Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2022**; 27(9): 264
https://doi.org/10.31083/j.fbl2709264

Original Research

Diagnostic Potential of the Serum lncRNAs HOTAIR, BRM and ICR for Hepatocellular Carcinoma

Zhong-Han Lou[1,†], Ke-Yang Xu[2,†], Liang Qiao[3,†], Xiao-Qian Su[1,†], Yuan Ou-Yang[4], Liang-Bin Miao[5], Fang Liu[5], Yi Wang[5], Ai Fu[5], Xian-Hua Ren[5], Jing-Song Huang[5,*], Zhao-Yi Li[5,*], Jian-Feng Bao[5,*]

[1]Forth Clinical School of Zhejiang Chinese Medical University, 310053 Hangzhou, Zhejiang, China
[2]Centre for Cancer and Inflammation Research, School of Chinese Medicine, Hong Kong Baptist University, 999077 Hong Kong, China
[3]Storr Liver Centre, Westmead Institute for Medical Research, The University of Sydney and Westmead Hospital, Westmead, NSW 2145, Australia
[4]The Third Affiliated Hospital of Zhejiang Chinese Medicine University, 310013 Hangzhou, Zhejiang, China
[5]Hangzhou Xixi Hospital Affiliated to Zhejiang Chinese Medical University, 310023 Hangzhou, Zhejiang, China
*Correspondence: zjbjf1972@aliyun.com (Jian-Feng Bao); 13306817568@163.com (Jing-Song Huang); emily.alice@163.com (Zhao-Yi Li)
[†]These authors contributed equally.

Academic Editor: Graham Pawelec
Submitted: 12 July 2022 Revised: 22 August 2022 Accepted: 1 September 2022 Published: 27 September 2022

Abstract

Background: Long noncoding RNAs (lncRNAs) are closely associated with the initiation, progression, metastasis, and recurrence of hepatocellular carcinoma (HCC). They could therefore serve as markers for the early diagnosis and for the prognosis of HCC patients. **Methods**: This was an observational prospective cohort study. A total of 101 participants were included, comprising patients with HCC (n = 61), liver cirrhosis (LC) (n = 20), or healthy controls (HC) (n = 20). The baseline characteristics of participants in each group were compared. Serum levels of the lncRNAs HOTAIR, BRM and ICR were determined in each group by reverse transcription and quantitative real-time polymerase chain reaction (qRT-PCR). Correlations between the serum levels of the three lncRNAs and multiple clinical parameters were analysed. The receiver operating characteristic (ROC) curve was used to assess the diagnostic potential for HCC of each lncRNA individually, or in combination with AFP. Multivariate Cox regression analysis was used to evaluate the accuracy of these lncRNAs for predicting the outcome and survival of HCC patients. **Results**: The serum levels of HOTAIR, BRM and ICR were significantly higher in HCC patients compared to LC patients and healthy subjects. The HOTAIR level was positively correlated to tumour-node metastasis (TNM), Barcelona Clinic Liver Cancer (BCLC) stage, extrahepatic metastasis, vascular invasion, portal vein tumour thrombus (PVTT), and tumour size. The BRM level was positively associated with TNM stage, BCLC stage, vascular invasion, PVTT, and tumour size, while the ICR level was positively correlated with PVTT. A combination of the three lncRNAs and AFP showed the highest diagnostic accuracy for HCC, with an AUC of 0.998, sensitivity of 98.4%, and specificity of 100.0%. This combination showed a better diagnostic accuracy than the individual lncRNAs or AFP alone. Serum levels of the HOTAIR and ICR lncRNAs decreased significantly following surgery. **Conclusions**: Serum levels of the HOTAIR, BRM and ICR lncRNAs are potential prognostic markers for HCC. Upregulation of HOTAIR, BRM and ICR may facilitate early diagnosis and indicate poor prognosis for HCC. These lncRNAs could potentially serve as therapeutic targets for HCC. Combination of the three lncRNAs with AFP may increase the diagnostic accuracy for HCC. Further studies in larger cohorts of patients are needed to validate these findings.

Keywords: hepatocellular carcinoma; long noncoding RNAs; serum biomarker; diagnosis; HOX transcript antisense RNA; lncRNA for association with Brahma; ICAM-1-related long noncoding RNA

1. Introduction

Globally, hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths [1]. HCC often exhibits an insidious onset and rapid progression making early detection and intervention difficult [2]. At present, the most commonly used diagnostic markers for HCC include Alpha-fetoprotein (AFP), Des-γ-Carboxy Prothrombin, AFP Lens Culinaris Agglutin-3 and Golgi Protein [3]. However, these traditional serum biomarkers show low sensitivity and variable specificity for HCC. For example, elevated levels of AFP may not be present in many HCC patients [4], and it has low sensitivity and specificity (58.1% and 29.0%, respectively) [5]. The GALAD scoring algorithm (combination of AFP, DCP and AFP-L3) can significantly improve the early diagnosis of HCC [6], but measurement of AFP-L3 is complex, time-consuming and relatively expensive [7]. As a consequence, AFP-L3 is not widely used in routine clinical practice. Clearly, novel biomarkers with high specificity and sensitivity are urgently needed for the early diagnosis and prognosis of liver cancer.

Long noncoding RNAs (lncRNAs) regulate multiple biological processes and play important roles in the development and progression of various cancer types [8]. Aberrant levels of lncRNAs have been found in the tumour

Table 1. Demographic and clinical features of all participants.

Categories	HC (n = 20)	LC (n = 20)	HCC (n = 61)
Age (years)	41.65 ± 9.90	55.31 ± 12.28	55.96 ± 12.34
Gender			
Male/Female	13/7	14/6	50/11
TNM stage			
I/II/III/IV			31/5/19/5
BCLC stage			
0/A/B/C/D			5/17/14/20/5
ALT (IU/L)	16.5 (12.0–22.0)	24.5 (15.2–39.0)	31.0 (21.0–54.5)*
AFP (ng/mL)	2.30 (1.47–3.34)	3.17 (1.94–9.94)*	54.39 (9.25–1291.50)*#

*: $p < 0.01$, vs. HC; #: $p < 0.01$, vs. LC; HC, healthy controls; LC, Liver cirrhosis; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; AFP, alpha fetoprotein; TNM, Tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer.

tissues and body fluids of patients with various cancers, and lncRNAs have therefore been investigated as candidate early diagnostic markers for cancers including HCC [9]. However, most of the lncRNAs studied so far in liver cancer have shown limited sensitivity and specificity as diagnostic markers. For example, the lncRNA UCA1 has low sensitivity (81.4%) and specificity (75.3%) for HCC diagnosis [10]. However, when combined with other markers such as Linc00152 and AFP, the diagnostic accuracy of this panel for HCC was significantly enhanced [11].

HOTAIR (homeobox transcript antisense intergenic RNA) is a lncRNA located in the Homeobox C gene cluster. It regulates epigenetic modification of multiple genes [12] and is involved in tumorigenesis, metastasis, and drug resistance in various cancer types [13]. The lncRNAs BRM (lncRNA for association with Brahma) and ICR (ICAM-1-related long noncoding RNA) are highly expressed in HCC tumours and in liver CSCs [14,15]. Since lncBRM is necessary for the maintenance and self-renewal of liver CSCs and tumour initiation [14], it has been suggested as a potential marker for early diagnosis and progression of HCC. Increased expression of lncRNA ICR has been closely linked to the development of portal vein tumour thrombus (PVTT), HCC metastasis, and poor clinical outcomes in HCC patients [15]. Thus, lncRNA ICR may be a predictive marker for HCC metastasis.

Based on these earlier findings, we hypothesized that serum levels for the lncRNAs HOTAIR, BRM and ICR could comprise a useful panel for early diagnosis, monitoring of disease progression, and for predicting the outcome of HCC patients.

2. Materials and Methods

2.1 Patients

A total of 61 HCC patients (50 men and 11 women) who attended HangZhou Xixi Hospital from April 2019 to November 2019 were included in this study. Diagnosis of HCC was based on clinical symptoms, serum AFP levels, imaging studies (ultrasound, CT, and MRI) and histopathological examinations. Patients with a history of other tumours, and those receiving radiotherapy or chemotherapy were excluded from the study. Control groups included patients with liver cirrhosis (diagnosed by ultrasound, CT and MRI) with or without complicating portal hypertension and hypersplenism (LC group, n = 20), and healthy subjects identified by physical examination at the Hangzhou Xixi Hospital (HC group, n = 20). Detailed clinical features of the study subjects are shown in Table 1. The clinical staging of HCC patients was determined by TNM and BCLC classification systems [16]. Serum samples were collected from 34 HCC patients before and one week after surgery (27 men, 7 women; mean age: 54.88 ± 12.57 years). The three study groups were balanced for age and sex. The study was approved by the Ethics Committee of Hangzhou Xixi Hospital (Approval NO. 20181228Y22) and all subjects signed informed consent forms.

Table 2. Primer sequences used in qRT-PCR.

Genes	Sequence (5'-3')
HOTAIR	F: 5'-AAACAGAGTCCGTTCAGTGTC-A-3'
	R: 5'-TTCTTAAATTGGGCTGGGTC-3'
BRM	F: 5'-GAGGAGAGAAGTCACTGAAATGG-3'
	R: 5'-CTC-TTCAAAGCAGACCCTCTAC-3'
ICR	F: 5'-CCC-AGAAGGTCATAGAAAGTCCGA-3'
	R: 5'-TCTAAGCAGCCACAGCC-TGAT-3'
RNA 18S	F: 5'-CAGCCACCCGAGATT-GAGCA-3'
	R: 5'-TAGTAGCGACGGGCGGTGTG-3'

Abbreviations: F, forward; R, reverse.

2.2 Serum Collection

Five millilitres of venous blood were collected from each donor, centrifuged at 1000 *g* for 10 min, and the serum collected into a 1.5 mL centrifuge tube and stored at –80 °C until use.

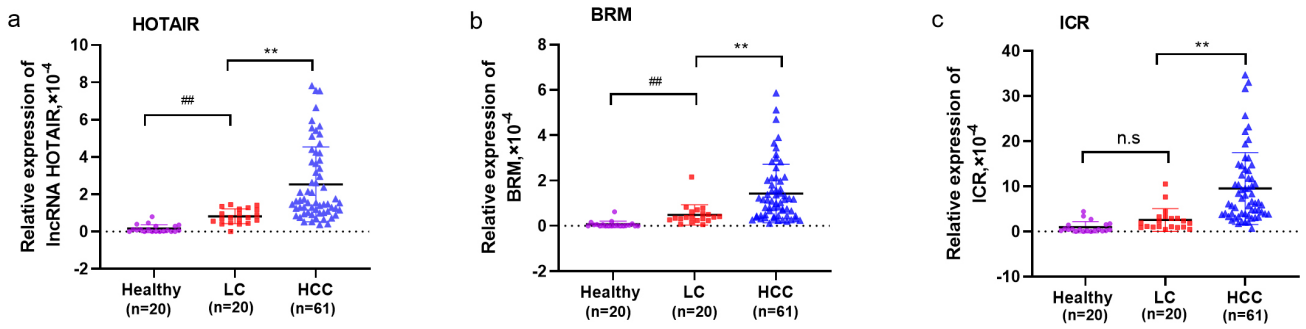

Fig. 1. Compariso of serum lncRNA levels in patients with HCC, LC, and HC. Serum level of lncRNAs HOTAIR (a), BRM (b) and ICR (c) in HCC patients, LC patients, and healthy controls. **: $p < 0.01$, HCC vs. LC; ##: $p < 0.01$, LC vs. Healthy controls. n.s, no significant difference.

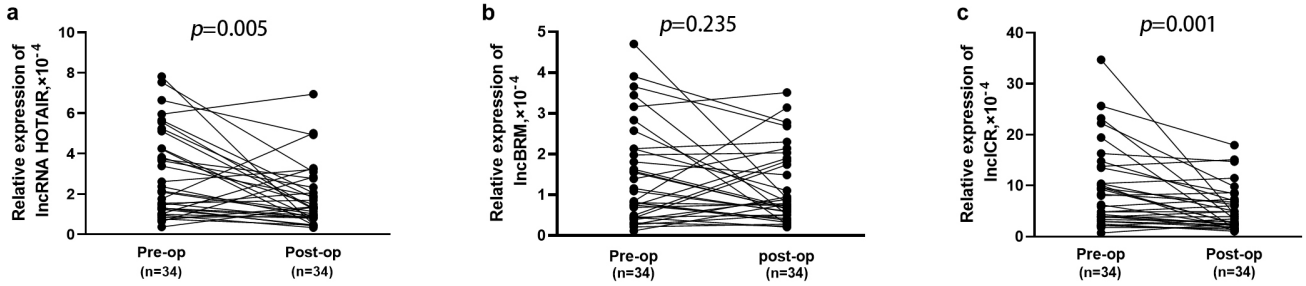

Fig. 2. Comparison of serum Levels of HOTAIR, BRM and ICR in Pre- and Postoperative HCC Patients. Serum levels of three lncRNAs HOTAIR (a), BRM (b) and ICR (c) in the preoperative (Pre-op) and postoperative (Post-op) serum samples of HCC patients (n = 34).

2.3 RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

All lncRNA assays were carried out in the HangZhou Adicon Clinical Laboratory using qRT-PCR. Total RNA was extracted using the miRcute miRNA isolation Kit (DP501; Beijing, China) according to the manufacturer's protocol. Briefly, complementary DNA (cDNA) was synthesized using the TIANGEN lnRcute lncRNA cDNA kit (KR202; Beijing, China). One hundred ng of total extracted serum RNA was converted into cDNA. The relative expression of various genes was examined using the SYBR Green Realtime PCR Master Kit (QPK-201; Shanghai, China) and a PCR ABI 7500 Sequence Detection System. Each reaction mixture consisted of 2 μL of cDNA, 12.5 μL of mastermix and 1 μL of each primer, with the total volume adjusted to 25 μL using RNase-free water. The housekeeping gene 18S was used as the internal control. Primer sequences for the lncRNAs HOTAIR, BRM and ICR, and for 18S mRNA were designed and synthesized by the HangZhou Adicon Clinical Laboratory. The primer sequences for qRT-PCR are shown in Table 2.

2.4 Serum AFP Determination

The serum AFP levels were determined by chemiluminescence enzyme immunoassay (CLEIA).

2.5 Patient Follow-Up

A total of 47 patients were followed up till December 2021. Follow-up data were obtained through telephone calls and hospital records, and were updated every 3~6 months. Clinical information recorded in the follow up database included sex, age, AFP, Child-Pugh, Performance Status (PS), HBsAg, PVTT, tumour size, tumour number, type of treatment, tumour recurrence and metastasis. Patients who died from unexpected events or other diseases were excluded from the study.

2.6 Statistical Analyses

All statistical analyses were performed using SPSS software (version 22.0, IBM Inc., Chicago, IL, USA). Non-parametric variables are presented as median and interquartile ranges, and were analysed using the Mann-Whitney U test and Kruskal-Wallis H test. Categorical variables were compared using chi square tests. To determine the accuracy of the lncRNAs HOTAIR, BRM and ICR as biomarkers for HCC, ROC (Receiver Operating Characteristic) curves were constructed and AUC (Area Under the Curve) values were estimated. Multivariate analysis was performed to identify independent prognostic factors. A p value of <0.05 was considered statistically significant.

Table 3. The relationship between the serum level of lncRNAs HOTAIR, BRM and ICR and clinicopathological features in HCC patients (n = 61, level of lncRNAs is expressed as $\times 10^{-4}$).

Factors	N	HOTAIR	*p*	BRM	*p*	ICR	*p*
Age (y)			0.575		0.859		0.707
>60	24	1.47 (0.96, 3.59)		1.13 (0.44, 1.51)		5.79 (3.30, 15.80)	
≤60	37	1.63 (1.20, 4.04)		1.03 (0.27, 2.07)		6.69 (3.98, 12.20)	
Gender			0.599		0.985		0.113
Male	50	1.55 (1.14, 3.93)		1.01 (0.44, 2.16)		7.84 (3.99, 14.61)	
Female	11	1.68 (0.73, 3.93)		1.16 (0.44, 1.99)		4.84 (3.34, 8.05)	
TNM stage			0.007		0.007		0.188
I+II	38	1.36 (0.91,2.44)		0.71 (0.35, 1.42)		5.81 (3.79, 10.90)	
III+IV	23	2.96 (1.54, 2.44)		1.44 (0.81, 2.83)		9.62 (4.03, 16.4)	
BCLC stage			0.015		0.009		0.822
0–A	22	1.27 (0.85, 2.18)		0.56 (0.27, 1.25)		6.59 (3.79, 12.43)	
B–D	39	2.10 (1.30, 4.40)		1.23 (0.67, 2.13)		6.27 (3.92, 13.50)	
PS			0.293		0.786		0.119
0~1	50	1.47 (0.98, 4.23)		1.01 (0.43, 2.14)		5.81 (3.52, 12.20)	
2~4	11	2.15 (1.54, 3.39)		1.16 (0.46, 1.81)		10.40 (7.35, 13.50)	
Child-Pugh			0.810		0.781		0.215
A	39	1.63 (1.04, 4.25)		1.03 (0.36, 2.57)		7.35 (4.06, –14.80)	
B + C	22	1.55 (1.22, 3.46)		1.15 (0.68, 1.48)		5.50 (3.27, 11.08)	
AFP (g/L)			0.658		0.848		0.315
≤20	24	1.89 (1.02, 4.79)		1.04 (1.02, 4.79)		8.67 (4.13, 14.40)	
>20	37	1.55 (1.14, 3.42)		1.14 (0.43, 2.13)		6.14 (3.68, 11.75)	
Extrahepatic metastasis			0.043		0.102		0.605
Yes	12	3.60 (1.26, 5.88)		1.48 (0.72, 3.08)		10.01 (3.02, 19.40)	
No	49	1.54 (1.03, 3.28)		1.03 (0.41, 1.70)		6.27 (3.92, 11.75)	
Vascular invasion			0.022		0.011		0.714
Yes	19	4.22 (1.30, 5.56)		1.63 (0.81, 2.84)		9.54 (3.29, 16.30)	
No	42	1.46 (1.01, 2.62)		0.79 (0.35, 1.47)		6.25 (3.92, 12.23)	
PVTT			0.004		0.005		0.023
Yes	11	4.25 (1.55, 5.65)		2.57 (1.16, 3.91)		10.40 (9.29, 16.30)	
No	50	1.46 (1.01, 2.71)		0.81 (0.43, 1.47)		5.81 (3.52, 11.73)	
HBV			0.338		0.691		0.894
Yes	57	1.63 (1.10, 4.02)		1.03 (0.44, 2.07)		6.27 (3.89, 13.65)	
No	4	1.14 (0.91, 3.10)		1.15 (0.62, 1.35)		7.70 (4.34, 10.79)	
Tumor size (cm)			0.039		0.036		0.239
≤5	40	1.43 (1.00, 2.62)		0.79 (0.37, 1.52)		5.81 (3.88, 11.40)	
>5	21	2.96 (1.42, 4.97)		1.39 (0.81, 2.70)		9.62 (3.90, 14.95)	
Tumor number			0.398		0.337		0.884
Single	41	1.55 (1.03, 3.69)		0.84 (0.38, 2.00)		6.49 (3.97, 11.20)	
Multiple	20	1.61 (1.26, 4.36)		1.16 (0.67, 2.05)		7.95 (3.22, 16.00)	

TNM, Tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer; PS, Performance Status; AFP, Alpha fetoprotein; HBV, Hepatitis B Virus; PVTT, portal vein tumor thrombus.

3. Results

3.1 Serum lncRNA Levels in Patients with HCC, LC, and HC

Serum levels for the lncRNAs HOTAIR, BRM and ICR were all significantly higher in HCC patients compared to LC patients and healthy controls (Fig. 1, $p < 0.01$). Moreover, the serum level of the lncRNA HOTAIR was significantly higher in LC patients than in healthy controls ($p < 0.05$). No significant difference in the serum level of the lncRNA ICR was seen between LC patients and healthy controls (n.s, Fig. 1c).

3.2 Correlation of the lncRNAs HOTAIR, BRM and ICR with Clinical Features

As shown in Table 3, the serum level of lncRNA HOTAIR was closely associated with TNM stage ($p = 0.007$), BCLC stage ($p = 0.015$), extrahepatic metastasis ($p = 0.043$), vascular invasion ($p = 0.022$), PVTT ($p = 0.004$),

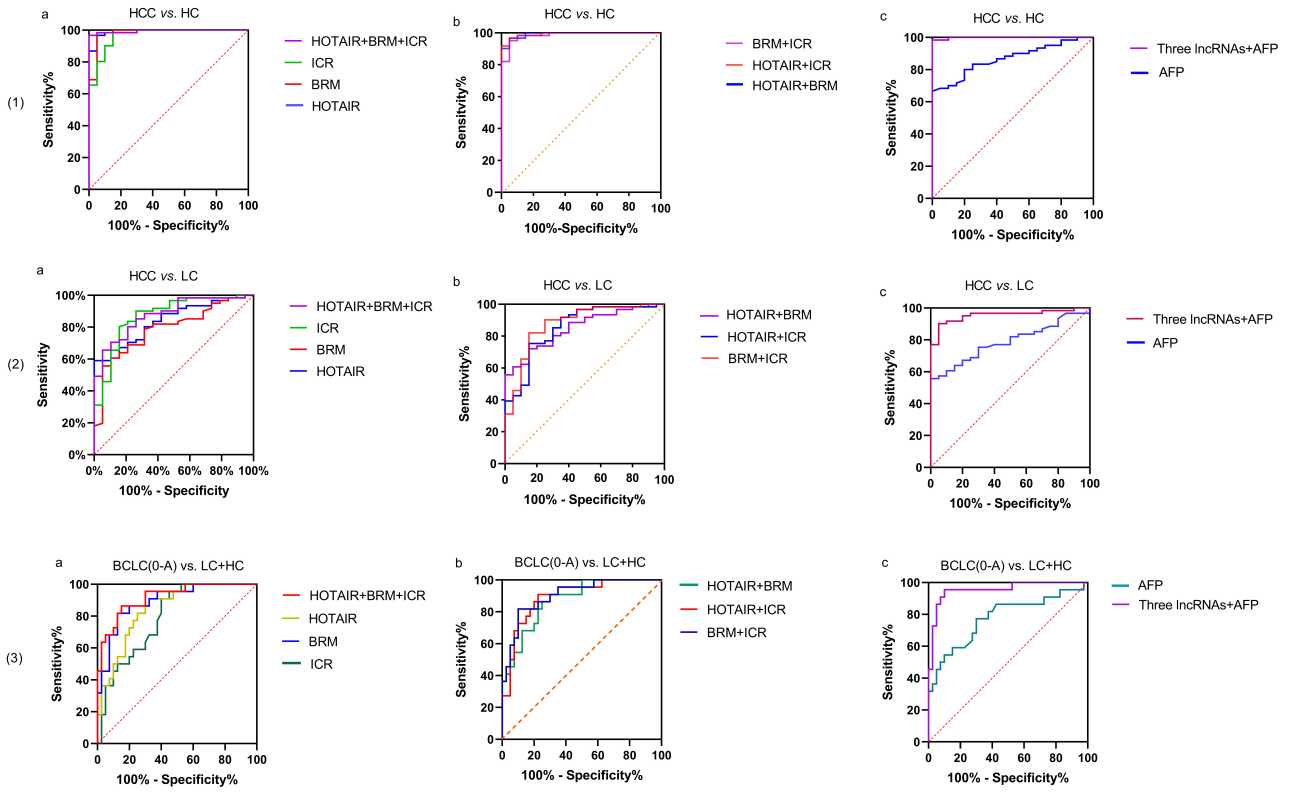

Fig. 3. Diagnostic efficiency of individual serum lncRNAs orAFP or their combinations in HCC patients. (1) ROC curve analysis for the individual lncRNAs (a, b) or AFP (c) or their combinations in distinguishing HCC patients from healthy controls. (2) ROC curve analysis for the individual lncRNAs (d, e) or AFP (f) or their combinations in distinguishing HCC patients from LC patients. (3) ROC curve analysis for the individual lncRNAs or AFP or their combinations in distinguishing HCC patients with early BCLC stage (n = 22) from healthy controls and LC patients.

and tumour size (p = 0.039). The serum level of lncRNA BRM was significantly correlated with TNM stage, BCLC stage, vascular invasion, PVTT, and tumour size (p = 0.007, p = 0.009, p = 0.011, p = 0.005, p = 0.039, p = 0.036, respectively). The serum level of lncRNA ICR was closely correlated with portal venous invasion (p = 0.023). No significant associations were found between these lncRNAs and gender, age, PS, Child-Pugh score, serum HBsAg level, serum AFP level, and tumour number.

3.3 Serum Levels of HOTAIR, BRM and ICR in Pre- and Postoperative HCC Patients

The serum levels of the three lncRNAs in 34 HCC patients were measured on day one of their hospital visit (pre-operative) and one week after tumour resection (post-operative). A significant decrease in the serum level after surgery was observed for the lncRNAs HOTAIR and ICR, but not for BRM (Fig. 2).

3.4 Diagnostic Accuracy of Serum lncRNAs in HCC Patients

To assess the diagnostic value of the three lncRNAs HOTAIR, BRM and ICR for HCC, ROC curve analysis was performed to determine their AUC, sensitivity, and specificity. AFP is widely used as a serum marker for HCC, hence this marker was analysed alone and in combination with the lncRNAs for the diagnosis of HCC. The three lncRNAs alone showed higher accuracy for identifying HCC compared to AFP (Fig. 3 and Table 4). Notably, lncRNA HOTAIR showed the highest AUC for distinguishing HCC patients from healthy controls, while lncRNA ICR showed better ability for distinguishing HCC from LC patients.

Based on the above results, we next used binary logistic regression to assess the diagnostic accuracy of lncRNA-based panels. As shown in Table 4 and Fig. 3, combination of the three lncRNAs resulted in better diagnostic power than the individual lncRNAs. The diagnostic accuracy of the three lncRNAs for HCC was further improved by combining with AFP, as indicated by the higher AUC values for distinguishing HCC patients from healthy controls and from LC patients (Table 4; Fig. 3(1c); Fig. 3(2c)). Thus, combination of the three lncRNAs with AFP resulted in the best diagnostic accuracy for HCC patients.

In the 22 HCC patients (22/61, 36%) with early-stage disease (BCLC stage 0+A), the diagnostic accuracy of the combined panel of three lncRNAs and AFP was also significantly better compared to each lncRNA alone or to AFP alone (Table 4, Fig. 3).

Table 4. The diagnostic efficiency of serum lncRNAs and AFP in distinguishing HCC from HC and LC.

Method	AUC (95% CI)	SEN (%)	SPE (%)	Cut-off	*p*-value
HCC vs. HC					
HOTAIR	0.991 (0.976–1.000)	96.7	95.0	0.49×10^{-4}	<0.001
BRM	0.983 (0.952–1.000)	98.4	95.0	0.20×10^{-4}	<0.001
ICR	0.966 (0.925–1.000)	98.4	85.0	1.76×10^{-4}	<0.001
HOTAIR+BRM	0.992 (0.979–1.000)	96.7	95.0	0.597	<0.001
HOTAIR+ICR	0.992 (0.979–1.000)	91.8	100.0	0.863	<0.001
BRM+ICR	0.985 (0.964–1.000)	95.1	95.0	0.650	<0.001
HOTAIR+BRM+ICR	0.994 (0.983–1.000)	96.7	100.0	0.708	<0.001
AFP	0.854 (0.774–0.934)	65.6	100.0	16.75	<0.001
Three lncRNAs+AFP	0.998 (0.994–1.000)	98.4	100.0	0.641	<0.001
HCC vs. LC					
HOTAIR	0.811 (0.712–0.911)	59.0	100.0	1.45×10^{-4}	<0.001
BRM	0.749 (0.621–0.877)	55.7	94.7	0.83×10^{-4}	<0.001
ICR	0.850 (0.750–0.950)	80.3	84.2	3.46×10^{-4}	<0.001
HOTAIR+BRM	0.850 (0.766,0.934)	72.1	85.0	0.761	<0.001
HOTAIR+ICR	0.855 (0.759–0.951)	75.4	85.0	0.671	<0.001
BRM+ICR	0.877 (0.786–0.968)	82.0	85.0	0.647	<0.001
HOTAIR+BRM+ICR	0.884 (0.807–0.960)	65.6	94.7	0.849	<0.001
AFP	0.788 (0.691–0.884)	55.7	100.0	45.5	<0.001
Three lncRNAs+AFP	0.955 (0.911–0.999)	90.2	95.0	0.611	<0.001
BCLC (0–A) vs. LC+HC					
HOTAIR	0.848 (0.753–0.942)	90.9	67.5	0.64×10^{-4}	<0.001
BRM	0.792 (0.682–0.902)	90.9	60.0	0.25×10^{-4}	<0.001
ICR	0.894 (0.814–0.975)	81.8	87.5	3.49×10^{-4}	<0.001
HOTAIR+BRM	0.873 (0.787–0.959)	86.4	75.0	0.294	<0.001
HOTAIR+ICR	0.892 (0.810–0.974)	90.9	77.5	0.246	<0.001
BRM+ICR	0.903 (0.827–0.980)	81.8	90.0	0.358	<0.001
HOTAIR+BRM+ICR	0.918 (0.849–0.988)	86.4	85.0	0.338	<0.001
AFP	0.778 (0.647–0.909)	77.3	70.0	8.12	<0.001
Three lncRNAs+AFP	0.955 (0.900–1.000)	95.5	90.0	0.307	<0.001

AUC, Area under the curve; SEN, sensitivity; SPE, specificity; HC, healthy controls; LC, Liver cirrhosis; PLC, Primary liver carcinoma; AFP, Alpha fetoprotein; BCLC, Barcelona Clinic Liver Cancer.

Table 5. Cox regression analysis for the prognostic value of three lncRNAs in HCC patients (n = 47).

Variables	HR	95% CI	*p*
HOTAIR level (low, high)	3.311	1.697–15.726	0.032
BRM level (low, high)	2.090	1.120–10.892	0.038
ICR level (low, high)	1.437	1.003–4.338	0.006

HR, Hazard Ratio; CI, Confidence Interval.

These data suggest that a combined panel comprising three lncRNAs and AFP could be a potential biomarker for HCC.

3.5 Correlation of the Serum Level of Three lncRNAs with Overall Survival

The prognostic value of the lncRNAs HOTAIR, BRM and ICR was evaluated in 47 HCC patients (survival time 19~25 days, median 23 days) by Cox regression analysis. Serum levels of the lncRNAs HOTAIR, BRM and ICR were significantly correlated with HCC prognosis (Table 5).

4. Discussion

Accurate early diagnosis and prediction of outcome for HCC patients remains a major clinical challenge worldwide. Previous studies have implicated the lncRNAs HOTAIR, BRM and ICR in liver cancer and LCSCs. However, the clinical value of these lincRNAs in the management of HCC patients has yet to be explored. The present study is the first to assess the diagnostic value of the serum lncRNAs HOTAIR, BRM and ICR in HCC patients. Elevated serum levels of the three lncRNAs were found to be accurate diagnostic markers for HCC, as well as prognostic biomarkers for these patients. A significant decrease in the serum level of HOTAIR and ICR was observed following surgical resection in HCC patients. Hence, the lncRNAs HOTAIR and ICR may be useful markers for monitoring the outcome of surgical resection in HCC patients. Another novelty of this study was the finding that combination of the three lncRNAs with the classical tumour marker AFP resulted in improved accuracy for HCC diagnosis.

An increase in the HOTAIR level has been reported to promote the malignant transformation of normal liver cells, including normal liver stem cells (NLSCs), via epithelial-mesenchymal transition (EMT) [17]. Hence, the serum level of HOTAIR may be useful for the early diagnosis of HCC. Indeed, elevated levels of HOTAIR have been observed in many cancer types and a high level could therefore facilitate their diagnosis and perhaps also predict poor many cancer types including HCC [18–21]. In these aspects, our data are consistent with the previously published data [22] in that elevated serum HOTAIR level significantly correlated with TNM stage, BCLC stage, extrahepatic metastasis, vascular invasion, portal vein tumour thrombus, and tumour size.

The oncogenic role of BRM has been reported in multiple cancers including colorectal [23], ovarian [24] and liver [14]. Previous studies have also shown that the lncRNAs BRM and ICR were highly expressed in liver CSCs and HCC tumours [14,15], and that ICR could regulate liver CSC properties and contribute to PVTT development [15]. In particular, BRM promotes the self-renewal of liver CSCs and initiates tumour propagation via YAP1 signalling, while the serum level of BRM was positively correlated to the disease severity of HCC patients [14]. In the current study, an increased serum level of BRM was significantly correlated with advanced TNM stage, BCLC stage, vascular invasion, portal venous invasion, and tumour size. Furthermore, the serum level of the three lncRNAs decreased significantly following surgical resection of HCC tumours. These data provide support for the potential application of three lncRNAs (HOTAIR, BRM and ICR) for the early diagnosis, prediction of survival outcome, and monitoring of disease progression in HCC patients. A novel finding of our study was that combination of these three lncRNAs with the classical HCC marker AFP further increased the diagnostic value of the lncRNAs. Additional studies in large patient cohorts are needed to validate our findings.

Considering the known oncogenic roles of the lncRNAs studied here, we speculate they could also serve as therapeutic targets for HCC. For example, an RNA-based dominant negative molecule that counteracts HOTAIR function (HOTAIR-SNAIL-binding domain, HOTAIR-sbid) has been shown to inhibit mobility, invasiveness and EMT in HCC cells [25]. The potential application of these lncRNAs as therapeutic targets for liver cancer warrants further investigation.

A major limitation of this study was the small number of patients analysed and the short follow up time. Further studies in larger cohorts of patients should be conducted to confirm our findings. These should include patients with various pre-malignant conditions (e.g., hepatitis, fatty liver disease), as well as patients who have been treated with surgery or other approaches (e.g., regional ablation).

5. Conclusions

Increased serum levels of the lncRNAs HOTAIR, BRM and ICR could be used in a panel of markers for early diagnosis of HCC. Combining these lncRNAs with AFP improves the diagnostic accuracy of HCC even further, while also providing a useful tool for monitoring therapeutic effects in HCC.

Data Availability Statement

All data generated or analysed during this study are included in this published article. Additional data/files would be available from the corresponding author upon reasonable request. The funding bodies played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Author Contributions

ZHL, KYX, and JFB designed and revised the manuscript. ZHL wrote the manuscript and drew figures. ZHL, XQS, and YOY prepared the patient samples. LQ, and KYX provided assistances with revising the manuscript. ZYL, JSH, and XHR conducted data collection. LBM, FL, YW, and AF conducted data analysis. All the authors read and approved the final version of the manuscript.

Ethics Approval and Consent to Participate

The protocal regarding human serum samples was approved by the Hangzhou Xixi Hospital and an agreement was signed in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all patients and healthy donors (Approval NO. 20181228Y22).

Acknowledgment

We thank anonymous reviewers for excellent criticism of the article and all the authors in the reference list.

Funding

This work has been supported by the Natural Science Foundation of Zhejiang Province (LSY19H030002), the project of Hangzhou Science and Technology Bureau (20181228Y22), the project of Yuhuan Science and Technology Bureau (2018075).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Yang W-S, Zeng X-F, Liu Z-N, Zhao Q-H, Tan Y-T, Gao J, *et al*. Diet and liver cancer risk: a narrative review of epidemiological evidence. British Journal of Nutrition. 2020; 124: 330–340.

[2] Anwanwan D, Singh SK, Singh S, Saikam V, Singh R. Challenges in liver cancer and possible treatment approaches.

Biochimica et Biophysica Acta - Reviews on Cancer. 2020; 1873: 188314.

[3] Gao Y-X, Yang T-W, Yin J-M, Yang P-X, Kou B-X, Chai M-Y, *et al*. Progress and prospects of biomarkers in primary liver cancer (Review). International Journal of Oncology. 2020; 57: 54–66.

[4] Luo P, Wu S, Yu Y, Ming X, Li S, Zuo X, *et al*. Current Status and Perspective Biomarkers in AFP Negative HCC: towards Screening for and Diagnosing Hepatocellular Carcinoma at an Earlier Stage. Pathology & Oncology Research. 2020; 26: 599–603.

[5] Qin Q, Weng J, Xu G, Chen C, Jia C. Combination of serum tumor markers dickkopf-1, DCP and AFP for the diagnosis of primary hepatocellular carcinoma. Asian Pacific Journal of Tropical Medicine. 2017; 10: 409–413.

[6] Best J, Bilgi H, Heider D, Schotten C, Manka P, Bedreli S, *et al*. The GALAD scoring algorithm based on AFP, AFP-L3, and DCP significantly improves detection of BCLC early stage hepatocellular carcinoma. Zeitschrift für Gastroenterologie. 2016; 54: 1296–1305.

[7] Yen C, Kuo Y, Wang J, Chang K, Kee K, Hung S, *et al*. Did AFP-L3 save ultrasonography in community screening? The Kaohsiung Journal of Medical Sciences. 2018; 34: 583–587.

[8] Li Y, Jiang T, Zhou W, Li J, Li X, Wang Q, *et al*. Pan-cancer characterization of immune-related lncRNAs identifies potential oncogenic biomarkers. Nature Communications. 2020; 11: 1000.

[9] Lao Y, Li Q, Li N, Liu H, Liu K, Jiang G, *et al*. Long noncoding RNA ENST00000455974 plays an oncogenic role through up-regulating JAG2 in human DNA mismatch repair-proficient colon cancer. Biochemical and Biophysical Research Communications. 2019; 508: 339–347.

[10] Yao F, Wang Q, Wu Q. The prognostic value and mechanisms of lncRNA UCA1 in human cancer. Cancer Management and Research. 2019; 11: 7685–7696.

[11] Huang J, Zheng Y, Xiao X, Liu C, Lin J, Zheng S, *et al*. A Circulating Long Noncoding RNA Panel Serves as a Diagnostic Marker for Hepatocellular Carcinoma. Disease Markers. 2020; 2020: 5417598.

[12] Qu X, Alsager S, Zhuo Y, Shan B. HOX transcript antisense RNA (HOTAIR) in cancer. Cancer Letters. 2019; 454: 90–97.

[13] Rajagopal T, Talluri S, Akshaya RL, Dunna NR. HOTAIR LncRNA: a novel oncogenic propellant in human cancer. Clinica Chimica Acta. 2020; 503: 1–18.

[14] Zhu P, Wang Y, Wu J, Huang G, Liu B, Ye B, *et al*. LncBRM initiates YAP1 signalling activation to drive self-renewal of liver cancer stem cells. Nature Communications. 2016; 7: 13608.

[15] Guo W, Liu S, Cheng Y, Lu L, Shi J, Xu G, *et al*. ICAM-1–Related Noncoding RNA in Cancer Stem Cells Maintains ICAM-1 Expression in Hepatocellular Carcinoma. Clinical Cancer Research. 2016; 22: 2041–2050.

[16] Ayuso C, Rimola J, Vilana R, Burrel M, Darnell A, García-Criado Á, *et al*. Diagnosis and staging of hepatocellular carcinoma (HCC): current guidelines. European Journal of Radiology. 2018; 101: 72–81.

[17] Ye P, Wang T, Liu W, Li X, Tang L, Tian F. Enhancing HOTAIR/MiR-10b Drives Normal Liver Stem Cells toward a Tendency to Malignant Transformation through Inducing Epithelial- to-Mesenchymal Transition. Rejuvenation Research. 2015; 18: 332–340.

[18] GAO J, LI J, DU J, LI X. Long non-coding RNA HOTAIR is a marker for hepatocellular carcinoma progression and tumor recurrence. Oncology Letters. 2016; 11: 1791–1798.

[19] Qin W, Kang P, Xu Y, Leng K, Li Z, Huang L, *et al*. Long noncoding RNA HOTAIR promotes tumorigenesis and forecasts a poor prognosis in cholangiocarcinoma. Scientific Reports. 2018; 8: 12176.

[20] Wu Z, Wang X, Tang H, Jiang T, Chen J, Lu S, *et al*. Long noncoding RNA HOTAIR is a powerful predictor of metastasis and poor prognosis and is associated with epithelial-mesenchymal transition in colon cancer. Oncology Reports. 2014; 32: 395–402.

[21] Loewen G, Jayawickramarajah J, Zhuo Y, Shan B. Functions of lncRNA HOTAIR in lung cancer. Journal of Hematology & Oncology. 2014; 7: 90.

[22] Zhong DN, Luo YH, Mo WJ, Zhang X, Tan Z, Zhao N, *et al.* High expression of long noncoding HOTAIR correlated with hepatocarcinogenesis and metastasis. Molecular Medicine Reports. 2018; 17: 1148–1156.

[23] Li R, Zhu H, Yang D, Xia J, Zheng Z. Long noncoding RNA lncBRM promotes proliferation and invasion of colorectal cancer by sponging miR-204-3p and upregulating TPT1. Biochemical and Biophysical Research Communications. 2019; 508: 1259–1263.

[24] Xi J, Feng J, Zeng S. Long noncoding RNA lncBRM facilitates the proliferation, migration and invasion of ovarian cancer cells via upregulation of Sox4. The American Journal of Cancer Research. 2017; 7: 2180–2189.

[25] McDonald OG, Wu H, Timp W, Doi A, Feinberg AP. Genome-scale epigenetic reprogramming during epithelial-to-mesenchymal transition. Nature Structural & Molecular Biology. 2011; 18: 867–874.