Small Nucleolar RNA Host Gene 18 Acts as a Tumor Suppressor and a Diagnostic Indicator in Hepatocellular Carcinoma

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Abstract

Background: Noncoding RNAs are crucial regulators acting as either tumor suppressor genes or oncogenes in human cancer progression. The aberrant expression of noncoding RNAs has been confirmed in different kinds of cancers. Hepatocellular carcinoma is one of the most common malignant tumors worldwide, characterized by insidious onset, great malignancy, and high rates of recurrence and metastasis. Due to lack of early predictive markers, numerous patients are diagnosed in the late stages. As therapeutic options for advanced patients are quite limited, great efforts have been made to screen patients at early stages. A previous study reported that small nucleolar RNA host gene 18 played crucial role in glioma. However, its functions and roles in hepatocellular carcinoma are unknown. **Purpose:** To explore its functional role and diagnostic value in hepatocellular carcinoma, we investigated its expression level. **Methods:** We performed real-time quantitative polymerase chain reaction in tumor tissues and adjacent noncancerous tissues derived from patients with hepatocellular carcinoma as well as in plasma, including samples from the healthy control, patients with hepatitis B, cirrhosis, and hepatocellular carcinoma. **Results:** Small nucleolar RNA host gene 18 was downregulated in liver tissues compared to paired adjacent noncancerous tissues ($P < .0001$). Meanwhile, plasma small nucleolar RNA host gene 18 showed a relatively high sensitivity and specificity (75.61% and 73.49%) for distinguishing patients with hepatocellular carcinoma whose α-fetoprotein levels were below 200 ng/mL from the healthy controls. **Conclusion:** Our study suggested that small nucleolar RNA host gene 18 might act as a tumor suppressor gene in hepatocellular carcinoma and potentially a diagnostic indicator to distinguish hepatocellular carcinoma from the healthy control and cirrhosis.

Keywords

noncoding RNA, HCC, SNHG18, tumor suppressor gene, diagnostic marker

Abbreviations

AFP, α-fetoprotein; CSC, caner stem cell; EMT, epithelial–mesenchymal transition; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; RT-qPCR, real-time quantitative polymerase chain reaction; SNHG18, small nucleolar RNA host gene 18

Introduction

Hepatocellular carcinoma (HCC) accounts for the most common form of primary liver cancer[1] and ranks the second most common cause of deaths related to cancers.[2] Viral infection, alcoholic cirrhosis, and fatty liver are the common risk factors for HCC.[3] Chronic inflammatory conditions of the biliary tree, genetic disorders, and carcinogens can also increase the risk of developing into HCC.[4] It is widely accepted that the

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development of HCC is a multistep process. Over the past 2 decades, the incidence of HCC is increasing at a rate second to thyroid disease in the United States.[5-7] Notably, HCC represents a serious health crisis worldwide. Currently, the diagnosis of HCC mostly relies on imaging studies and laboratory tests. Ultrasonography, computed tomography scanning, and magnetic resonance imaging are the widely used imaging methods in the diagnosis, treatment assessment, and prognosis prediction of HCC. With respect to laboratory tests, α-fetoprotein (AFP) is the most frequently used marker. Disappointingly, due to the absence of sensitive imaging methods and biomarkers, the patients with HCC are mostly detected at advanced stages. In addition, HCC is inclined to recur and metastasize, so the 5-year survival rate remains far from satisfactory.[8] Therefore, it is in an urgent need to explore the potential mechanisms underlying liver cancer and find out sensitive biomarkers to screen out high-risk patients.

The development of high-throughput RNA sequencing technology makes it possible to discover noncoding RNA genes in great numbers. In fact, the numbers of noncoding RNA genes are much more greater than the coding transcripts.[9] Noncoding RNAs include classical ribosomal RNA, small nucleolar RNA, small nuclear RNA, transfer RNA, and the most-studied microRNA and long noncoding RNA.[10] Instead of being "junk RNA," accumulating evidence indicates that noncoding RNAs are of great significance in the physiological and pathological processes.[11] Although noncoding RNAs are unable to code proteins, they play critical roles in cellular processes such as proliferation, differentiation, apoptosis, via pre and posttranscriptional regulation.[12] Increasing studies have reported that noncoding RNAs are deregulated in cancers, which suggests that noncoding RNAs are strongly related to the development and progression of cancers.[13]

A recent study showed that upregulation of small nucleolar RNA host gene 18 (SNHG18) could promote radio resistance of glioma by repressing Semaphorin 5A,[14] demonstrating that SNHG18 was related to cancer development. Possible mechanism was that SNHG18 could interact with microRNA-binding sites and thus downregulate the expression of Semaphorin 5A. Noncoding RNAs could act as competing endogenous RNAs (ceRNAs), called ceRNA regulation mode, which was confirmed in various cancers.[15] Until now, little is known about the relationship between SNHG18 and HCC. In our study, we aim to explore the expression pattern of SNHG18 in the tissues of HCC and in the plasma of related diseases and further evaluate whether it can be a screening biomarker for HCC.

Materials and Methods

Tissue and Plasma Samples

We collected 71 paired HCC tissues in the Zhongnan Hospital of Wuhan University from April 2016 to July 2017. All patients had been pathologically diagnosed as HCC, none of who had previously undergone radiotherapy or chemotherapy treatment. Blood samples were obtained from the Zhongnan Hospital of Wuhan University, including 4 groups: 80 preoperative samples of HCC, 83 samples of cirrhosis, 60 samples of chronic hepatitis B, and 83 healthy control (samples collected from the Physical Examination Center of the Zhongnan Hospital of Wuhan University). All healthy controls were excluded from hepatitis, hepatic diseases, or aberrant indicators related to the liver function. We collected blood samples into the EDTA anticoagulant tubes. All blood samples were centrifuged at 2000g for 5 minutes at 4°C to separate the blood cells. The supernatants were then transferred to microcentrifuge tubes and centrifuged at 12 000g for 5 minutes at 4°C to completely remove cell debris. Tissue and plasma samples were stored at −80°C until use.

RNA Extraction and Reverse Transcription

The total tissue RNA was extracted by Trizol reagent (Invitrogen, California). Separate extraction kit (Biotake, Beijing, China) was used to extract the RNA of plasma according to the manufacturer's instruction. We used NanoDrop ND2000 (Thermo, California) to quantify the concentration and purity of extracted RNA. Complementary DNA was synthesized using PrimeScript RT reagent Kit with genomic DNA Eraser (Takara, Japan).

Real-Time Quantitative Polymerase Chain Reaction Analysis

To quantify the expression levels of SNHG18, real-time quantitative polymerase chain reaction (RT-qPCR) assay was performed on the Bio-Rad CFX96 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using SYBR-Green I Premix EXTaq. The cycling program for amplification was set for initial stage at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, 61.4°C for 30 seconds, and 72°C for 30 seconds. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the results of RT-qPCR. The synthesized primers were as follows: SNHG18 (forward: 5′-GACCTGGACCT-CACCTAA-3′ and reverse: 5′-GCTGCTTCCTTGAACTTG-3′); GAPDH (forward: 5′-AGAAGGCTGGGGCTCATTTG-3′ and reverse: 5′-GCAGGAGGCATTGCTGATGAT-3′). All reactions were run in duplicate. Relative gene expression levels were calculated in −log2$^{\Delta Ct}$.

Statistical Analysis

All statistical analyses were carried out using the SPSS version 23.0 (SPSS, Inc Chicago, Illinois) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, California). The Shapiro-Wilk test was used to check whether the data were normally distributed. Normal distribution data were presented as mean ± standard deviation (mean ± SD). Nonnormal distribution data were described by quartiles. The differences between normally distributed numeric variables within the 2 groups were evaluated by Student's t test, meanwhile nonnormally distributed numeric variables were analyzed by Mann-Whitney

U test. If the variance was homogeneous, 1-way ANOVA was used for the comparison among multiple groups, whereas nonnormally distributed variables were evaluated by Kruskal-Wallis variance analysis. χ^2 test was adopted to analyze the categorical variables. $P < .05$ was considered to be statistically significant.

Results

Correlation Between SNHG18 and Clinical Variables

The main demographic and clinical characteristics of the patients included are shown in Table 1. No statistical significance was found in gender, age, smoking, alcoholism, cirrhosis, hepatitis B virus (HBV) DNA, and other biochemistry indexes. However, SNHG18 levels were correlated with the tumor size and levels of serum AFP in the tissues.

The Expression Level of SNHG18 was Significantly Downregulated in HCC Tissue Specimens

The expression level of SNHG18 was measured by RT-qPCR in 71 paired clinical HCC tissues and adjacent normal liver tissues. Expression of SNHG18 relative to GAPDH in tumor tissues was significantly downregulated compared with nontumor tissues ($P < .0001$; Figure 1A and B). Furthermore, the expression levels of SNHG18 were correlated with the tumor sizes ($P = .028$) and levels of the serum AFP ($P = .025$; Figure 1C and D).

The Expression Level of SNHG18 in Plasma Among Subgroups

The main demographic and clinical characteristics of studied patients were shown in Table 2. No difference was observed in important risk factors including gender, age, smoking, and alcoholism in the 4 groups. There was a significant difference in the AFP, alanine aminotransferase, and aspartate aminotransferase among the groups.

To observe the diagnostic value of SNHG18 as biomarkers, we detected the expression level of SNHG18 in plasma by RT-qPCR. The results indicated that the expression of SNHG18 in HCC was lower than in healthy controls and patients with cirrhosis (HCC vs cirrhosis: $P < .001$; HCC vs the control: $P < .001$). When comparing the expression level of hepatitis B, cirrhosis, and the healthy control, the former was lower than the latter two. However, no significance was found between patients with HCC and patients with hepatitis B and cirrhosis and healthy controls (Figure 2). The proportion of each group according to the quartiles of the SNHG18 expression level is shown in Table 3.

Diagnostic Value of SNHG18 in Plasma

To assess whether plasma SNHG18 could be used as a potential diagnostic marker for HCC, receiver–operating characteristic curve (ROC) was constructed by 5 models: HCC versus the

Table 1. Association of SNHG18 Expression With Clinical Parameters in HCC.

Characteristic	n	SNHG18 relative expression ($-\log2^{\Delta Ct}$), mean (SD)	t	P
Gender				.598
Male	66	2.10 (1.42)	1.26	
Female	5	1.50 (1.42)		
Age				.057
<55	43	1.88 (1.09)	−1.93	
≥55	28	2.35 ± 0.86		
Smoking				.437
Negative	22	1.92 (0.92)	−0.782	
Positive	49	2.13 (1.07)		
Alcoholism				.938
Negative	42	2.07 (1.19)	0.078	
Positive	29	2.05 (0.74)		
Tumor size				.028[a]
<10 cm	25	2.26 (0.96)	−2.241	
≥10 cm	46	1.70 (1.05)		
Tumor nodes				.770
Single	35	2.10 (0.90)	0.29	
Multi	36	2.03 (1.14)		
TNM				.18
I–II	27	2.28 (1.09)	1.356	
III–IV	44	1.93 (0.97)		
HBV-DNA				.374
<500	29	2.11 (1.02)	−0.899	
≥500	13	1.82 (0.71)		
Cirrhosis				.453
Negative	37	2.15 (1.15)	0.755	
Positive	34	1.97 (0.88)		
AFP				.025[a]
<200	40	1.82 (1.10)	−2.294	
≥200	31	2.37 (0.85)		
ALT				.758
<46	40	2.10 (1.06)	0.310	
≥46	31	2.02 (1.00)		
AST				.8
<46	37	2.04 (1.12)	−0.227	
≥46	34	2.09 (0.93)		

Abbreviations: AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TNM, tumor node metastasis.
Data are mean ± SD.
[a]$P < .05$.

healthy control, HCC with AFP levels below 200 ng/mL versus the healthy control, HCC versus cirrhosis, HCC with AFP below 200 ng/mL versus cirrhosis with AFP also below 200 ng/mL, and hepatitis B versus the healthy control (Figure 3).

From Table 4, we could know that the area under curve (AUC) of SNHG18 was greater than that of AFP. In addition, SNHG18 showed a relatively high AUC in distinguishing HCC with AFP levels below 200 ng/mL from the healthy controls (AUC = 0.7459, 95% CI: 0.6530-0.8427) and from patients with cirrhosis whose AFP levels were also <200 ng/mL (AUC = 0.7527, 95% CI: 0.6482-0.8573). The sensitivity of SNHG18 would be improved if we combined AFP with SNHG18 for
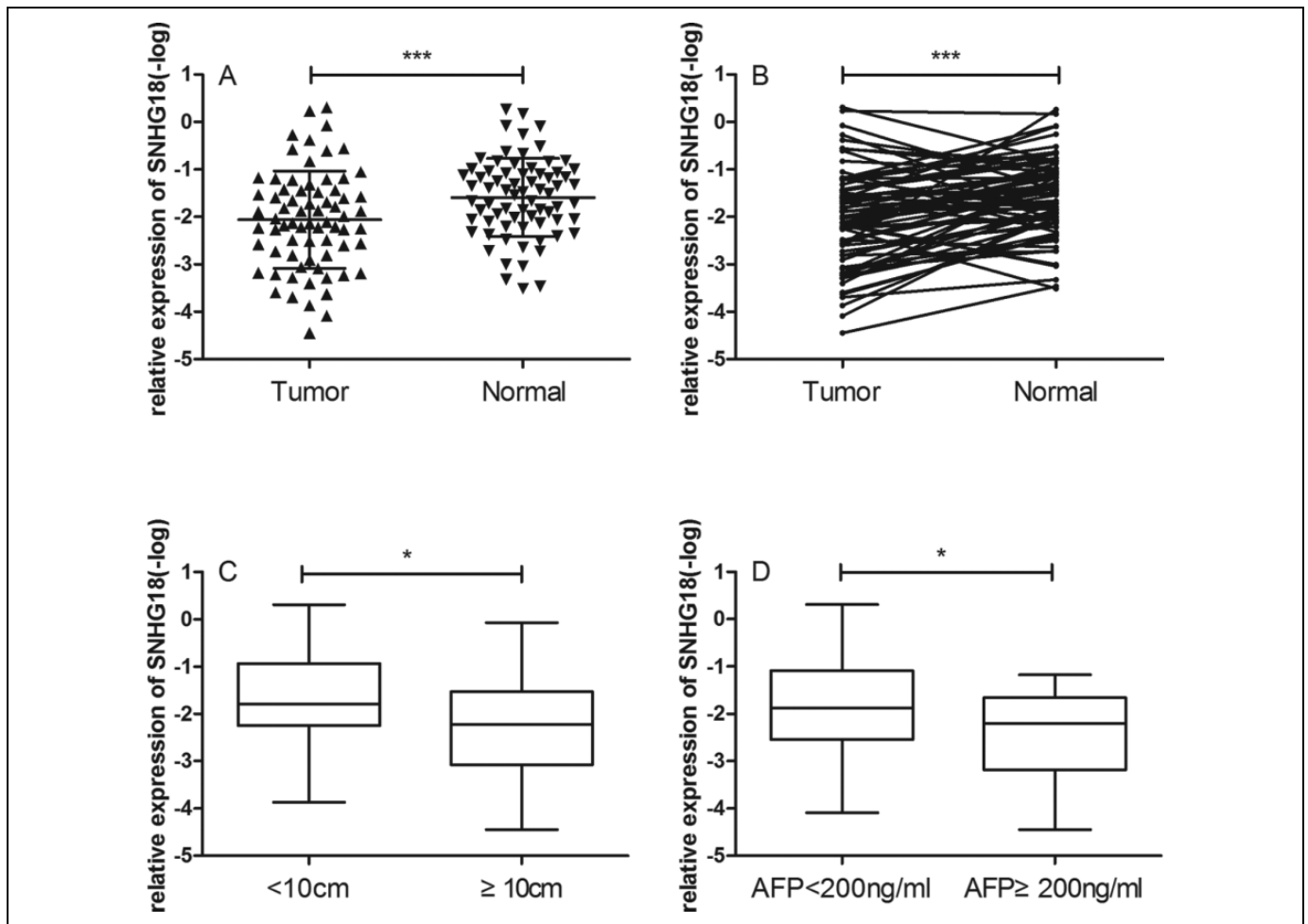
Figure 1. SNHG18 expression in hepatocellular carcinoma tissues and adjacent normal liver tissues. A and B, SNHG18 expression levels in tumor tissues were significantly lower than in nontumor tissues ($P < .0001$). C, SNHG18 expression levels were associated with the tumor size ($P = .028$). D, SNHG18 expression levels were associated with the levels of α-fetoprotein ($P = .025$). *$P < .05$, ***$P < .001$.

Table 2. Characteristics of the Studied Subjects.

Characteristic	HCC (n = 80)	Hepatitis B (n = 60)	Cirrhosis (n = 82)	Control (n = 83)	P
Gender					.358[a]
Male	63	45	63	56	
Female	17	15	19	27	
Age					.192[a]
<50	23	21	21	34	
≥50	57	39	61	49	
Smoking					.628[a]
Negative	36	27	34	26	
Positive	44	33	48	47	
Alcoholism					.722[a]
Negative	34	23	32	28	
Positive	46	37	50	55	
AFP, ng/mL[b]	46 (3,950)	12 (3, 102)	4 (2, 20)	5 (4, 7)	<.001[c]
ALT, U/L[b]	40 (25, 69)	69 (26, 150)	26 (18, 54)	21 (18, 27)	<.001[c]
AST, U/L[b]	65 (36, 123)	77 (36, 161)	44 (28, 76)	22 (19, 26)	<.001[c]

Abbreviations: AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
[a]χ^2 test.
[b]Median (25 percentiles, 75 percentiles), some data are missing.
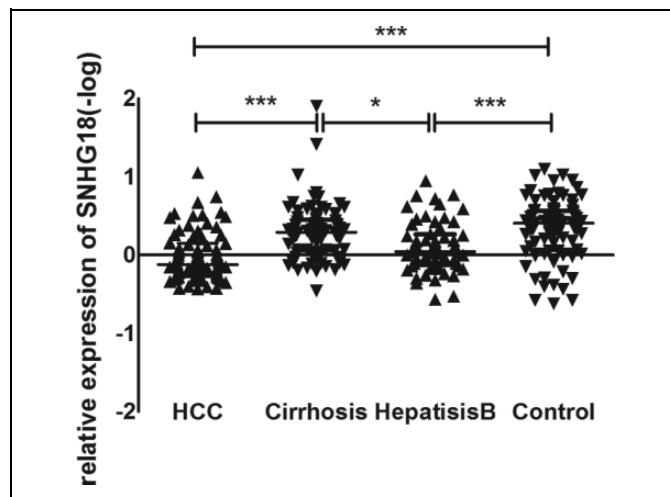[c]Kruskal-Wallis.

Figure 2. SNHG18 levels in plasma among subgroups. The data are analyzed using Kruskal-Wallis variance analysis. *P < .05, ***P < .001.

those whose AFP levels were below the diagnostic standard. So, it was necessary to combine SNHG18 with AFP to screen out patients with HCC.

Discussion

Noncoding RNAs are frequently reported to be aberrant in various kind of cancers, which can be involved in apoptosis, proliferation, metastasis, angiogenesis, and other processes.[2] Therefore, understanding the relationship between noncoding RNAs and the development of cancer can lay the foundation to discover novel approaches to diagnosis and therapy of tumors. Hepatocellular carcinoma is the most common type of solid tumors worldwide with high invasiveness and poor prognosis. Once related symptoms appear, the patients are mostly in the late stages.[16] For last 40 years, AFP has been used to screen HCC, whose sensitivity and specificity were 39% to 65% and 76% to 94%, respectively.[17,18] Additionally, data suggested that AFP would be seldomly elevated for tumorous mass <2 cm in diameter.[19] Thus, the utility of AFP to detect HCC at early stages has been challenged. Hence, exploring novel biomarkers for differentiating HCC from other liver diseases at early stage is urgently needed. Over the past decade, considerable studies were undertaken to explore the biomarker for HCC. Amounts of attention were focused on noncoding RNAs. In 2016, Zheng et al[14] revealed that the expression of SNHG18 was abnormal in the glioma tissue specimens. Furthermore, high expression of SNHG18 was associated with the progression of primary glioma. Little is known about the role of SNHG18 in HCC. Therefore, it is potentially prospective to explore the value of SNHG18 in HCC.

In the present study, we investigated the clinical and diagnostic value of SNHG18 in patients with HCC for the first time and found that SNHG18 was significantly downregulated in HCC tissues compared to the corresponding noncancerous tissues. Our results also indicated that the levels of SNHG18 were related to the levels of AFP and tumor sizes. By detecting the

expression pattern of SNHG18 in plasma, levels of SNHG18 in HCC group were found lower than that of both healthy control and the cirrhosis, consistent with the results in tissues. It was widely known that the development of HCC was a multifactor, multistep, and complex process.[20,21] Unfortunately, despite remarkable difference was found between patients with HCC and patients with cirrhosis, there was no statistical difference between patients with hepatitis B and patients with HCC and cirrhosis and the healthy controls. We may find significant difference by enlarging sample size. Therefore, further researches are necessary. Our present study supports that SNHG18 can help to screen out patients with HCC from the healthy controls and patients with cirrhosis.

Increasing studies in the field of diagnosis and treatment of HCC suggested that circulating noncoding RNAs derived from tumor tissues might be relevant to the tumor and could have the potential value to be used for early diagnosis or survival prediction for HCC.[22-27] More importantly, the detection of circulating noncoding RNAs in body fluids was considered as a nonintrusive procedure with limited side effects. We assessed the expression of SNHG18 in plasma to analyze the diagnostic value for the first time. The area under the ROC indicated that SNHG18 was helpful for differentiating patients with HCC from the healthy control, with AUC of 0.7694. Itis widely accepted that HBV and cirrhosis constituted important risk factors for developing into HCC.[28] The AUC of SNHG18 for differentiating HCC from cirrhosis was 0.7702. These data showed that SNHG18 could be a good marker in screening HCC. Importantly, it could yield a relatively high sensitivity and specificity to combine SNHG18 with AFP in distinguishing HCC with AFP levels below 200 ng/mL from the healthy controls (80.49% and 73.49%) and patients with cirrhosis (73.17% and 75.86%) whose AFP levels were also <200 ng/mL. This might be useful for diagnosing patients with HCC whose levels of AFP were lower than the standard. However, the limitation of our research was that the sample size was relatively small. Further researches are necessary to verify our results.

A deeper understanding of the mechanism of noncoding RNA in HCC would be helpful to determine new diagnostic markers and therapeutic intervention. Katrin Panzitt et al found that noncoding RNA HULC (highly upregulated in liver cancer) could mediate epithelial–mesenchymal transition (EMT) by upregulating epithelial markers (E-cadherin and β-catenin) and downregulating mesenchymal markers (Vimentin and N-cadherin) in HCC.[29] Thus, HULC could induce HCC cells to activate EMT and then promote tumor progression and metastasis. Caner stem cells (CSCs) were a population of cancer cells, characterized by the properties of self-renewal and differentiation, which had the great potential to form tumors.[30] Studies pointed out that up to 40% of HCC developed from clonal populations originated from hepatic CSCs.[31] Wang et al suggested that noncoding RNA plasmacytoma variant translocation could contribute to the CSC phenotype of liver cancer cells.[32] Lan et al revealed that SNHG12 functioned as an endogenous sponge for miR-199a/b-5p to regulate the

Table 3. The Proportion of Each Group According to the Quartiles of the SNHG18 Expression Level.[a]

Subgroups	Quartiles of Relative SNHG18 Expression (−log), N = 306				P
	1st, <−0.11	2nd, −0.111–0.19	3rd, 0.19–0.44	4th, 0.44–1.91	
HCC	41 (53.25%)	20 (26.32%)	10 (13.16%)	9 (11.69%)	<.001
Cirrhosis	9 (11.69%)	17 (22.37%)	35 (46.05%)	22 (28.57%)	
HBV	16 (20.78%)	25 (32.90%)	9 (11.84%)	10 (12.99%)	
Control	11 (14.28%)	14 (18.53%)	22 (28.95%)	36 (46.75%)	

Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNHG18, small nucleolar RNA host gene 18.
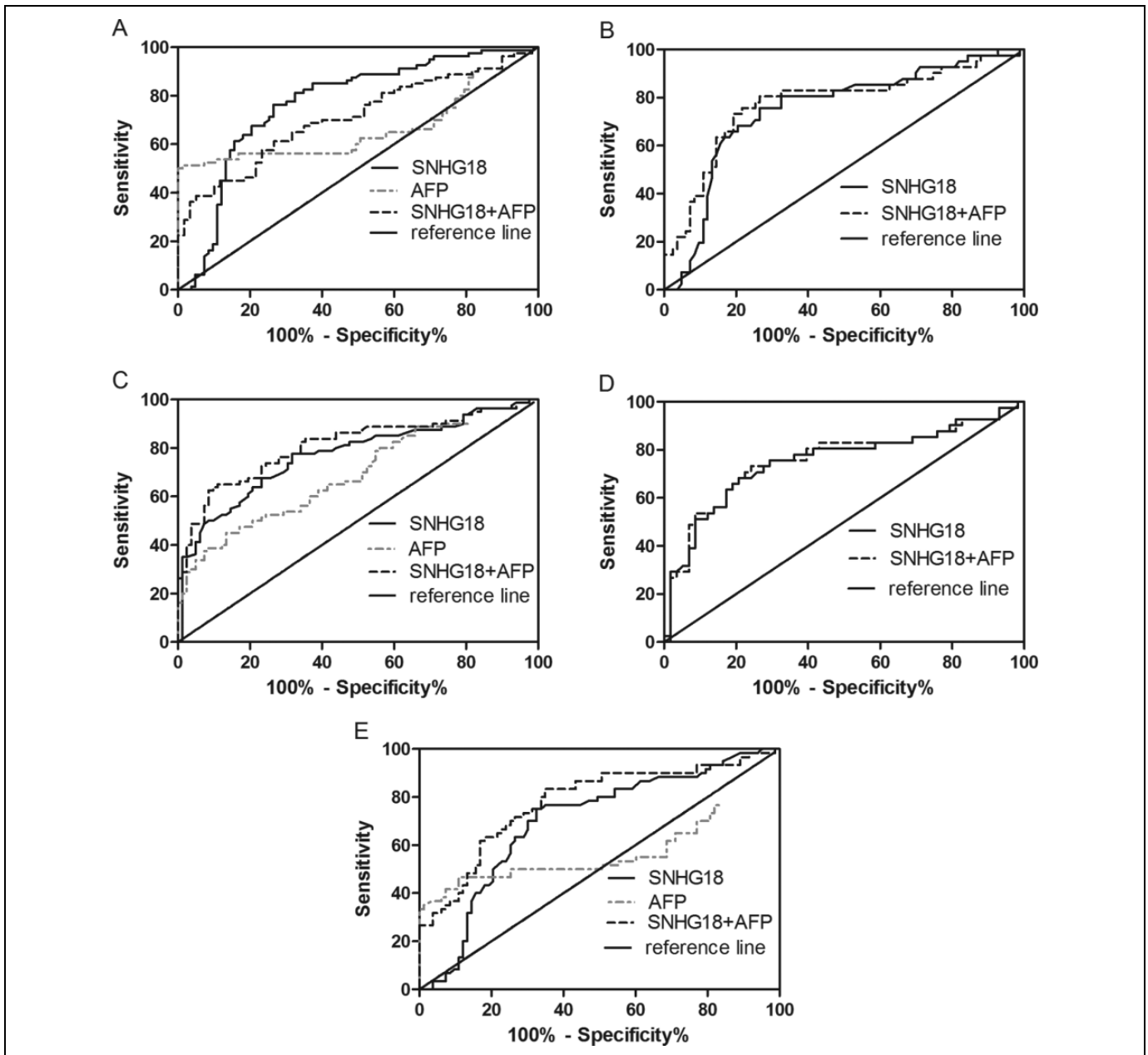[a]The data are analyzed by using Chi-square test.

Figure 3. Receiver–operating characteristic curves. A, Hepatocellular carcinoma (HCC) versus the healthy control; B, HCC with the α-fetoprotein levels less than 200 ng/mL versus the healthy control with the AFP levels also less than 200 ng/mL; C, HCC versus cirrhosis; D, HCC whose AFP levels below 200 ng/mL versus cirrhosis whose AFP Levels also below 200 ng/mL; E, Hepatitis B versus the healthy control.

Table 4. Comparisons of the AUC of AFP, SNHG18, and Combining AFP with SNHG18 for Subgroups.

Group	Tumor Marker	AUC	95% CI	P	Se (%)	Sp (%)
HCC vs control	AFP	0.665	0.576-0.754	0.000277	51.25	98.8
	SNHG18	0.769	0.694-0.845	<.0001	76.25	73.49
	AFP + SNHG18	0.709	0.624-0.793	<.0001	61.25	73.33
HCC vs control[a]	SNHG18	0.748	0.653-0.843	<.0001	75.61	73.49
	AFP + SNHG18	0.776	0.681-0.870	<.0001	80.49	73.49
HCC vs cirrhosis	AFP	0.688	0.606-0.769	<.0001	45	86.59
	SNHG18	0.770	0.697-0.844	<.0001	77.5	68.29
	AFP + SNHG18	0.810	0.742-0.879	<.0001	65	89.02
HCC vs cirrhosis[b]	SNHG18	0.753	0.648-0.857	<.0001	68.29	79.31
	AFP + SNHG18	0.759	0.655-0.863	<.0001	73.17	75.86
HBV vs control	AFP	0.579	0.472-0.686	.1098	46.67	89.16
	SNHG18	0.697	0.608-0.785	<.0001	75	67.47
	AFP + SNHG18	0.778	0.699-0.857	<.0001	83.33	65.06

Abbreviations: AFR, α-fetoprotein; AUC, area under curve; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; Se, sensitivity; SNHG18, small nucleolar RNA host gene 18; Sp, specificity.
[a]Patients with HCC with AFP levels less than 200 ng/mL and the healthy control with AFP levels also below 200 ng/mL.
[b]Patiets with HCC with AFP levels below 200 ng/mL and cirrhosis with AFP levels also below 200 ng/mL.

expression of MLK3 and affect the nuclear factor κB pathway, through which SNHG12 was associated with tumor progression and metastasis.[15] Regarding SNHG18, it has been documented that upregulation of SNHG18 promoted radio resistance of glioma by repressing SEMA5A protein.[14] In human glioma, Zheng et al and Li et al also reported that SEMA5A protein could inhibit human cell motility and radio resistance of glioma.[14,33] Sadanandam et al previously suggested that SEMA5A protein was constitutively expressed in pancreatic tumors but not in normal pancreas. In addition, high Sema5A expression enhanced tumor cell invasion and aggregation.[34] The studies above suggested that SEMA5A protein could function as disparate roles in different cancer types. Further researches are necessary to explore the functional role of SNHG18 and its connection to SEMA5A protein in HCC. Potentially, SNHG18 can regulate the expression of various proteins via cis or trans regulation mode. The rapid development of RNA sequencing technology provides us an opportunity to explore the relationship between SNHG18 and target proteins in HCC. Additional studies involved in the molecular mechanism are urgently needed.

Authors' Note
Xue-Fang Liu and Khaing Zar contributed equally to this work.

Ethical approval
All tissue and plasma samples were collected after patients signed their informed consent according to institutional ethical guidelines. This study was approved by the Ethics Committee of Zhongnan Hospital of Wuhan University (Ethical Approval No. 2013059).

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References
1. Wang Y, He L, Du Y, et al. The long noncoding RNA lncTCF7 promotes self-renewal of human liver cancer stem cells through activation of wnt signaling. *Cell Stem Cell*. 2015;16(4):413-425.
2. Zhang A, Lakshmanan J, Motameni A, Harbrecht BG. MicroRNA-203 suppresses proliferation in liver cancer associated with PIK3CA, p38 MAPK, c-Jun, and GSK3 signaling. *Mol Cell Biochem*. 2017;441(1-2):89-98.
3. Goh GB, Chang P, Tan C. Changing epidemiology of hepatocellular carcinoma in Asia. *Best Pract Res Clin Gastroenterol*. 2015;29(6):919-928.
4. Burkhart RA, Ronnekleiv-Kelly SM, Pawlik TM. Personalized therapy in hepatocellular carcinoma: molecular markers of prognosis and therapeutic response. *Surg Oncol*. 2017;26(2):138-145.
5. Ladep NG. Incidence and mortality of primary liver cancer in England and Wales: changing patterns and ethnic variations. *World J Gastroenterol*. 2014;20(6):1544.
6. Kudo M, Izumi N, Ichida T, et al. Report of the 19th follow-up survey of primary liver cancer in Japan. *Hepatol Res*. 2016;46(5):372-390.
7. Mokdad AA, Lopez AD, Shahraz S, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC Med*. 2014;12:145.
8. Waghray A, Murali AR, Menon KN. Hepatocellular carcinoma: from diagnosis to treatment. *World J Hepatol*. 2015;7(8):1020.

9. Iyer MK, Niknafs YS, Malik R, et al. The landscape of long noncoding RNAs in the human transcriptome. *Nat Genet*. 2015; 47(3):199-208.

10. Seitz AK, Christensen LL, Christensen E, et al. Profiling of long non-coding RNAs identifies LINC00958 and LINC01296 as candidate oncogenes in bladder cancer. *Sci Rep*. 2017;7(1):395.

11. Klingenberg M, Matsuda A, Diederichs S, Patel T. Non-coding RNA in hepatocellular carcinoma: mechanisms, biomarkers and therapeutic targets. *J Hepatol*. 2017;67(3):603-618.

12. Liu X, Li M, Peng Y, et al. miR-30c regulates proliferation, apoptosis and differentiation via the Shh signaling pathway in P19 cells. *Exp Mol Med*. 2016;48(7):e248.

13. Valeri N, Braconi C, Gasparini P, et al. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. *Cancer Cell*. 2014;25(4):469-483.

14. Zheng R, Yao Q, Ren C, et al. Upregulation of long noncoding RNA small nucleolar rna host gene 18 promotes radioresistance of glioma by repressing semaphorin 5A. *Int J Radiat Oncol Biol Phys*. 2016;96(4):877-887.

15. Lan T, Ma W, Hong Z, Wu L, Chen X, Yuan Y. Long non-coding RNA small nucleolar RNA host gene 12 (SNHG12) promotes tumorigenesis and metastasis by targeting miR-199a/b-5p in hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2017;36(1):11.

16. Jing W, Luo P, Zhu M, Ai Q, Chai H, Tu J. Prognostic and diagnostic significance of sdpr-cavin-2 in hepatocellular carcinoma. *Cell Physiol Biochem*. 2016;39(3):950-960.

17. Trevisani F, D'Intino PE, Morselli-Labate AM, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol*. 2001;34(4):570-575.

18. Alpert ME, Uriel J, de Nechaud B. Alpha1 fetoglobulin in the diagnosis of human hepatoma. *New Engl J Med*. 1968;278(18):984-986.

19. Zhang B, Yang B. Combined alpha fetoprotein testing and ultrasonography as a screening test for primary liver cancer. *J Med Screen*. 1999;6(2):108-110.

20. Riazalhosseini B, Mohamed R, Apalasamy YD, Langmia IM, Mohamed Z. Circulating microRNA as a marker for predicting liver disease progression in patients with chronic hepatitis B. *Rev Soc Bras Med Trop*. 2017;50(2):161-166.

21. Lin C, Kao J. Natural history of acute and chronic hepatitis B: the role of HBV genotypes and mutants. *Best Pract Res Clin Gastroenterol*. 2017;31(3):249-255.

22. Li J, Wang X, Tang J, et al. HULC and linc00152 act as novel biomarkers in predicting diagnosis of hepatocellular carcinoma. *Cell Physiol Biochem*. 2015;37(2):687-696.

23. El-Tawdi AH, Matboli M, El-Nakeep S, Azazy AE, Abdel-Rahman O. Association of long noncoding RNA and c-JUN expression in hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol*. 2016;10(7):869-877.

24. Kamel MM, Matboli M, Sallam M, Montasser IF, Saad AS, El-Tawdi AHF. Investigation of long noncoding RNAs expression profile as potential serum biomarkers in patients with hepatocellular carcinoma. *Transl Res*. 2016;168:134-145.

25. Xie H, Ma H, Zhou D. Plasma HULC as a promising novel biomarker for the detection of hepatocellular carcinoma. *Biomed Res Int*. 2013;2013:1-5.

26. Ma W, Wang H, Jing W, et al. Downregulation of long noncoding RNAs JPX and XIST is associated with the prognosis of hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol*. 2017; 41(2):163-170.

27. Jing W, Gao S, Zhu M, et al. Potential diagnostic value of lncRNA SPRY4-IT1 in hepatocellular carcinoma. *Oncol Rep*. 2016;36(2): 1085-1092.

28. Xu W, Yu J, Wong VW. Mechanism and prediction of HCC development in HBV infection. *Best Pract Res Clin Gastroenterol*. 2017;31(3):291-298.

29. Panzitt K, Tschernatsch MMO, Guelly C, et al. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. *Gastroenterology*. 2007; 132(1):330-342.

30. Tarlow BD, Pelz C, Naugler WE, et al. Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. *Cell Stem Cell*. 2014;15(5):605-618.

31. Mishra L, Banker T, Murray J, et al. Liver stem cells and hepatocellular carcinoma. *Hepatology*. 2009;49(1):318-329.

32. Wang F, Yuan JH, Wang SB, et al. Oncofetal long noncoding RNA PVT1 promotes proliferation and stem cell-like property of hepatocellular carcinoma cells by stabilizing NOP2. *Hepatology*. 2014;60(4):1278-1290.

33. Li X, Lee AYW. Semaphorin 5A and Plexin-b3 inhibit human glioma cell motility through RhoGDIα-mediated inactivation of rac1 GTPase. *J Biol Chem*. 2010;285(42):32436-32445.

34. Sadanandam A, Sidhu SS, Wullschleger S, et al. Secreted semaphorin 5A suppressed pancreatic tumour burden but increased metastasis and endothelial cell proliferation. *Br J Cancer*. 2012;107(3):501-507.