SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 16: 1593-1601, 2018

Serum long noncoding RNA LRB1 as a potential biomarker for predicting the diagnosis and prognosis of human hepatocellular carcinoma

ZHI-FENG WANG*, RUI HU*, JIAN-MIN PANG, GUI-ZHEN ZHANG, WEI YAN and ZENG-NING LI

Department of Gastroenterology, The First Hospital of Hebei Medical University, Shijiazhuang, Hebei 050030, P.R. China

Received November 8, 2016; Accepted November 20, 2017

DOI: 10.3892/ol.2018.8825

Abstract. Hepatocellular carcinoma (HCC) is a type of malignant tumor with a high mortality rate. Long non-coding RNAs (lncRNAs) serve important roles in cellular processes and gene regulation. Identifying novel prognostic biomarkers is important for the monitoring and treatment of HCC. However, only a limited number of biomarkers with high sensitivity and specificity have been determined and are used in clinical practice. The aim of the present study was to investigate the use of serum lncRNA uc007biz.1 (LRB1) expression levels as a novel non-invasive biomarker for the monitoring and diagnosis of HCC. The expression levels of LRB1 were detected in 326 patients with HCC and 73 healthy volunteers by using lncRNA expression microarrays and reverse transcription quantitative polymerase chain reaction analysis, and the associations between LRB1 expression and clinical parameters were analyzed. The results indicated that the serum LRB1 levels in patients with HCC were significantly increased compared with healthy volunteers. The serum LRB1 levels were positively associated with α-fetoprotein (AFP) expression, large tumor sizes, tumor stage (tumor-node metastasis or Barcelona Clinic Liver Cancer stage) and venous invasion, and were negatively associated with overall survival. Additionally, the use of a combination of LRB1, AFP and des-γ-carboxy prothrombin (DCP) markers for the diagnosis of HCC, the diagnostic accuracy was increased compared with using LRB1 alone. LRB1 may act as an important regulator in the progression of HCC, and LRB1 may be considered as a novel biomarker for diagnosis and prediction of prognosis of HCC, additionally complementing the accuracy of AFP and DCP.

Correspondence to: Dr Zeng-Ning Li, Department of Gastroenterology, The First Hospital of Hebei Medical University, 89 Donggang Road, Shijiazhuang, Hebei 050030, P.R. China
E-mail: 15632352072@163.com

*Contributed equally

Key words: hepatocellular carcinoma, long non-coding RNA uc007biz.1, α-fetoprotein, des-γ-carboxy prothrombin

Introduction

Hepatocellular carcinoma (HCC) is the fifth-most common malignant tumor with poor prognosis globally in the previous 10 years, and also is the second most common cause of cancer-associated mortalities in China in the previous 10 years (1,2). For patients with HCC who undergo surgery, radiotherapy and chemotherapy treatment, the median survival rate is ~40% (range, 15-62%) after 5 years. If patients are diagnosed early and treated appropriately, the 5-year survival rate may increase >70% (3). To improve prognosis and diagnosis, predictive biomarkers for HCC are required, allowing screening of high risk patients in order to provide timely and suitable treatment (4). To date, certain oncofetal proteins are used in clinical settings as diagnostic markers for a wide variety of tumors and to monitor recurrence following treatment (5). However, the accuracy, sensitivity and the detection thresholds for these diagnostic markers are low. Therefore, novel biomarkers with high specificities and sensitivities are necessary to ensure that the optimal clinical decisions for patients with HCC are available.

Over the previous decades, a number of tissue-specific and circulating biomarkers of HCC have been identified from retrospective studies, but the lack of clinical validation has limited their use (6-8). The serum levels of α-fetoprotein (AFP) and lens culinaris lectin-reactive AFP, and imaging techniques, including ultrasonography, magnetic resonance imaging or computer tomography are the gold standard to identify suspected cases of HCC (7,8). However, many early-stage HCC (<2 cm) patients have a low level of AFP, and the detection of AFP can not effectively predict the HCC leading to high false-negative rates (>30%) (9,10). Therefore, novel diagnostic biomarkers with high specificity and sensitivity are required.

Long noncoding RNAs (lncRNAs) are a type of noncoding RNAs with >200 nucleotides, which act as the regulatory molecules and may serve central roles in a variety of diseases through complex mechanisms (11,12). Therefore, identifying HCC-associated oncofetal lncRNAs may be important for the diagnosis and treatment of HCC. Additionally, previous studies have demonstrated that specific lncRNAs are associated with cancer development, and are detectable in plasma, highlighting the convenience and speed of detection of these biomarkers (13,14). For example, the lncRNA SOCS2-antisense RNA 1 is positively associated with castration-resistant prostate cancer, whereas lncRNA neuroblastoma associated transcript 1

is negatively associated with neuroblastoma in stage 2A (15-17). Therefore, the levels of lncRNAs in plasma may act as potential biomarkers for cancer detection, diagnosis, tumor grade screening and recurrence monitoring during clinical treatment.

In the present study, the abnormal expression of serum LRB1 was identified in patients with HCC. The potential value of LRB1 as a biomarker for the detection and monitoring of prognosis of patients with HCC was investigated, and the diagnostic accuracy of LRB1 in sera was compared to des-γ-carboxy prothrombin (DCP) and AFP, respectively and in different combinations, in patients with HCC, consequently providing the optimal combination of biomarker indices for HCC detection.

Materials and methods

Patients and serum samples. The present study was approved by Human Research Ethics Committee of The First Hospital of Hebei Medical University (Shijiazhuang, China), and all patients provided written informed consent. The serum samples were collected from 326 patients with HCC who underwent primary surgery treatment without radiotherapy or preoperative chemotherapy treatment at The First Hospital of Hebei Medical University between March 2011 and March 2015 (251 males and 75 females, 162 patients >60 years old and 164 patients ≤60 years old) and 73 healthy volunteers were recruited from the health examination center of The First Hospital of Hebei Medical University between March 2011 and March 2015 (43 males and 30 females, 32 volunteers >60 years old and 31 volunteers ≤60 years old). The median expression level of LRB1 was used as the cut-off value, and all of the patients with HCC were divided into two groups according to the cut-off value of LRB1.Firstly, peripheral blood was collected from each patient with HCC, then a second blood sample was obtained 10 days after surgery using BD Vacutainer® sodium heparin tubes (BD Biosciences, Franklin Lakes, NJ, USA). The blood samples were centrifuged at 4˚C with 800 x g for 20 min, 2,000 x g for 10 min and then 5,000 x g for 5 min for the prevention of contamination with nucleic acids. Then, the serum samples were transferred into 1.5 ml tubes and stored at -80˚C until use.

HCC diagnosis and grade determination. The HCC diagnoses were determined according to the American Association for the Study of Liver Diseases practice guidelines (18). The direct HCC diagnosis gold standard is pathological detection, and the tumor tissues are obtained through surgical resection or percutaneous biopsy. Alternatively, blood biochemical detection and radiological diagnosis are indirect methods of detection. For tumor stage determination, Tumor Node Metastasis (TNM) stage was assessed postoperatively according to the 7th edition of American Joint Commission on Cancer (19), and the clinical definition of HCC stage was determined based on the Barcelona Clinic Liver Cancer (BCLC) classification (20).

Clinical chemistry and detection of AFP and DCP. Blood samples were collected for detection of the biomarker indices using the SpotChem EZ clinical chemistry analyzer (ARKRAY Inc., Kyoto, Japan). The levels of serum AFP were analyzed by Human α-Fetoprotein Quantikine® ELISA Kit (cat. no. DAFP00; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. The serum DCP concentrations were detected by Lumipulse G1200 auto-analyzer (FUJIREBIO Inc., Tokyo, Japan) according to the manufacturer's protocol. All the experiments were repeated three times, and the average values were calculated.

RNA extraction and reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from serum samples using the RNA Isolation kit (Axygen Scientific, Inc., Union City, CA, USA) according to the manufacturer's protocol. Triplicates of each gene and each specimen were used, with GAPDH as an internal standard. The single-strand cDNA for PCR template was synthesized from 10 μg of total RNA by ReverTra Ace qPCR RT kit (cat. no. FSQ-101; Toyobo Life Science, Osaka, Japan) from the extracted total RNA. StepOne™ Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used in the RT-PCR assay. The RT-PCR was performed with a total reaction volume of 20 μl, including 10 μl Power SYBR Green PCR Master mix (Roche Diagnostics, Indianapolis, IN, USA), 5 pmol of forward and reverse primer respectively and 2 μl of cDNA. Quantification cycle (Cq) was observed in the amplification with 35 cycles of 1 min at 95˚C, 1 min at 58˚C, and 1 min at 72˚C. The results were normalized to GAPDH, which served as the endogenous control, and the relative expression of LRB1 was quantified using the $2^{-\Delta\Delta Cq}$ method (21). The PCR primer sequences for LRB1 and GAPDH were as follows: LRB1 forward, 5'-TCATGCGATAGCTGAACGCTA-3' and reverse, 5'-GAGGCCGGTAGTCGTAACT-3'; GAPDH forward, 5'-ATTCCACCCATGGCAAATTC-3' and reverse, 5'-TGGGATTTCCATTGATGACAAG-3'.

Microarray analysis of lncRNAs. Total RNA was extracted from serum samples using an RNA Isolation kit (Axygen Scientific, Inc.) according to the manufacturer's protocol., the extracted RNA was amplified and transcribed into fluorescence-labeled cDNA using Quick Amp Labeling kit (Agilent Technologies, Inc., Santa Clara, CA, USA), the labeled cDNA was hybridized onto a lncRNA Array 2.0 (8x60 K array; ArrayStar, Inc., Rockville, MD, USA) for lncRNA expression detection. The array was scanned using Agilent Scanner G2505C (Agilent Technologies, Inc.), and the array images were obtained by Agilent Feature Extraction software (version 11.0.1.1; Agilent Technologies, Inc.). Accessible raw and normalization data was processed using GeneSpring software (version GX v12.1, Agilent Technologies, Inc.). Subsequently, functional annotation was performed on the samples using the gene set enrichment analysis (GSEA) method (22), GSEA was supported by the Broad Institute website (http://www.broadinstitute.org/gsea/index.jsp), and was performed using the GSEA software (version 2.2.2; Broad Institute, Inc., Massachusetts Institute of Technology, and Regents of the University of California).

Statistical analysis. All data were presented as the mean ± standard deviation, and were analyzed using SPSS (version 20.0; IBM Corp., Armonk, NY, USA). The differences between two groups were analyzed using Pearson's χ^2 test, paired Student's t-test, Wilcoxon test or Fisher's exact test. For comparisons between more than two groups, the differences were estimated

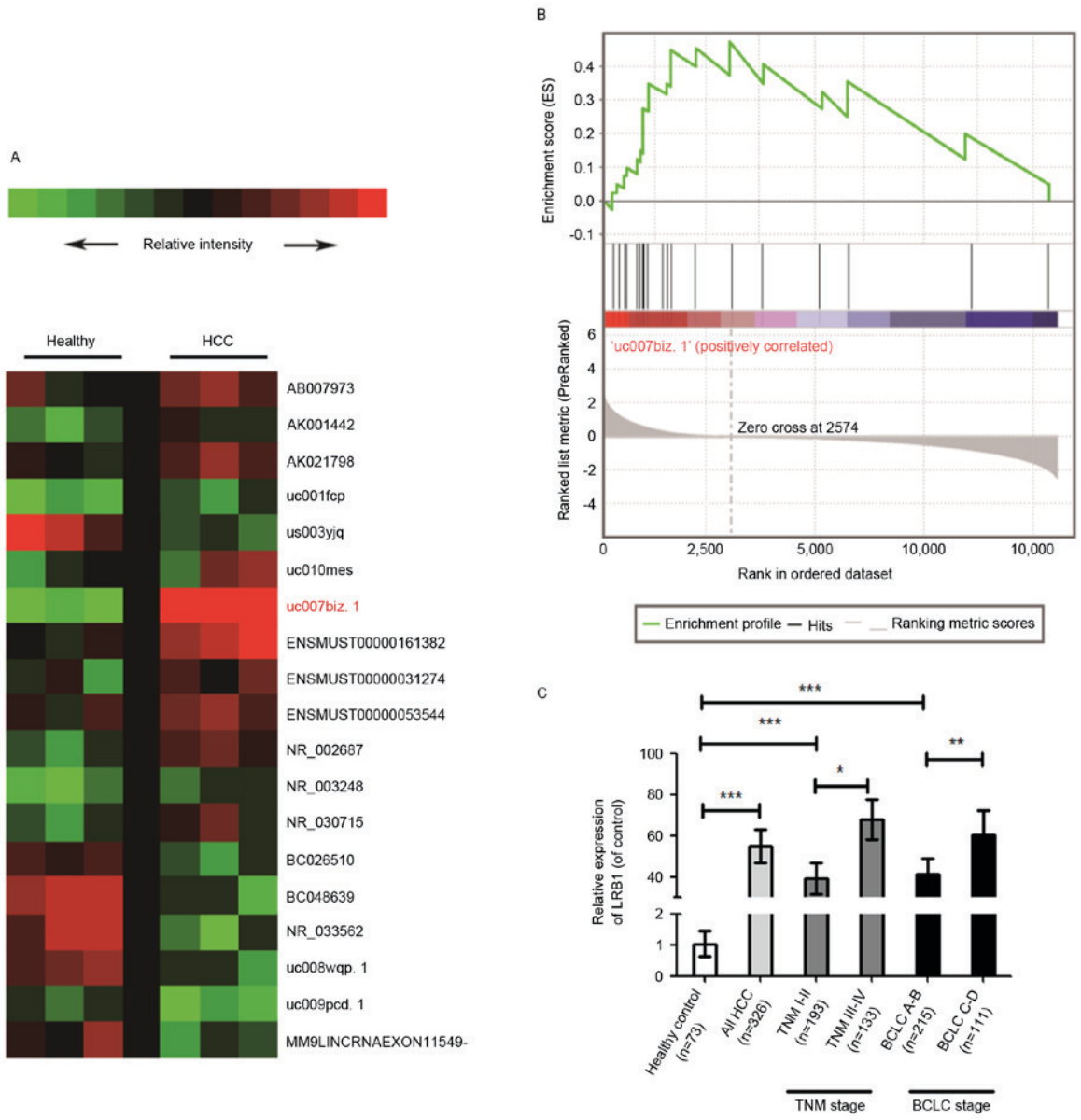

Figure 1. Expression levels of serum lncRNAs in patients with HCC. (A) The expression level of LRB1 was significantly upregulated (P<0.001) in patients with HCC compared with healthy volunteers. Green represents low expression levels and red represents high expression levels. (B) The serum LRB1 expression level was associated with HCC as indicated by gene set enrichment analysis. (C) Levels of LRB1 expression in patients with HCC were significantly increased compared with healthy volunteers, and also significantly increased in patients with late-stage disease compared with early-stage disease. Staging of the disease was determined using TNM and BCLC staging systems. *P<0.05, **P<0.01 and ***P<0.005. lncRNAs, long non-coding RNAs; LRB1, lncRNA uc007biz.1; HCC, hepatocellular carcinoma; TNM, tumor-node metastasis; BCLC, Barcelona Clinic Liver Cancer.

using Kruskal-Wallis test, followed by Bonferroni post hoc testing. Pearson's correlation coefficient was used to analyze the correlation between LRB1 and AFP as well DCP respectively in diagnosis of HCC. Receiver operator characteristic (ROC) curve and area under the curve (AUC) analyses were used to detect the accuracy of markers with a 95% confidence interval (CI). The sensitivity represents the true positive rate and was used as the y-axis, (1-specificity) represents the false positive rate and was used as the x-axis. The value of AUC is the size of the area under the ROC curve and is between 0.5 and 1, and the closer the AUC is to 1, the better the diagnosis. The Kaplan-Meier method was used to analyze the postoperative survival rate, and the log-rank test was used to assess the difference of survival rate in different groups. The correlation between serum LRB1 level with pathological and clinical characteristics was analyzed with the Spearman rank correlation. P<0.05 was considered to indicate a statistically significant difference.

Results

Evaluation of serum LRB1 levels in patients with HCC. In order to investigate the hypothesis that the serum level of LRB1 is a potential biomarker for the diagnosis of HCC, an lncRNA expression microarray and RT-qPCR for the lncRNA expression detection was performed with samples from 326 patients with HCC and 73 healthy volunteers. The results of the lncRNA expression microarray indicated that the serum LRB1 expression was significantly increased (P<0.001) in patients with HCC compared with healthy volunteers (Fig. 1A), and

Table I. Levels of LRB1, AFP and DCP expression.

Parameters	LRB1 (ng/ml)	AFP (ng/ml)	DCP (mAU/ml)
Control (n=73)	1.05±0.40	13.96±0.83	30.27±4.22
HCC (n=326)	54.83±8.21	1,046.72±135.18	5,934.72±416.73
P-value (control vs. HCC)	<0.0001	<0.0001	<0.0001
TNM stage			
TNM I-II (n=193)	39.25±7.76	946.17±159.33	4,352.33±496.04
TNM III-IV (n=133)	68.03±9.66	1,191.25±218.04	7,130.25±569.23
P-value (control vs. TNM I-II)	<0.001	<0.001	<0.001
P-value (TNM I-II vs. TNM III-IV)	0.027	0.074	0.037
BCLC stage			
BCLC A-B (n=215)	41.32±7.64	1,002.33±132.57	4,342.86±672.03
BCLC C-D (n=111)	60.31±11.77	1,160.27±194.28	7,361.07±548.28
P-value (control vs. BCLC A-B)	<0.001	<0.001	<0.001
P-value (BCLC A-B vs. BCLC C-D)	0.007	0.088	0.031

Data are presented as the mean ± standard deviation. LRB1, lncRNA uc007biz.1; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; TNM, tumor-node metastasis; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.

the results of GSEA suggested that the expression of LRB1 was associated with HCC (Fig. 1B). The RT-qPCR assay also confirmed that the expression of serum LRB1 in patients with HCC was significantly increased compared with healthy volunteers, and that the expression of LRB1 exhibited a positive association with HCC stage (Fig. 1C). Concurrently, the expression of AFP and DCP was analyzed, and the results were similar to those of LRB1. The expression levels of AFP and DCP in patients with HCC were significantly increased compared with healthy volunteers (Table I).

Association between serum LRB1 expression levels and clinicopathological factors in patients with HCC. In order to detect whether the serum level of LRB1 was associated with the clinicopathological factors in patients with HCC, 326 patients with HCC were enrolled in the present study. The serum samples were divided into two independent groups according to the median expression level (47.24 ng/ml) of LRB1 in HCC patients. The results indicated that the serum LRB1 expression level was associated with AFP expression, larger tumor size, tumor stage and venous invasion (Table II). However, there was no significant association with sex, age and hepatitis B virus infection (Table II). These results suggest that LRB1 may serve an important role in hepatocarcinogenesis and tumor progression.

Serum LRB1 levels in HCC tumors may be used for prediction of tumor prognosis according to tumor stage. In order to determine the association between serum LRB1 level and HCC progression, patients with HCC were divided into several groups according to TNM or BCLC stages, and the serum LRB1 levels were presented in Fig. 1C and Table I. The results suggested that the LRB1 levels in patients with HCC were significantly increased compared with the healthy controls. In patients with TNM stages I-II, the levels of LRB1 were significantly decreased compared with patients with TNM stages III-IV. Similarity, the levels of LRB1 in patients with BCLC stages A-B were significantly decreased compared with patients with BCLC stages C-D, where BCLC A-B and BCLC C-D represent early-stage and represents late-stage disease, respectively. Concurrently, the DCP and AFP levels were also detected in patients with HCC. When stratified by TNM or BCLC stages, the levels of DCP in patients with TNM I-II or BCLC A-B were significantly decreased compared with patients with TNM III-IV or BCLC C-D (Table I). However, no statistically significant differences in AFP levels between patients with different TNM or BCLC stages were observed (Table I). Compared with the healthy control group, LRB1, DCP and AFP levels in patients with early stage HCC were significantly increased compared with the healthy group (Table I). Subsequently, survival analysis of the follow-up data of patients with HCC was performed. As indicated by Kaplan-Meier survival analysis, patients with HCC with low serum LRB1 levels exhibited improved overall survival compared with patients with high serum LRB1 levels (Fig. 2A). These results indicate that the serum level of LRB1 in patients with HCC was associated with tumor prognosis, and may be used as an indicator of tumor stage detection.

Accuracy of LRB1, AFP and DCP markers for the diagnosis of HCC. Based on the ROC curve analysis (Fig. 2B), the optimum cut-off values of LRB1, AFP and DCP were calculated as 3.481, 9.36 ng/ml and 41.26 mAU/ml, respectively. The results for AUC, 95% CI, sensitivity and specificity values for all biomarkers were calculated (Table III). In present clinical detection, the threshold values of AFP and DCP were 10 ng/ml and 35 mAU/ml, respectively, and the threshold values of AFP and DCP in our research were 9.36 ng/ml and 41.26 mAU/ml, respectively; therefore, the clinical examination threshold is notably similar to the threshold in our experiment. ROC analysis was performed to assess the ability

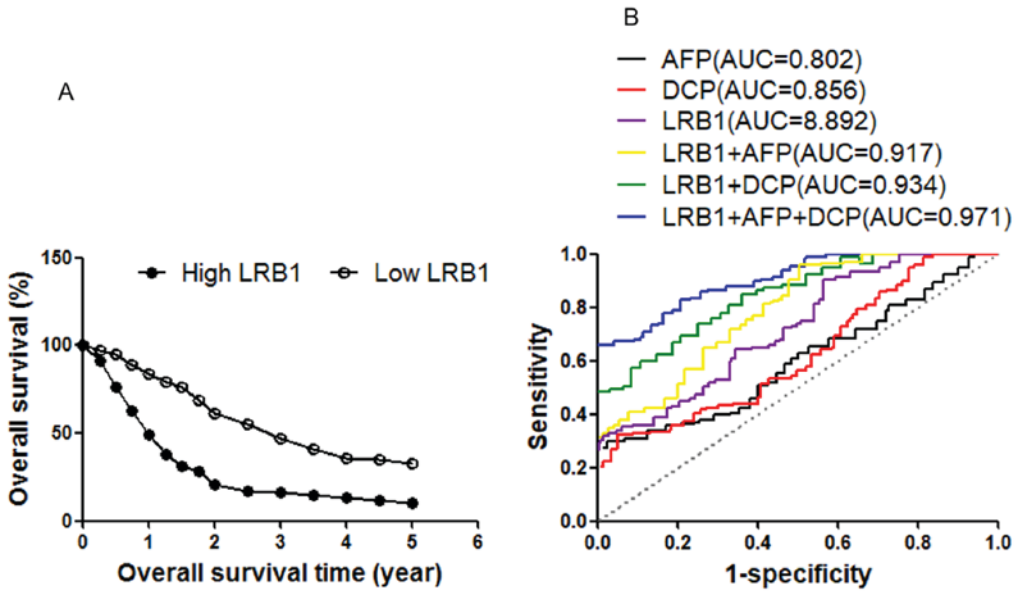

Figure 2. Overall survival curves and ROC curves. (A) Overall survival curves of patients with HCC with high and low levels of serum LRB1 expression. The patients with low serum LRB1 levels exhibited improved overall survival compared with patients with high serum LRB1 levels with the threshold value of 47.24 ng/ml (P=0.001). (B) ROC analysis of AFP, DCP and LRB1 markers to distinguish patients with HCC from healthy controls. LRB1, lncRNA uc007biz.1; HCC, hepatocellular carcinoma; ROC, receiver operator characteristic; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; AUC, areas under the curve.

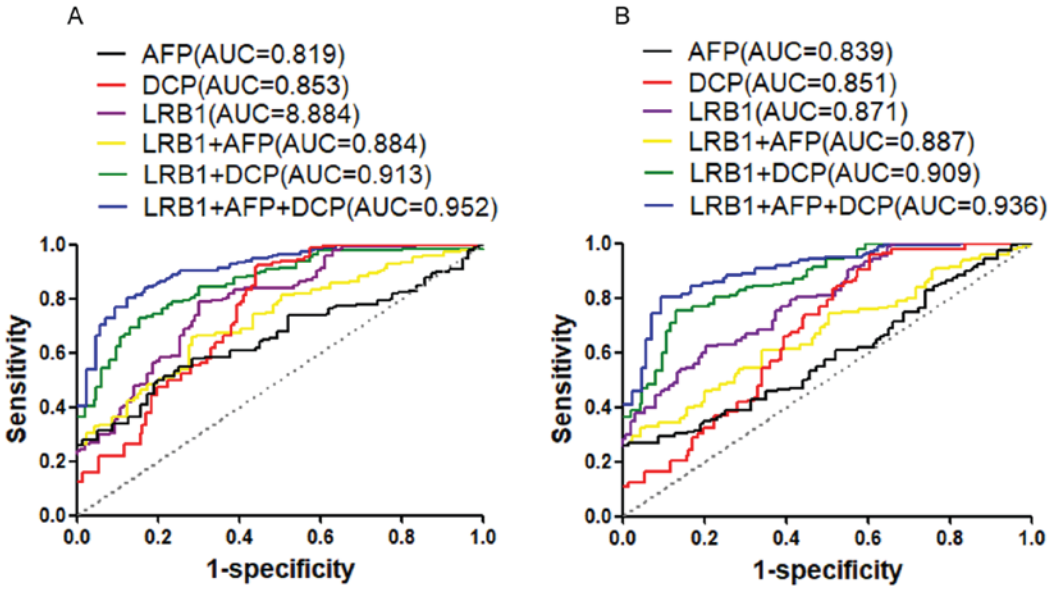

Figure 3. ROC curves of LRB1, AFP and DCP and combinations of these markers for the diagnosis of early-stage HCC. (A) ROC curve analysis to distinguish HCC patients with TNM stages I-II from healthy controls (B) and to distinguish HCC patients with BCLC stages A-B from healthy controls. LRB1, lncRNA uc007biz.1; HCC, hepatocellular carcinoma; ROC, receiver operator characteristic; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; AUC, areas under the curve; TNM, tumor-node metastasis; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.

of the markers in distinguishing between patients with HCC and healthy controls. The results indicated that the AUC value for LRB1 marker was higher compared with the values for AFP or DCP (Fig. 2B; Table III). According to Pearson's correlation analysis, the results indicated that there were no statistically significant correlations between LRB1 and AFP or DCP.

Therefore, it was subsequently evaluated if the combination of these three HCC markers may improve the accuracy of diagnosis. The results indicated that the diagnostic accuracy of LRB1 combined with AFP or DCP were markedly increased compared with the accuracy of using LRB1 alone (Fig. 2B; Table III). In addition, the diagnostic accuracy may be optimized when a combination of all three markers, LRB1, AFP and DCP, was employed. (Fig. 2B; Table III).

The accuracy of using each of the markers (LRB1, AFP and DCP) alone and a combination of these markers for the diagnosis of early-stage HCC (TNM stages I-II or BCLC stages A-B) was also assessed. The results indicated that the AUC value of the LRB1 marker was higher compared with

Table II. Clinicopathological association of LRB1 expression levels in patients with HCC.

Clinicopathological characteristics	LRB1		χ^2	P-value[b]
	Low	High[a]		
All cases	159	167		
Age			0.361	0.537
>60	77	85		
≤60	82	82		
Sex			0.243	0.674
Male	121	130		
Female	38	37		
HBs antigen			2.094	0.157
Present	83	90		
Absent	76	77		
HBe antigen			2.993	0.131
Present	73	79		
Absent	86	88		
Liver cirrhosis			0.405	0.339
Present	79	86		
Absent	80	81		
AFP, ng/ml			10.329	0.002
>20	92	155		
≤20	67	12		
Tumor size, cm			11.905	0.001
>5	33	129		
≤5	126	38		
TNM stage			14.551	0.002
I	61	7		
II	62	53		
III	32	64		
IV	4	43		
BCLC stage			14.229	0.001
A	62	4		
B	73	59		
C	21	66		
D	3	38		
Venous invasion			6.327	0.018
Present	26	108		
Absent	133	59		
Tumor microsatellite			0.975	0.328
Present	83	89		
Absent	76	78		
Tumor encapsulation			1.704	0.115
Present	93	98		
Absent	66	69		

[a]The median expression level of LRB1 was used as the cut-off value. 159 patients with HCC were identified as exhibiting low LRB1 expression, and 167 patients with HCC was identified as high LRB1 expression [b]Pearson's χ^2 test was used to analyze the association between LRB1 and clinical parameters. LRB1, lncRNA uc007biz.1; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B envelope antigen; AFP, α-fetoprotein; TNM, tumor-node metastasis; BCLC, Barcelona Clinic Liver Cancer.

AFP and DCP for TNM stages I-II (Fig. 3A; Table IV) or BCLC A-B (Fig. 3B; Table V). In addition, when a combination of the three markers was employed for the diagnosis of TNM stages I-II vs. control (Fig. 3A; Table IV) or BCLC stages A-B vs. control (Fig. 3B; Table V), the AUC value was the highest. These results suggested that serum LRB1 has a high accuracy for the diagnosis of HCC and early-stage of the disease, and the combination of LRB1, AFP and DCP may increase the accuracy of diagnosis of HCC and early-stage of the disease.

Discussion

HCC is one of the common types of malignant tumor that is associated with a poor prognosis, and the second most common cause of cancer-associated mortality in China (1-3). HCC frequently occurs in patients with liver cirrhosis, and the low rates of early diagnosis and high recurrence rates result in a poor prognosis (23). Detection of early-stage HCC increases the availability of appropriate treatment, including local ablative therapy, resection or liver transplantation. These treatments may prolong survival (24,25). Although the markers DCP and AFP are widely used for the detection of HCC, the sensitivity and specificity values are not optimum (9,26,27). Consequently, novel molecular biomarkers for the diagnosis of HCC and monitoring of therapy are urgently required, particularly in early-stage cancer screening protocols.

Previous studies investigating the human genome and transcriptome sequencing revealed that only a minority of gene transcripts encodes protein. The majority of the genome that is transcribed does not encode protein (12). lncRNAs are a type of noncoding RNA molecules with >200 nucleotides (12). Previous studies have demonstrated that dysregulation of lncRNAs may alter epigenetic information and promote cell growth, resulting in tumor growth and progression (28,29). The survival of cancer cells and proliferation is associated with the expression level of lncRNAs, which is associated with cancer diagnosis and prediction of tumor prognosis (30,31). Therefore, the identification of cancer-associated lncRNAs and their clinical functions is important, and may contribute to the identification of novel cancer biomarkers, and clarify oncogenic and tumor networks. In previous studies, serum lncRNAs have been used as biomarkers to identify various types of cancer, including lung, gastric and breast cancer (32-34). However, a small number of studies demonstrated the potential use of serum lncRNAs in the diagnosis and prediction of prognosis of HCC (35). Therefore, there may be a novel lncRNA biomarker for the diagnosis of early-stage HCC.

In the present study, a lncRNA expression microarray, GSEA and RT-qPCR analyses were conducted in 326 patients with HCC and 73 healthy volunteers, the results of which suggested that LRB1 is significantly associated with a risk of HCC, and that the serum LRB1 level is associated with HCC early diagnosis and tumor prognosis. The serum LRB1 levels in patients with HCC were significantly increased compared with the healthy volunteers. In current clinical HCC detection, AFP and DCP are widely used as biomarkers, but the associated high false-positive rates limit the use of these markers.

In the present study, the results indicated that LRB1 level was positively associated with AFP expression, large tumor

Table III. Accuracy of LRB1, AFP and DCP markers for the diagnosis of HCC.

HCC vs. control	AUC	95% CI	Sensitivity, %	Specificity, %	PPV, %	NPV, %	+LR	-LR
LRB1	0.892	0.843-0.922	92.43	71.85	81.37	82.19	3.97	0.29
AFP	0.802	0.769-0.834	61.72	83.63	87.02	76.32	7.42	0.37
DCP	0.856	0.773-0.879	63.08	89.41	94.26	77.22	10.83	0.41
LRB1+AFP	0.917	0.869-0.938	79.32	79.38	84.52	84.91	4.62	0.21
LRB1+DCP	0.934	0.893-0.953	81.69	83.25	92.19	87.36	8.44	0.17
LRB1+AFP+DCP	0.971	0.942-0.988	86.33	87.64	93.04	89.08	9.01	0.11

Realizing the sum of sensitivity and specificity maximizing and the overall error minimizing, so that the best predicted probabilities of LRB1, AFP, DCP, and their different combinations are obtained. LRB1, lncRNA uc007biz.1; HCC, hepatocellular carcinoma; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio.

Table IV. Accuracy of LRB1, AFP and DCP markers for the diagnosis of early-stage (TNM I-II) HCC.

Early stage of HCC (TNM I-II) vs. control	AUC	95% CI	Sensitivity, %	Specificity, %	PPV, %	NPV, %	+LR	-LR
LRB1	0.884	0.775-0.892	89.76	73.66	71.26	83.26	2.57	0.17
AFP	0.819	0.761-0.887	67.54	87.93	86.88	77.46	6.35	0.41
DCP	0.853	0.796-0.894	68.37	91.65	92.35	73.49	9.35	0.45
LRB1+AFP	0.884	0.825-0.916	84.29	77.44	79.43	80.68	4.11	0.19
LRB1+DCP	0.913	0.881-0.947	79.05	89.47	89.68	79.49	8.95	0.25
LRB1+AFP+DCP	0.952	0.919-0.978	86.33	90.28	92.33	82.18	9.07	0.18

Realizing the sum of sensitivity and specificity maximizing and the overall error minimizing, so that the best predicted probabilities of LRB1, AFP, DCP, and their different combinations are obtained. LRB1, lncRNA uc007biz.1; HCC, hepatocellular carcinoma; TNM, tumor-node metastasis; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; AUC, areas under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio.

Table V. Accuracy of LRB1, AFP and DCP markers for the diagnosis of early-stage (BCLC A-B) HCC.

Early stage of HCC (BCLC A-B) vs. control	AUC	95% CI	Sensitivity, %	Specificity, %	PPV, %	NPV, %	+LR	-LR
LRB1	0.871	0.847-0.896	88.38	76.79	73.42	85.47	2.13	0.23
AFP	0.839	0.819-0.895	68.47	88.26	87.39	79.33	5.61	0.42
DCP	0.851	0.824-0.879	69.35	92.47	93.15	75.79	9.85	0.38
LRB1+AFP	0.887	0.837-0.926	82.83	81.16	82.16	83.41	3.64	0.18
LRB1+DCP	0.909	0.874-0.933	81.49	88.35	90.43	81.26	7.06	0.24
LRB1+AFP+DCP	0.936	0.908-0.954	87.06	91.56	92.75	84.63	9.18	0.16

Realizing the sum of sensitivity and specificity maximizing and the overall error minimizing, so that the best predicted probabilities of LRB1, AFP, DCP, and their different combinations are obtained. LRB1, lncRNA uc007biz.1; HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; AUC, areas under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio.

size, tumor stage (TNM or BCLC stage) and venous invasion. Additionally, the association between LRB1 level and clinical outcomes were detected. The Kaplan-Meier analysis suggested that LRB1 level was significantly associated with overall survival of patients with HCC, and higher LRB1 levels were associated with poorer recurrence-free survival rates. In addition, in the present study, the AUC and sensitivity values for LRB1 for distinguishing between patients with HCC and healthy controls were higher compared with DCP or AFP, but the specificity value was lower for LRB1 compared with DCP

or AFP. When a combination of all three markers (LRB1, AFP and DCP) was employed for the diagnosis of HCC, the AUC and specificity values were markedly higher compared with using LRB1 alone. However, the sensitivity value of using a combination of all three markers was lower compared with using LRB1 alone.

These results indicated that there is a high expression of LRB1 in HCC and therefore this may be used to may predict the prognosis and overall survival of patients with HCC. Consequently, serum LRB1 may be a novel biomarker for HCC diagnosis and prediction of prognosis.

Previous studies have demonstrated that circulating lncRNAs are associated with tumor dynamics (11-15). In the present study, serum LRB1 levels demonstrated clinical significance in the diagnosis of HCC and prediction of tumor prognosis, as high serum LRB1 levels were associated with higher degrees of malignancy of HCC. These findings suggested that higher serum LRB1 level may be an independent prognostic factor of poor prognosis of patients with HCC, and that serum LRB1 level was associated with the early stages of HCC. Taken together, serum LRB1 level may be used for monitoring, diagnosis and prediction of prognoses of HCC, and employing a combination of LRB1, AFP and DCP may increase diagnostic accuracy.

In summary, to the best of our knowledge, the present study was the first to reveal the use of serum LRB1 level in cancer diagnosis and prognosis. LRB1 may act as an important key regulator in HCC progression and be a potential therapeutic target for treatment of HCC. Although the sample size of the present study was small, the data suggested that serum LRB1 may serve as a useful biomarker for the diagnosis of HCC and prediction of prognosis. When a combination of LRB1, DCP and AFP was employed, the diagnostic accuracy was markedly increased. Large-scale prospective studies are required to validate the accuracy and effectiveness of LRB1 as a biomarker of HCC, including the use of a combination of LRB1, DCP and AFP markers.

Competing interests

The authors declare that they have no competing interests.

References

1. Forner A, Llovet JM and Bruix J: Hepatocellular carcinoma. Lancet 379: 1245-1255, 2012.
2. Trépo C, Chan HL and Lok A: Hepatitis B virus infection. Lancet 384: 2053-2063, 2014.
3. Lu LC, Cheng AL and Poon RT: Recent advances in the prevention of hepatocellular carcinoma recurrence. Semin Liver Dis 34: 427-434, 2014.
4. Wang X, Zhang A and Sun H: Power of metabolomics in diagnosis and biomarker discovery of hepatocellular carcinoma. Hepatology 57: 2072-2077, 2013.
5. Zucman-Rossi J, Villanueva A, Nault JC and Llovet JM: Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology 149: 1226-1239, 2015.
6. Bruix J, Gores GJ and Mazzaferro V: Hepatocellular carcinoma: Clinical frontiers and perspectives. Gut 63: 844-855, 2014.
7. Bruix J, Reig M and Sherman M: Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. Gastroenterology 150: 835-853, 2016.
8. Frau M, Biasi F, Feo F and Pascale RM: Prognostic markers and putative therapeutic targets for hepatocellular carcinoma. Mol Aspects Med 31: 179-193, 2010.
9. Sauzay C, Petit A, Bourgeois AM, Barbare JC, Chauffert B, Galmiche A and Houessinon A: Alpha-foetoprotein (AFP): A multi-purpose marker in hepatocellular carcinoma. Clin Chim Acta 463: 39-44, 2016.
10. Cheng J, Wang W, Zhang Y, Liu X, Li M, Wu Z, Liu Z, Lv Y and Wang B: Prognostic role of pre-treatment serum AFP-L3% in hepatocellular carcinoma: Systematic review and meta-analysis. PLoS One 9: e87011, 2014.
11. Schmitt AM and Chang HY: Long noncoding rnas in cancer pathways. Cancer Cell 29: 452-463, 2016.
12. Batista PJ and Chang HY: Long noncoding RNAs: Cellular address codes in development and disease. Cell 152: 1298-1307, 2013.
13. Mohankumar S and Patel T: Extracellular vesicle long noncoding RNA as potential biomarkers of liver cancer. Brief Funct Genomics 15: 249-256, 2016.
14. Bolha L, Ravnik-Glavač M and Glavač D: Long noncoding RNAs as biomarkers in cancer. Dis Markers 2017: 7243968, 2017.
15. Misawa A, Takayama K, Urano T and Inoue S: Androgen-induced long noncoding RNA (lncRNA) Socs2-As1 promotes cell growth and inhibits apoptosis in prostate cancer cells. J Biol Chem 291: 17861-1780, 2016.
16. Xue S, Li QW, Che JP, Guo Y, Yang FQ and Zheng JH: Decreased expression of long non-coding RNA NBAT-1 is associated with poor prognosis in patients with clear cell renal cell carcinoma. Int J Clin Exp Pathol 8: 3765-3774, 2015.
17. Pandey GK, Mitra S, Subhash S, Hertwig F, Kanduri M, Mishra K, Fransson S, Ganeshram A, Mondal T, Bandaru S, *et al*: The risk-associated long noncoding RNA NBAT-1 controls neuroblastoma progression by regulating cell proliferation and neuronal differentiation. Cancer Cell 26: 722-737, 2014.
18. Bruix J, Sherman M and Practice Guidelines Committee: American association for the study of liver diseases, management of hepatocellular carcinoma. Hepatology 42: 1208-1236, 2005.
19. Chun YH, Kim SU, Park JY, Kim DY, Han KH, Chon CY, Kim BK, Choi GH, Kim KS, Choi JS and Ahn SH: Prognostic value of the 7th edition of the AJCC staging system as a clinical staging system in patients with hepatocellular carcinoma. Eur J Cancer 47: 2568-2575, 2011.
20. Forner A, Reig ME, de Lope CR and Bruix J: Current strategy for staging and treatment: The BCLC update and future prospects. Semin Liver Dis 30: 61-74, 2010.
21. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
22. Subramanian A, Kuehn H, Gould J, Tamayo P and Mesirov JP: Gsea-p: A desktop application for gene set enrichment analysis. Bioinformatics 23: 3251-3253, 2007.
23. Singal AG, Pillai A and Tiro J: Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: A meta-analysis. PLoS Med 11: e1001624, 2014.
24. Poon D, Anderson BO, Chen LT, Tanaka K, Lau WY, Van Cutsem E, Singh H, Chow WC, Ooi LL, Chow P, *et al*: Management of hepatocellular carcinoma in Asia: Consensus statement from the asian oncology summit 2009. Lancet Oncol 10: 1111-1118, 2009.
25. Guo Y, Wang J, Zhang L, Shen S, Guo R, Yang Y, Chen W, Wang Y, Chen G and Shuai X: Theranostical nanosystem-mediated identification of an oncogene and highly effective therapy in hepatocellular carcinoma. Hepatology 63: 1240-1255, 2016.
26. Cui SX, Zhang YS, Chu JH, Song ZY and Qu XJ: Des-gamma-carboxy prothrombin (DCP) antagonizes the effects of gefitinib on human hepatocellular carcinoma cells. Cell Physiol Biochem 35: 201-212, 2015.
27. Hiraoka A, Ishimaru Y, Kawasaki H, Aibiki T, Okudaira T, Toshimori A, Kawamura T, Yamago H, Nakahara H, Suga Y, *et al*: Tumor Markers AFP, AFP-L3, and DCP in hepatocellular carcinoma refractory to transcatheter arterial chemoembolization. Oncology 89: 167-174, 2015.
28. Sun M and Kraus WL: From discovery to function: The expanding roles of long noncoding RNAs in physiology and disease. Endocr Rev 36: 25-64, 2015.
29. Knoll M, Lodish HF and Sun L: Long non-coding RNAs as regulators of the endocrine system. Nat Rev Endocrinol 11: 151-160, 2015.
30. Okugawa Y, Grady WM and Goel A: Epigenetic alterations in colorectal cancer: Emerging biomarkers. Gastroenterology 149: 1204-1225, 2015.

31. Martens-Uzunova ES, Böttcher R, Croce CM, Jenster G, Visakorpi T and Calin GA: Long noncoding RNA in prostate, bladder, and kidney cancer. Eur Urol 65: 1140-1151, 2014.
32. Tantai J1, Hu D1, Yang Y1 and Geng J1: Combined identification of long non-coding RNA XIST and HIF1A-AS1 in serum as an effective screening for non-small cell lung cancer. Int J Clin Exp Pathol 8: 7887-7895, 2015.
33. Jin C, Shi W, Wang F, Shen X, Qi J, Cong H, Yuan J, Shi L, Zhu B, Luo X, *et al*: Long non-coding RNA HULC as a novel serum biomarker for diagnosis and prognosis prediction of gastric cancer. Oncotarget 7: 51763-51772,2016.
34. Amorim M, Salta S, Henrique R and Jerónimo C: Decoding the usefulness of non-coding RNAs as breast cancer markers. J Transl Med 14: 265, 2016.
35. Chauhan R and Lahiri N: Tissue- and serum-associated biomarkers of hepatocellular carcinoma. Biomark Cancer 8: 37-55, 2016.