Potential diagnostic value of lncRNA SPRY4-IT1 in hepatocellular carcinoma

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Abstract. The manifestation of hepatocellular carcinoma (HCC) involves a multi-factor, multi-step and complex process. Due to the lack of early prediction indices, numerous patients are diagnosed in their late stage. Recently, research in the field of non-coding RNAs (ncRNAs) has changed the original idea that ncRNA genes are just 'noise'. Cumulative evidence shows that long non-coding RNAs (lncRNAs) among ncRNAs play an increasingly important role in epigenetics, pre-transcription and post-transcription. In the present study, we focused on the expression pattern of lncRNA SPRY4 intronic transcript 1 (SPRY4-IT1) and its clinical significance in HCC diagnostics. We analyzed the expression, its association with clinical characteristics and the diagnostic value of SPRY4-IT1 using HCC tissues, cell lines and plasma. The levels of SPRY4-IT1 were upregulated in HCC and were associated with tumor differentiation (r=0.249, p=0.039), tumor size (r=0.258, p=0.024) and tumor-node-metastasis (TNM) stage (r=0.287, p=0.015). Meanwhile, the sensitive of SPRY4-IT1 was 87.3% in differentiating HCC patients from controls. Our data suggest that SPRY4-IT1 plays a critical role in HCC tumorigenesis and may be considered as a potential diagnostic indicator in HCC.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer among males, and it is the ninth leading cause of cancer-related deaths in females worldwide (1). HCC is the most common form of liver cancer, accounting for between 85 and 90% of all primary liver cancers (2). The leading risk factor for developing HCC is cirrhosis and the rate is 80-90% (3). It has been reported that chronic hepatitis B virus (HBV) infection is the cause of the majority of cirrhosis cases (4). Despite the fact that patients can be successfully treated by surgery, liver transplantation, chemotherapy and interventional therapy (5,6), HCC is commonly diagnosed in the advanced stage after related symptoms appear, and the 5-year survival rate remains at 7% (7). During the past 20 years, the mortality rate associated with HCC has significantly increased and epidemiologic evidence indicates that the burden on medical care costs may significantly increase during the next decades (8). The serum α-fetoprotein (AFP) level is used as a diagnostic marker for HCC, yet AFP results may be negative in as many as 40% of cases presenting with early stage HCC. Even in 15-30% of advanced patients, AFP levels remain normal and imaging examination must be utilized to discriminate between tumor and non-neoplastic lesions (9). The lack of knowledge regarding the mechanisms of the tumorigenesis of HCC results in ineffective therapy and a high probability of relapse after treatment (10). Therefore, identification of reliable diagnostic markers for HCC is urgently needed.

Long non-coding RNAs (lncRNAs), which were first described by Brockdorff *et al* in 1992 (11), are molecules with a length longer than 200 bp that cannot code protein products. Khachane and Harrison (12) demonstrated that the proportion of lncRNAs associated with cancer was 2-fold higher than that of protein coding genes in the human genome. Increasing evidence has pointed to a relationship between lncRNAs and cancers, including metastasis, migration, apoptosis and clinical outcome (13). For example, highly upregulated in liver cancer (HULC) is a specific gene which is markedly upregulated both in tissue and plasma in HCC (5). The H19 lncRNA was found to be associated with tumorigenesis and invasion, partially via the regulation of carcinogenic miRNA-675 which locates in its first exon (14). lncRNA-ROR can downregulate miR-145, causing cancer progression and drug resistance (15). Urothelial carcinoma-associated 1 (UCA1) can directly bind to miR-216b, and the abnormal expression of UCA1 in HCC is correlated with tumor-node-metastasis (TNM) stage, metastasis, survival and AFP level (16). Unfortunately, the functional role of lncRNAs in HCC remains largely unexplored.

lncRNA SPRY4 intronic transcript 1 (SPRY4-IT1) is a 703-bp molecule which maps to chromosome 5q31.3. This transcript may mediate cell growth, proliferation, apoptosis and was found to be upregulated in several different tumors, including glioma, prostate, breast, non-small cell lung and

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esophageal squamous cell cancer (17-21). Mazar *et al* (22) found that SPRY4-IT1 was upregulated in melanoma cells, and demonstrated that RNAi-mediated knockdown of SPRY4-IT1 may induce apoptosis via lipin 2-mediated alterations in lipid metabolism leading to cellular lipotoxicity. Peng *et al* (23) indicated that upregulation of SPRY4-IT1 expression was significantly correlated with tumor size, depth of invasion, distant metastasis and TNM stage in gastric cancer, and knockdown of SPRY4-IT1 expression suppressed cell migration, invasion, proliferation and colony formation capabilities. Zhang *et al* (24) showed that overexpression of SPRY4-IT1 was associated with the progression and development of clear cell renal carcinoma. Much research concerning SPRY4-IT1 in cancers has been carried out. However, the clinical and prognostic significance of lncRNA SPRY4-IT1 expression in HCC has not been reported.

The aim of the present study was to investigate the gene expression of lncRNA SPRY4-IT1 in HCC patients and cell lines, and then analyze the correlation between clinical characteristics and SPRY4-IT1 levels. We also evaluated the diagnostic value of SPRY4-IT1 in plasma. Moreover, we assessed whether SPRY4-IT1 could serve as a new biomarker for HCC.

Materials and methods

Tissue and blood samples. We recruited 87 patients (81 men and 6 women, mean age, 55±10) with HCC who underwent surgery without preoperative chemotherapy or radiotherapy from 2011 to 2015 at Zhongnan Hospital of Wuhan University, Wuhan, China. Tumor tissue specimens and corresponding adjacent non-tumor tissues were collected and stored at -80˚C until use.

Whole blood samples of 145 patients were obtained from Zhongnan Hospital of Wuhan University during 2015. The samples were classified into three groups: pre-operation (48 men and 12 women; mean age, 57±12), 2 weeks after surgery (48 men and 12 women; mean age, 57±12), patients with hepatitis B and cirrhosis (63 men and 22 women; mean age, 54±12), which were collected in EDTA tubes and centrifuged at 2,000 x g for 5 min at 4˚C to spin down the blood cells. The supernatants were transferred to microcentrifuge tubes and centrifuged at 12,000 x g for 5 min at 4˚C. The plasma was then stored at -80˚C until use. Patients who underwent previous preoperative chemotherapy or radiotherapy were excluded from the study. Then, from the Physical Examination Center, we collected 63 controls (50 men and 13 women; mean age, 54±11), who were without hepatitis, hepatic diseases and abnormal liver biochemical outcomes.

Ethical approval. Tissue and plasma specimens were collected after obtaining informed consent of the patients in accordance with the institutional ethical guidelines and approved by the Ethics Committee of Zhongnan Hospital of Wuhan University (Wuhan, China) for the use of these clinical materials.

Cell culture. The human HCC Hep-3B and HepG2 cell lines were obtained from the China Center for Type Culture Collection (CCTCC; Wuhan, China). HuH-7 and the normal human hepatocyte cell line L-02 were purchased from the Procell Inc. (Wuhan, China). MHCC97-L and HCCLM-9 cells were maintained at our laboratory. MHCC97-L cells were cultured in Dulbecco's modified Eagle's medium (DMEM), and the other cell lines were cultured in RPMI-1640 medium (both from Gibco, Grand Island, NY, USA). All cells were cultured at 37˚C in 5% CO_2 in culture media containing 10% fetal bovine serum (FBS; Gibco).

RNA extraction. Total RNA was extracted from tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and we used total RNA separate extraction kit (BioTeke, Beijing, China) for plasma samples. RNA was reverse transcribed to cDNA using PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Japan). The conditions were as follows: 42˚C for 2 min, and then 37˚C for 15 min, and 85˚C for 5 sec.

Real-time PCR analysis. The expression of SPRY4-IT1 was determined on the Bio-Rad CFX96 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using SYBR-Green I Premix Ex Taq according to the manufacturer's instructions. The reactions started at 95˚C for 5 min, followed by 45 cycles of 95˚C for 30 sec, 61˚C for 30 sec and 72˚C for 30 sec. In order to normalize the results for the qPCR, expression of 18s was used. The synthesized primers were as follows: SPRY4-IT1 sense, 5'-GTTTTTGCTGAGCTGGTGGTT-3' and antisense, 5'-ATGGCTCCACTGGGCATATT-3'; 18s sense, 5'-CAGCC ACCCGAGATTGAGCA-3' and antisense, 5'-TAGTAGCGAC GGGCGGTGTG-3'. The relative gene expression level was calculated using the comparative Ct method formula $2^{-\Delta Ct}$. All experiments were carried out in duplicate and each data point represents the mean results of the duplicate experiments.

Statistical analysis. All statistical analyses were carried out using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). The data in the present study were presented as mean ± standard deviation (SD) or median (25 and 75 percentiles). $p<0.05$ was considered to indicate a statistically significant result. The Shapiro-Wilk test was carried out to check the normality of the distribution. The normally distributed numeric variables were evaluated by Student's t-test, while non-normally distributed variables were analyzed by Kruskal-Wallis variance analysis. One-way ANOVA was used to validate the different expression levels of SPRY4-IT1 among subgroups. Chi-square test was used to analyze the categorical variables. To estimate the diagnostic value of the biomarkers, area under the corresponding ROC curve analysis was performed. Finally, correlations were analyzed using Pearson correlation.

Results

SPRY4-IT1 expression is upregulated in HCC tissues and cell lines. The relative expression levels of SPRY4-IT1 were assessed by RT-qPCR. In 87 HCC and adjacent normal liver tissues, the SPRY4-IT1 level in the tumor tissues was significantly upregulated when compared to the level in the non-tumor tissues ($p<0.01$; Fig. 1A). Expression of SPRY4-IT1 relative to 18s in HCC cell lines was compared with a normal human hepatocyte cell line. The results showed that the expression of SPRY4-IT1 was much higher in the HCC cell

Table I. Association of SPRY4-IT1 expression with clinical parameters in HCC.

Characteristics	n	SPRY4-IT1 relative expression (-log) Mean ± SD	t	P-value
Tissue			-3.060	0.003[b]
HCC	87	5.92±0.93		
Adjacent non-cancerous liver	87	6.19±0.80		
Gender			0.394	0.694
Male	81	5.93±0.89		
Female	6	5.78±1.39		
Age (years)			-0.509	0.612
<55	40	5.87±0.96		
≥55	47	5.97±0.90		
Smoking status			0.488	0.627
Negative	52	5.96±0.89		
Positive	35	5.86±0.99		
Alcoholism			1.569	0.122
Negative	70	6.00±0.94		
Positive	17	5.61±0.81		
Differentiation			2.106	0.039[a]
High	10	6.51±0.97		
Moderate/low	59	5.89±0.86		
Tumor size (cm)			2.298	0.024
<10	55	6.13±0.95		
≥10	21	5.61±0.59		
Tumor nodes			0.678	0.500
Single	66	5.97±0.99		
Multi	10	5.77±0.66		
TNM stage			2.514	0.015[a]
I-II	35	6.26±1.00		
III-IV	41	5.75±0.73		
HBV DNA (IU/ml)			1.490	0.147
<500	11	6.12±0.78		
≥500	21	5.69±0.78		
Cirrhosis			-1.129	0.262
Negative	39	5.80±0.85		
Positive	48	6.02±0.98		
AFP (ng/ml)			0.085	0.932
<200	38	5.98±0.88		
≥200	31	5.96±0.95		
CEA (μg/l)			0.471	0.640
<7.2	44	5.94±0.79		
≥7.2	3	5.72±0.71		
ALT (U/l)			-0.385	0.701
<46	49	5.89±0.85		
≥46	38	5.97±1.00		
AST (U/l)			0.676	0.501
<46	45	5.99±0.87		
≥46	42	5.85±1.00		
GGT (U/l)			0.967	0.337
<55	34	6.03±0.85		
≥55	44	5.83±0.94		
5'-NT (U/l)			0.482	0.631
<10	61	5.87±0.90		
≥10	2	5.56±0.81		
GLU (mmol/l)			-0.054	0.957
<6.2	64	5.84±0.85		
≥6.2	23	5.86±0.68		

Data are expressed as the mean ± SD. [a]p<0.05, [b]p<0.01. HCC, hepatocellular carcinoma; SPRY4-IT1, SPRY4 intronic transcript 1; TNM, tumor-node-metastasis; HBV, hepatitis B virus; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; 5'-NT, 5'-nucleotidase; GLU, glucose.

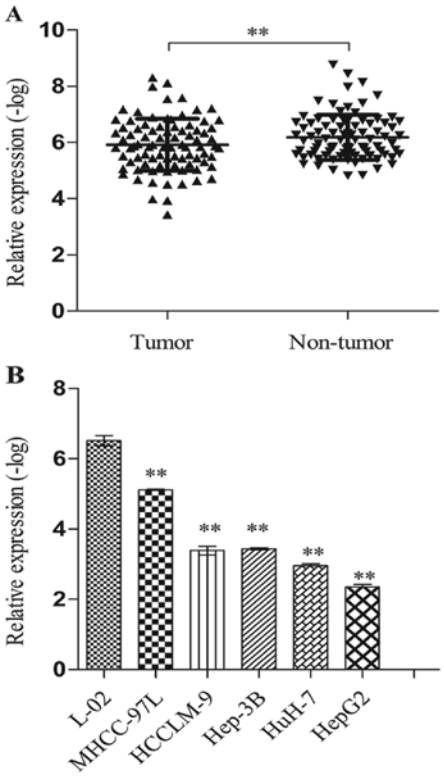

Figure 1. SPRY4-IT1 expression in HCC tissues and cell lines. The relative SPRY4-IT1 expression was determined using RT-qPCR. (A) SPRY4-IT1 levels in the tumor tissues were significantly higher than these levels in the non-tumor tissues. (B) The expression of SPRY4-IT1 was much higher in HCC cell lines than its expression in L-02 cells. Results are expressed as mean ± SD. All data were analyzed using Student's t-test. *p<0.05, **p<0.01.

lines than that in the L-02 cells (MHCC97-L vs. L-02, p<0.01; HCCLM-9 vs. L-02, p<0.01; Hep-3B vs. L-02, p<0.01; HuH-7 vs. L-02, p<0.01; HepG2 vs. L-02, p<0.01; Fig. 1B).

Table II. Correlation analysis of SPRY4-IT1 in relation to clinical parameters of the HCC cases.

Characteristics	Correlation coefficient
Differentiation (high vs. moderate/low)	0.249
Size (<10 vs. ≥10)	0.258
TNM stage (I-II vs. III-IV)	0.287

SPRY4-IT1, SPRY4 intronic transcript 1; TNM, tumor-node-metastasis.

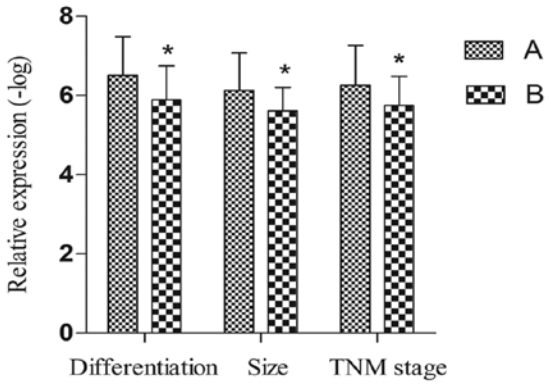

Figure 2. Association of SPRY4-IT1 expression with clinical parameters in HCC. Differentiation: A, high; B, moderate/low; size: A, <10 cm; B, ≥10 cm; TNM stage: A, I-II; B, III-IV. Results are expressed as mean ± SD. All data were analyzed using Student's t-test. *p<0.05.

Correlation between SPRY4-IT1 and clinical variables. We detected the correlation between SPRY4-IT1 and clinical parameters. As shown in Tables I and II, the level of SPRY4-IT1 expression was significantly correlated with differentiation (r=0.249, p=0.039), tumor size (r=0.258, p=0.024) and TNM stage (r=0.287, p=0.015) (Fig. 2). However, no correlations were found in regard to gender, age, smoking, alcoholism, cirrhosis, AFP, HBV-DNA and other biochemical indices.

SPRY4-IT1 levels in plasma among subgroups. The main demographic and clinical characteristics of the studied subjects were illustrated in Table III. No difference was observed in regard to important risk factors including gender, age, smoking, alcoholism and glucose (GLU) in the three groups. There was a significant difference in alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL) and γ-glutamyl transferase (GGT) among the groups.

To observe the value of SPRY4-IT1 as a biomarker, the levels of plasma target lncRNA in 60 HCC patients, 85 hepatitis B and cirrhosis patients, and 63 control cases were measured by RT-qPCR. The present study showed that the expression of SPRY4-IT1 at pre-operation was higher than that at post-operation, in hepatitis B and cirrhosis and the control groups (pre-operation vs. post-operation: p<0.01; pre-operation vs. hepatitis B and cirrhosis: p<0.05; pre-operation vs. the controls: p<0.001) (Fig. 3A). There were 60 paired plasma samples in

Table III. Characteristics of the studied subjects.

Characteristics	Pre-operation n=60	Hepatitis B and cirrhosis n=85	Control n=63	P-value
Gender				0.639[a]
Male	48	63	50	
Female	12	22	13	
Age (years)				0.424[a]
<55	14	28	20	
≥55	46	57	43	
Smoking				0.826[a]
Negative	33	50	38	
Positive	27	35	25	
Alcoholism				0.322[a]
Negative	42	55	48	
Positive	18	30	15	
ALT (U/l)	44 (27, 88)[c]	42 (24, 100)[c]	20 (16, 27)[c]	<0.001[b]
AST (U/l)	55 (30, 104)[c]	46 (30, 102)[c]	22 (20, 26)[c]	<0.001[b]
TBIL (μmol/l)	23 (16, 39)[c]	27 (16, 72)[c]	19 (15, 21)[c]	<0.001[b]
GGT (U/l)	78 (42, 204)[c]	59 (29, 98)[c]	17 (14, 24)[c]	<0.001[b]
GLU (mmol/l)	4.9 (4.6, 5.8)[c]	5.1 (4.4, 5.7)[c]	4.9 (4.5, 5.3)[c]	0.320[b]

[a]Chi-square test. [b]Kruskal-Wallis. [c]Median (25 and 75 percentiles). ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; GGT, γ-glutamyl transferase; GLU, glucose.

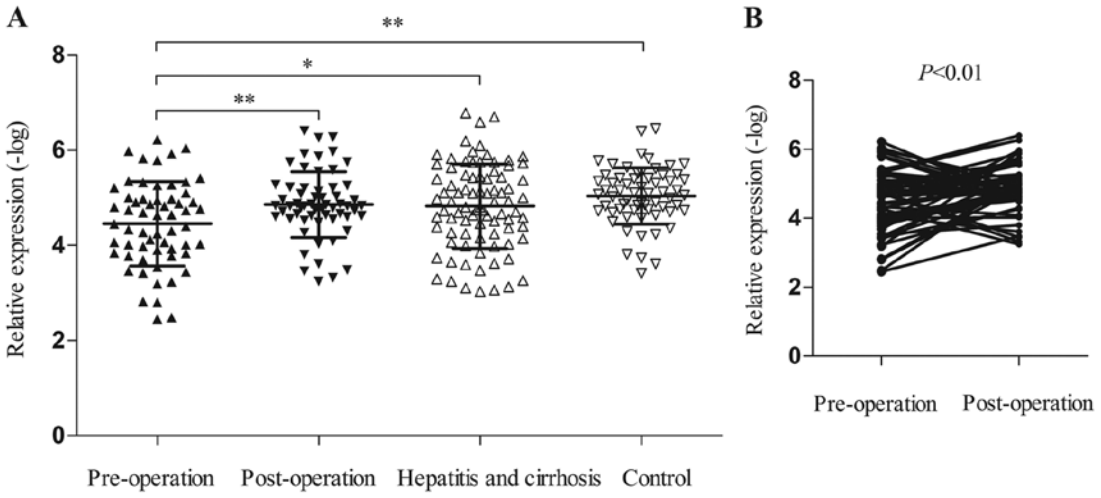

Figure 3. SPRY4-IT1 levels in plasma among subgroups. The relative SPRY4-IT1 expression was determined using RT-qPCR. (A) SPRY4-IT1 expression in pre-operation was higher than that in post-operation, hepatitis B and cirrhosis, and the control groups. No differences were observed among post-operation, hepatitis B and cirrhosis, and the control groups. (B) SPRY4-IT1 expression in pre-operation vs. post-operation. The data were analyzed using Student's t-test and one-way ANOVA. $^{*}p<0.05$, $^{**}p<0.01$.

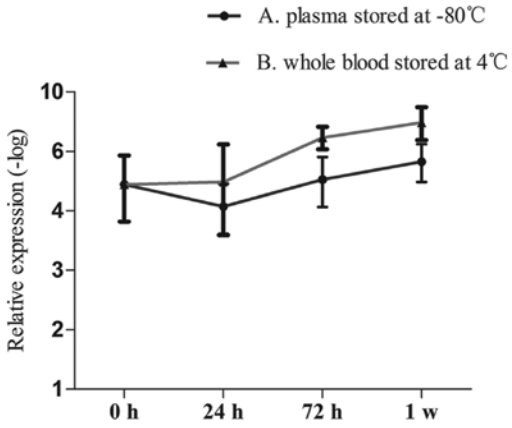

Figure 4. Stability detection of SPRY4-IT1 in human plasma. Human plasma was obtained from 5 healthy controls. A, Plasma was stored at -80˚C for 24 h, 72 h, and 1 week. B, Whole blood was incubated at 4˚C for 24 and 72 h, and 1 week. All data were analyzed using Student's t-test. No significant difference was observed in each group.

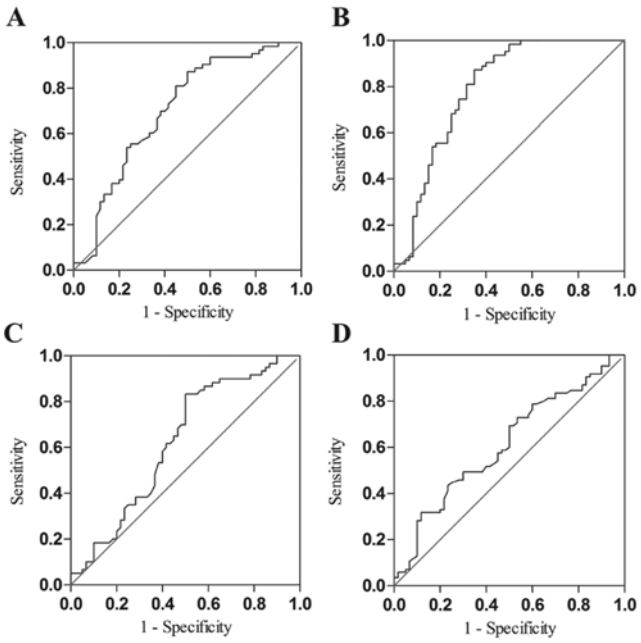

Figure 5. Receiver operating characteristic (ROC) curves. The ROC curves of the expression of SPRY4-IT1 for (A and B) pre-operation vs. controls, (C) pre-operation vs. post-operation and (D) pre-operation vs. hepatitis B and cirrhosis.

the present study, and the levels of SPRY4-IT1 were decreased in 40 of 60 HCC (66.7%) patients (Fig. 3B). However, upon comparison of the levels in the other three subgroups (post-operation, hepatitis B and cirrhosis, the controls), no marked differences were found.

Stability detection of SPRY4-IT1 in human plasma. The present study amplified SPRY4-IT1 in the plasma of HCC patients for the first time. Then, we detected the stability of this lncRNA. We collected plasma from 5 healthy individuals (4 men and 1 women) and stored the samples at -80˚C for 0, 24 and 72 h and 1 week. Meanwhile, we obtained whole blood from these individuals, and stored the samples at 4˚C. We then separated the plasma at 0, 24 and 72 h and 1 week. The expression of SPRY4-IT1 in the plasma was assessed by RT-qPCR. The results showed that the entire process had minimal effects on the levels of SPRY4-IT1 (Fig. 4).

Diagnostic value analysis. To estimate the diagnostic value of SPRY4-IT1 in plasma, ROC was constructed using 3 models: pre-operation vs. the controls, and pre-operation vs. post-operation, and pre-operation vs. hepatitis B and cirrhosis. The area under the ROC (AUCROC) indicated that SPRY4-IT1 had adequate diagnostic value for differentiating HCC patients from the controls (Fig. 5A). Combination of SPRY4-IT1 and AFP (the cut-off value of AFP was at 200 ng/ml) possessed a moderate ability for discrimination between HCC patients and controls; the area was equal to 0.80 (Fig. 5B). However, compared with the group of pre-operation vs. the controls, the

Table IV. Comparisons of the AUC of the expression of SPRY4-IT1 in the subgroups.

Group	AUC	95% CI	P-value	Se (%)	Sp (%)
Pre vs. C	0.702	0.609-0.796	<0.001	87.3	50.0
Pre vs. C[a]	0.800	0.706-0.874	<0.001	87.3	65.0
Pre vs. Post	0.624	0.523-0.726	0.019	83.3	49.0
Pre vs. HC	0.611	0.518-0.703	0.024	43.5	86.7

Pre, pre-operation; C, control; Post, post-operation; HC, hepatitis B and cirrhosis. AUC, area under the curve; Se, sensitivity; Sp, specificity; SPRY4-IT1, SPRY4 intronic transcript 1. [a]Combination of SPRY4-IT1 and AFP differentiated HCC patients from the controls.

diagnostic value of SPRY4-IT1 in the other two groups was not obvious (Fig. 5C and D and Table IV).

Discussion

For decades, much research has investigated potential biomarkers for hepatocellular carcinoma (HCC) (25). The prognosis of HCC remains quite poor, since most patients are diagnosed at an advanced stage, when treatments are less effective (26). Clinically, the most frequently used factor for the diagnosis of HCC is AFP, but the sensitivity is low (27). Using CT and MRI to diagnose the early stage of HCC, the sensitivity (55-91%) and specificity (77-96%) are higher (28). However, due to the different ability among doctors to identify the lesion, the accuracy is different. Recently, lncRNAs have been found to play an important role in the occurrence, invasion and metastasis of cancer (29). Only a few diverse hypothetical mechanisms have been presented to explain how lncRNAs exert their effects. These include interfering in the expression of the adjacent encoding protein gene (30); participating in transcription, chromatin-modifying and DNA methyltransferases to specific genomic (31); binding with functional protein (32); as precursors of miRNAs and affecting target genes of miRNAs (33,34); regulating signaling pathway via combining with chromosome (35,36). Bussemakers *et al* (37) demonstrated that lncRNA PCA3 is a prostate cancer-specific gene. In addition, HULC has been detected in the plasma of HCC patients with high expression (5). Thus, lncRNAs may be considered as novel diagnostic targets for HCC.

In the present study, for the first time, we investigated the clinical value of SPRY4-IT1 in HCC patients. We found that SPRY4-IT1 was upregulated in HCC tissues and cell lines to a greater extent than levels in corresponding non-cancerous tissues and normal human hepatocyte cell line L-02. Meanwhile, our data showed that SPRY4-IT1 expression was related to differentiation, tumor size and TNM stage. Kumarswamy *et al* (38) elaborated that circulating lncRNAs were from the tissues. Therefore, we also detected the expression of SPRY4-IT1 in plasma. The results showed that the levels of SPRY4-IT1 in HCC were much higher than levels in the controls, post-operation and hepatitis B and cirrhosis tissues. According to the present study, the expression of SPRY4-IT1 in plasma could be used to evaluate the effect after surgery in HCC. Due to a sharp decline in post-operative patients after 2 weeks, we supposed that the removal of tumor tissues did effect the contents of circulating lncRNAs. In 2014, Liu *et al* (39) found that plasma lncRNA FER1L4 levels underwent a sharp decline in GC patients 2 weeks after surgery (p=0.028). Therefore, we hypothesized that SPRY4-IT1 played a role in the prognostic evaluation after surgery in the plasma of HCC patients. The occurrence of HCC involves a multi-factor, multi-step and complex process. This concept was validated by the different expression levels of SPRY4-IT1 in the pre-operation group vs. the hepatitis B and cirrhosis group. However, the exact explanation needs further research.

Due to the existence of RNases, circulating lncRNAs are thought to be unstable (40). In the present study, we detected the expression of SPRY4-IT1 in plasma stored at 4 and -80˚C to assess the stability of SPRY4-IT1 in human plasma. No significant difference was observed in the process. Hu *et al* (41) also demonstrated the same result using another method. We confirmed that circulating lncRNAs were markedly stable.

For the first time, we detected the expression of SPRY4-IT1 in plasma to analysis the diagnostic value. The area under the AUC ROC showed that SPRY4-IT1 had a better diagnostic value to differentiate HCC patients from the control, but the diagnostic value of SPRY4-IT1 in the other two groups was not obvious. Further analysis showed that the combination of SPRY4-IT1 and AFP achieved a better diagnostic accuracy. Thus, SPRY4-IT1 may represent a promising target for HCC diagnosis. However, one limitation of the study was that the sample size was small, thus the present findings should be validated in trials with more cases.

Xie *et al* (42) indicated that SPRY4-IT1 led to gastric cancer cell metastasis partly via regulating epithelial-mesenchymal transition (EMT) and DNA methylation may be a key factor in controlling SPRY4-IT1 expression. Khaitan *et al* (43) found that SPRY4-IT1 could connect with multiple molecules in the mitogen-activated protein kinase (MAPK) signaling pathway promoting reduced apoptosis, induced proliferation and enhanced metastasis. In HCC, a number of studies reveal that EMT and the MAPK signaling pathway play important roles, thus SPRY4-IT1 may have an effect on the development of HCC (44,45). Lee *et al* (46) demonstrated that in prostate cancer, siRNA knockdown of SPRY4-IT1 in PC3 cells inhibited cell proliferation and invasion and increased cell apoptosis. These studies indicated that SPRY4-IT1 played an important role in tumorigenesis. Unfortunately, the exact function of SPRY4-IT1 in HCC is still unknown.

In conclusion, the present study provides insights into the expression levels of SPRY4-IT1 in HCC patients for the first time. We demonstrated that the expression of SPRY4-IT1

was significantly higher in HCC than that in corresponding non-cancerous tissues and was correlated to differentiation, tumor size and TNM stage. *In vitro*, our results also showed that SPRY4-IT1 was upregulated in HCC cell lines to a greater extent than that in a normal human hepatocyte cell line. We then detected the levels in HCC plasma, and found that SPRY4-IT1 expression was upregulated in the HCC group, and SPRY4-IT1 exhibited good diagnostic value. These findings suggest for the first time that the expression of SPRY4-IT1 could be used as a novel diagnostic marker for HCC.

Acknowledgements

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