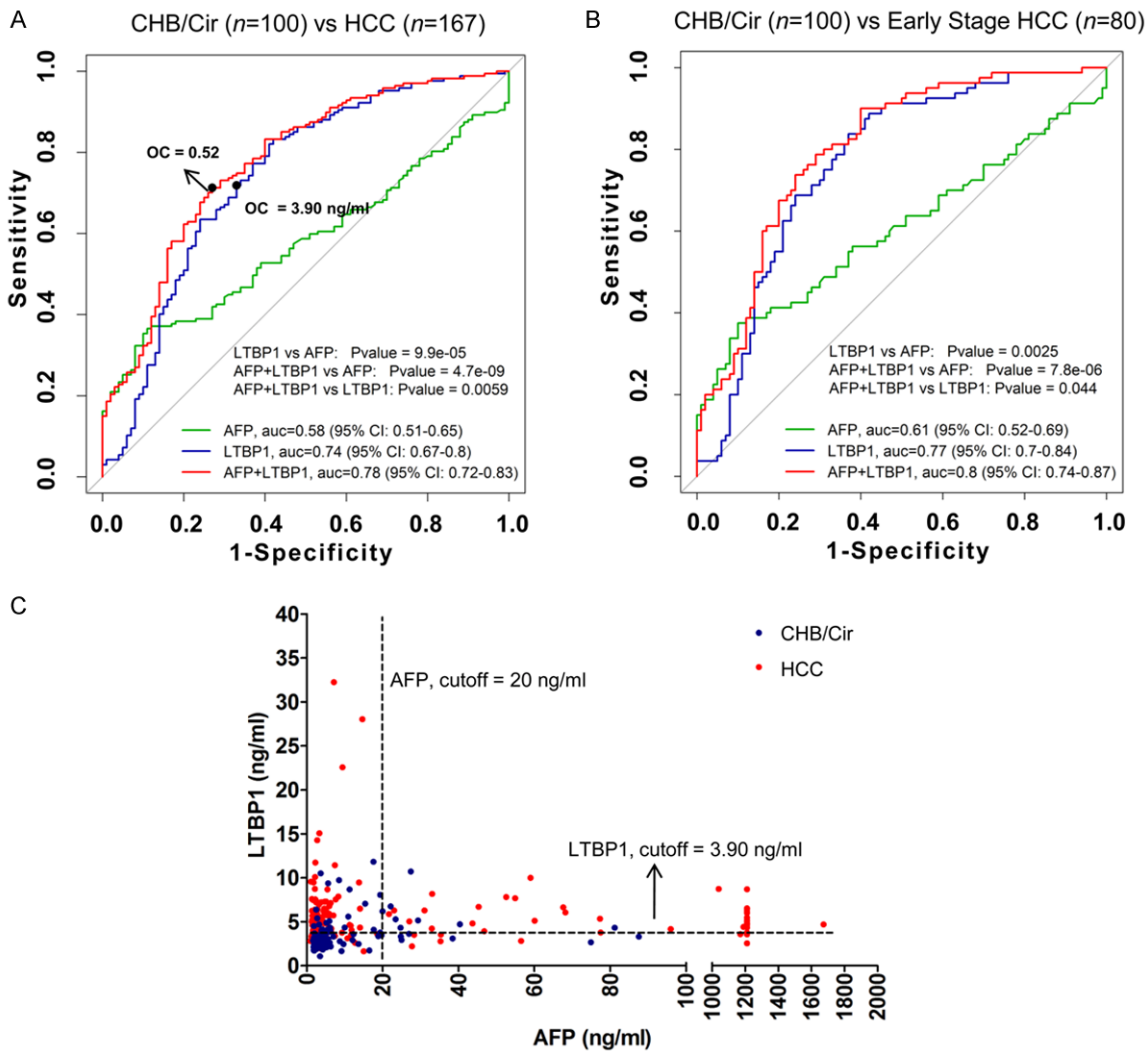

Figure 2. Diagnostic performance of LTBP-1 in discriminating HCC from CHB and liver cirrhosis patients. Receiver operating characteristics (ROC) curve analysis was performed in CHB and liver cirrhosis patients (n = 100) versus all HCC patients (A. n = 167) and early stage HCC patients (B. n = 80). ROC curves and area under the curves (AUC) with 95% confidence interval (CI) are plotted for AFP, LTBP1 and combination of the two proteins (probability of Logistic regression), respectively. Statistic comparison between two ROC curves was performed with "delong" method using the R package "pROC", P < 0.05 was considered to be significant. Optimal cut-offs (OC, the black points) of LTBP-1 and AFP + LTBP-1 were calculated by minimum distance to the top-left corner of the ROC curves in (A). (C) Scatter plot of AFP and LTBP-1 concentrations for each sample point indicates a good diagnostic performance of LTBP-1 in AFP-negative HCC patients, at the optimal cut-off in (A).

the LTBP-1 had a better area under curve (AUC = 0.74, 95% CI = 0.67-0.80) than AFP (AUC = 0.58, 95% CI = 0.51-0.65), with a statistical significance (P < 0.0001). Moreover, combination of the two biomarkers showed better diagnostic performance (AUC = 0.78, 95% CI = 0.72-0.83) than LTBP-1 (P = 0.006) or AFP (P < 0.0001) which were performed alone (**Figure 2A**). The optimal diagnostic cut-offs of LTBP1 (3.9 ng/ml) and LTBP-1 + AFP (probability of Logistic regression, 0.52) were calculated according to the ROC curves (**Figure 2A**), and the cut-off of AFP was set as routinely used 20 ng/ml. The diagnostic accuracy of AFP (sensitivity = 38.3%, specificity = 82.0%), LTBP-1 (sensitivity = 71.9%, specificity = 67.0%), and LTBP-1 + AFP (sensitivity = 70.7%, specificity = 73.0%) were 54.7%, 70.0% and 71.5%, respectively (**Table 3**).

In this study, 80 (47.9%) out of the 167 HCC cases were in early-stage. As data shown in

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Table 3. The diagnostic performance of AFP, LTBP-1, and the combination for HCC

†Cohorts	Classifiers	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
HCC vs. CHB/Cir				
	AFP	0.383 (0.313-0.459)	0.82 (0.733-0.883)	0.547 (0.487-0.605)
	LTBP-1	0.719 (0.646-0.781)	0.67 (0.573-0.754)	0.700 (0.643-0.752)
	AFP + LTBP-1	0.707 (0.634-0.770)	0.73 (0.636-0.807)	0.715 (0.658-0.766)
Early Stage HCC vs. CHB/Cir				
	AFP	0.412 (0.311-0.522)	0.82 (0.733-0.883)	0.639 (0.566-0.705)
	LTBP-1	0.775 (0.672-0.853)	0.67 (0.573-0.754)	0.717 (0.647-0.777)
	AFP + LTBP-1	0.750 (0.645-0.832)	0.73 (0.636-0.807)	0.739 (0.670-0.798)
HCC (AFP < 20 ng/ml) vs. CHB/Cir				
	AFP	-	-	-
	LTBP-1	0.689 (0.595-0.771)	0.67 (0.573-0.754)	0.680 (0.613-0.740)

†CHB, chronic hepatitis B; Cir, cirrhosis; HCC, hepatocellular carcinoma.

Figure 2B and **Table 3**, the concentrations of plasma LTBP-1 improved the diagnostic accuracy on this part of HCC (AUC = 0.77, 95% CI = 0.70-0.84; sensitivity = 77.5%, specificity = 67.0%, accuracy = 70.1%, when cut-off = 3.9 ng/ml) from the cirrhosis or CHB cases, comparing to AFP (AUC = 0.61, 95% CI = 0.52-0.69; sensitivity = 41.2%, specificity = 82.0%, accuracy = 63.9%, when cut-off = 20.0 ng/ml). Combination of the two proteins (AUC = 0.80, 95% CI = 0.74-0.87; sensitivity = 75.0%, specificity = 73.0%, accuracy = 73.9%) also showed better diagnostic result than any protein analyzed alone.

Complementary effect between LTBP-1 and AFP in HCC diagnosis

As what shown in **Figure 2C**, there was no significant correlation (Spearman's correlation rho = 0.029, P = 0.711) between the plasma protein levels of LTBP-1 and AFP in HCC, with only 49 out of 167 HCC patients were simultaneously detected by the two proteins at the current cut-offs. Among the 167 HCC cases investigated, 103 (61.7%) were AFP-negative according to the recommended clinical cut-off 20.0 ng/ml, which resulted in a diagnostic sensitivity of 38.3%. Whereas, in this AFP-negative HCC group, LTBP1 (cut-off = 3.9 ng/ml) remained a higher diagnostic sensitivity of 68.9%, specificity of 67.0%, and accuracy of 68.0% (**Table 3**). These results indicated that LTBP-1 was complementary to AFP in diagnosis of HCC.

Discussion

Transforming growth factor-β (TGF-β) is a multifunctional factor that is involved in cell growth and differentiation [19]. LTBPs (latent transforming growth factor-β binding proteins) were first purified by Kanzaki T et al. from human platelets (120-165 kDa) and fibroblast (170-190 kDa), and were found not to bind directly to active TGF-β1 [20]. The LTBPs are structural components of the extracellular matrix (ECM) microfibrils, which associate with elastic fibers. There are four isoforms of LTBPs (LTBP-1, -2, -3, -4), which are components of connective tissue microfibrils and regulators of TGF-β tissue deposition and signaling [21]. LTBP-1 is a secreted protein, and may have a structural role in the ECM. A previous study reported that LTBP-1 may be involved in epithelial-mesenchymal cell transformation (EMT) of embryonic heart, suggesting that LTBP-1 likely contributes to malignant transformation of cells [22].

Chronic hepatitis B (CHB) and subsequent liver cirrhosis resulting from Hepatitis B virus (HBV) infection is a major cause of developing hepatocellular carcinoma (HCC) in China [6]. Data obtained in this study show that the concentration of LTBP-1 protein in the circulating plasma is gradually increased from healthy individuals to CHB patients, to cirrhosis patients, and has the highest levels in HCC patients (**Figure 1B**). This indicates that LTBP-1 may play an important role in the process of HBV-induced HCC. Furthermore, LTBP-1 levels in the plasma displayed a good performance in distinguishing HCC from the healthy cohort, and significantly improved the diagnostic efficiency of the routinely used AFP when combining the two biomarkers (data not shown). Thus, LTBP-1 is proposed to be a potential novel biomarker for HCC diagnosis.

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To date, a great challenge is to screen HCC in the high-risk populations, such as liver cirrhosis and CHB patients, which is partially due to lack of effective biomarkers. Although raised AFP level in the plasma was a risk factor for HCC development [23, 24], it was unsuitable for HCC screening because of its poor diagnostic sensitivity of 39%-65% [5]. In the present study, the plasma levels of AFP had no statistic difference between the HCC and cirrhosis groups (P = 0.662), and thus couldn't distinguish HCC from the cirrhosis patients. In contrast, the plasma levels of LTBP-1 were significantly elevated in the HCC group than that in the cirrhosis group (P = 0.00015), and had a rising trend from CHB, cirrhosis to HCC patients (**Figure 1B**). Furthermore, the HCC patients with cirrhosis had higher AFP levels than those without (P = 0.017, **Table 2**), while the plasma levels of LTBP-1 in HCC patients were independent of the cirrhosis status. It has been reported that many non-malignant chronic liver disease patients with cirrhosis also have raised AFP concentrations in the serum [18, 25]. In the patients with chronic liver disease background, our data showed that LTBP1 performed well in discriminating HCC from the non-HCC patients (Supplementary Figure 1A, 1B).

Early detection was demonstrated to be helpful in improving the prognosis of primary liver cancer including HCC in China during the last decades [26]. Unfortunately, the biomarkers for early detection of HCC are greatly insufficient. Herein we found that the plasma LTBP-1 improved the diagnosis accuracy of AFP for the early-stage HCC, with an increased sensitivity from 41.2% to 75.0%, and overall accuracy from 63.9% to 73.9%. Moreover, among the HCC cases investigated in this study, about 60% were AFP-negative at the cut-off 20 ng/ml, similar with other published data [27]. Our results suggested that measurement of the plasma LTBP-1 could distinguish 68% of AFP-negative HCC cases from the cirrhosis and CHB patients. These data suggested that LTBP-1 could be complementary to AFP in diagnosis of HCC, especially for the cases at early stage.

In the clinical practice, the diagnosis of HCC tumors with small diameters is a prevalent problem currently. Solitary nodules without blood flow in the liver detected by ultrasonography (US) screening is usually less than 2 cm [28, 29], making a difficulty in HCC diagnosis. In the present study, despite an increase trend of LTBP-1 levels along with tumor size, there was no statistical difference between tumors less than 2 cm and those with diameters of 2-5 cm (Supplementary Figure 1C). Furthermore, the diagnostic performance of LTBP-1 remained better than AFP for HCC less than 2 cm (Supplementary Figure 1D). However, these observations need to be validated in large cohort of patients with small HCC.

In the present study, the plasma protein levels of AFP were higher in the female patients with HCC, similar results was also reported in a recently study based on a large cohort [30]. In addition, the LTBP-1 was correlated with age and sex, raising in elderly and female patients too (**Table 2**). However, there were no significant correlation between LTBP-1 and age in the healthy control, CHB nor cirrhosis patients (data not shown). Further, the diagnostic performance of LTBP-1 for HCC was independent of age and gender (Supplementary Figure 2). The research groups from Taiwan found that male gender and elder age were independently associated with a higher risk of HCC in a community-based cohort study [31, 32]. In mainland of China, HCC is two to three times more frequent in men than in women [26]. Coincidently, the expression of LTBP-1 was reported to be hormonally regulated [33], suggesting a potential biological explanation for the differences on HCC incidence between men and women. These observations suggest that the biological function of LTBP-1 in carcinogenesis of HCC needs to be further studied.

In summary, the protein levels of LTBP-1 in the circulating plasma exhibited a gradually increased trend in healthy individuals, CHB, liver cirrhosis and HCC patients. Combination of LTBP-1 and AFP performed better diagnostic ability than AFP in discriminating HCC from CHB or liver cirrhosis patients, especially in the AFP-negative or early-stage HCC cases. The LTBP-1 is proposed to be a promising diagnostic biomarker for HCC, which could complement some of the limitations of the AFP. The diagnostic value of LTBP-1, however, needs to be confirmed by large-scale and multicenter validation in future.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Yanning Gao and Ting Xiao, State Key Laboratory of Molecular Oncology, Department of Chemical Etiology and Carcinogenesis, Cancer Institute & Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, PO Box 2258, Beijing 100021, People's Republic of China. Tel: 86-10-8778 7363; Fax: 86-10-6776 6703; E-mail: yngao@cicams.ac.cn (YNG); xiaot@cicams.ac.cn (TX)

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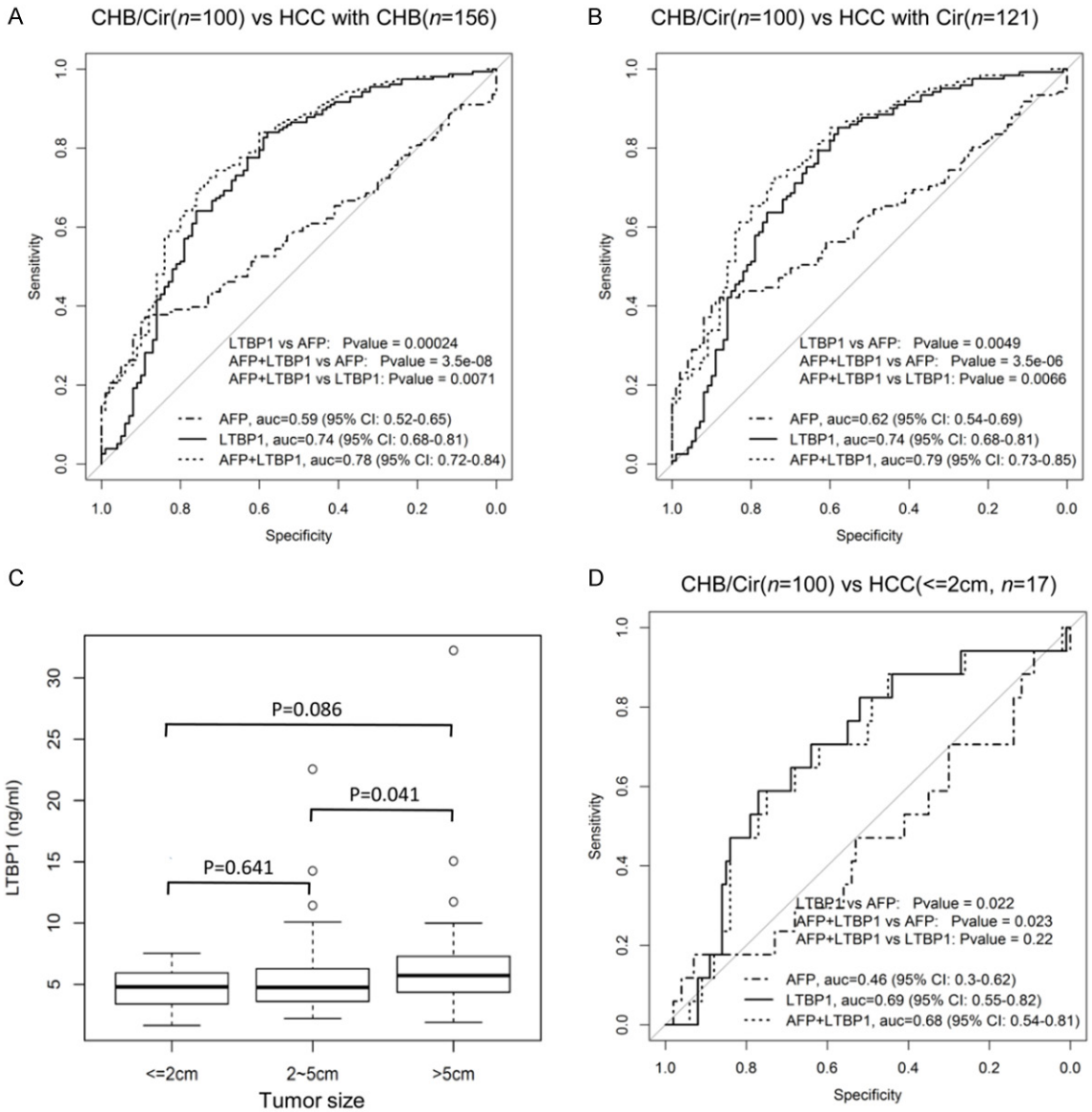
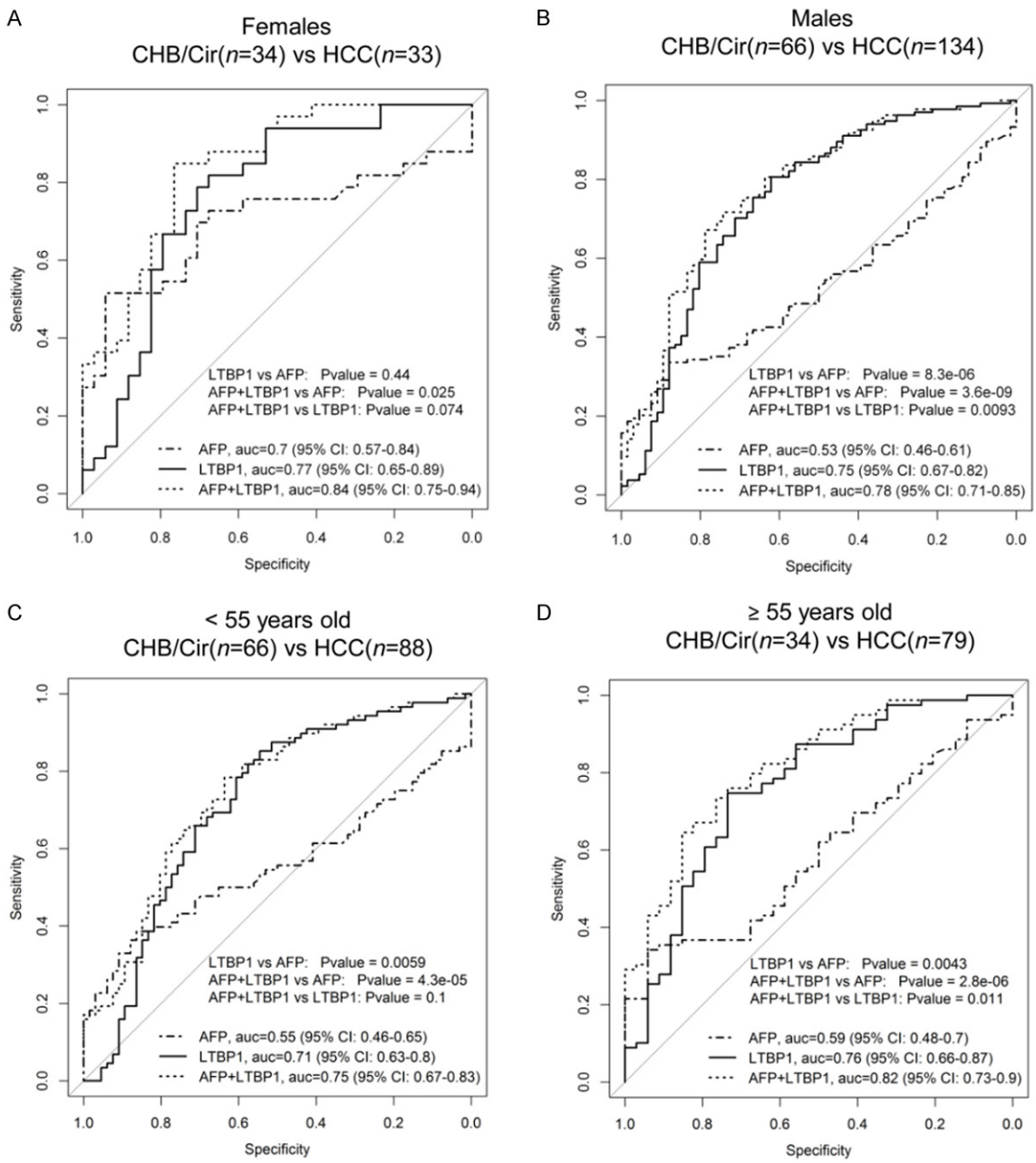


Supplementary Figure 1. HCC diagnostic performance of LTBP-1 in different sub-groups. Receiver operating characteristics (ROC) curve analysis was performed in CHB and liver cirrhosis patients versus HCC patients in different groups: A. HCC patients with CHB (n = 100 vs. n = 156); B. HCC patients with liver cirrhosis (n = 100 vs. n = 121); D. HCC patients with tumors ≤ 2 cm (n = 100 vs. n = 17). ROC curves and area under the curves (auc) with 95% confidence interval (CI) are plotted for AFP, LTBP-1 and combination of the two proteins (probability of Logistic regression), respectively. Statistic comparison between two ROC curves was performed with "delong" method using R package "pROC", P < 0.05 was considered to be significant. C. Box plot showing LTBP-1 levels in different HCC groups with diverse tumor size. Box refers to the 25th and 75th percentile values, with a line indicating median levels, whereas the 95% range is presented by short lines outside the box, points outside the 95% range are outliers. Statistic comparisons between each of two groups and all three groups were performed by two-sided Mann-Whitney and Kruskal-Wallis rank sum test, respectively.



Supplementary Figure 2. HCC diagnostic performance of LTBP-1 with stratification on gender and age. Receiver operating characteristics (ROC) curve analysis was performed in CHB and liver cirrhosis patients versus HCC patients in different groups: A. Females (n = 34 vs. n = 33); B. Males (n = 66 vs. n = 134); C. Individuals who < 55 years old (n = 66 vs. n = 88); D. Individuals who ≥ 55 years old (n = 34 vs. n = 79). ROC curves and area under the curves (AUC) with 95% confidence interval (CI) are plotted for AFP, LTBP1 and combination of the two proteins (probability of Logistic regression), respectively. Statistic comparison between two ROC curves was performed with "delong" method using R package "pROC", P < 0.05 was considered to be significant.