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lncRNA-D16366 Is a Potential Biomarker for Diagnosis and Prognosis of Hepatocellular Carcinoma

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Long non-coding RNAs (lncRTNAs) are a new focus in cancer research. Although lncRNAs have no protein coding capacity, they are important in epigenetics as well as in regulating gene expression, playing an important role in various cancers. In the current study, we investigated the roles of *lncRNA-D16366* in hepatocellular carcinoma (HCC) and expected to find a new biomarker for early detection and prognosis of the disease.

Material/Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression of *lncRNA-D16366* in tissue and serum samples. The relationship between *lncRNA-D16366* expression and clinicopathologic characteristics of patients with HCC was analyzed to estimate whether it was involved in malignancy development. Then, potential diagnostic and prognostic values were evaluated via receiver operating characteristic (ROC) curve, Kaplan-Meier and Cox regression analysis, respectively.

Results: *lncRNA-D16366* was proved to be decreased in the tissues and serum among patients with HCC compared with the corresponding controls. Its expression was influenced by tumor size, HbsAg, portal vein tumor thrombus, Child-Pugh score, therapies, and neoplasm metastasis. It had high diagnostic value, with an AUC of 0.752, accompanied by a sensitivity of 65.5% and a specificity of 84.6%. In addition, it was related to the prognosis of HCC.

Conclusions: *lncRNA-D16366* was decreased in HCC, and might be an independent diagnostic and prognostic indicator in the disease.

MeSH Keywords: Diagnosis • Liver Neoplasms • Prognosis • RNA Caps

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Background

Hepatocellular carcinoma (HCC) is one of the most common malignant cancers and has a high mortality rate [1,2]. The disease has steadily increasing incidence and mortality rates, especially in Asia and Africa [3]. Surgical resection is the most effective therapeutic method for HCC treatment, but high recurrence rate, early distant metastasis, frequent resistance to chemo and radiation therapies, and lack of symptoms in early stages lead to poor prognosis [4–6]. Therefore, exploring effective bio-markers is of great importance for early detection and improvement of prognosis in HCC.

Long non-coding RNAs (lncRNAs) are located in the cell nucleus or cytoplasm and have a length of more than 200 nucleotides [7]. Although lncRNAs have no protein coding capacity, they are important in epigenetics as well as in regulating gene expression [8,9]. It has been confirmed that lncRNAs take part in brain development, embryonic development, histological differentiation, and organogenesis [10,11]. Recently, studies demonstrated that lncRNAs are also involved in many complex cell processes in cancers [10–15]. For example, lncRNA-D16366 was confirmed to be decreased in HCC [16]. However, its role in HCC diagnosis and prognosis remains unclear.

Here, we assessed the expression of *lncRNA-D16366* and its correlation with clinicopathologic characteristics of patients with HCC. The diagnostic and prognostic values of *lncRNA-D16366* were also estimated through ROC curve, Kaplan-Meier analysis, and Cox regression analysis.

Material and Methods

Patients and samples

This study was conducted in Xianyang Central Hospital and was approved by the Research Ethics Committee of Xianyang Central Hospital. All patients had never received any therapies before sampling and signed written informed content in advance.

A total of 107 patients (62 males and 45 females) who were diagnosed with HCC via histopathological examination were recruited from Xianyang Central Hospital from September 2011 to July 2013. Paired tumor tissues and adjacent ones as well as serum samples were preserved at the same time. Serum samples from 58 patients, including 28 with hepatitis B virus (HBV), 18 with alcoholic liver disease (ALD), and 12 with fatty liver disease, were also obtained and taken as benign controls. Serum from 85 healthy people with matched age were taken as healthy controls. Tissues samples were frozen in liquid nitrogen while serum samples were immediately put into blood collection tubes containing EDTA. All samples were stored at –80°C for RNA extraction. The expression of *lncRNA-D16366* was detected through qRT-PCR analysis.

RNA extraction and qRT-PCR analysis

Total RNA was isolated from tumor tissues, adjacent tissues, and serum samples. Reverse transcription was conducted with a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) to synthesize the first chain of cDNA. Then, RT-PCR reaction was performed with β-actin as internal control in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The expression of *lncRNA-D16366* was evaluated using the comparative cycle threshold (CT).

Follow-up

A 5-year follow-up was conducted for 107 patients with HCC. Overall survival time referred to the duration between the day of diagnosis to the end of follow-up. Clinical information, including age, sex, tumor size, tumor site, HbsAg, AFP, portal vein tumor thrombus, Child-Pugh, therapies, and neoplasm metastasis were recorded in a database. The information of follow-up was obtained through telephone call or letter, and updated every 3 months. Patients who died from unexpected events or other diseases were excluded from our study.

Statistical analysis

Statistical analysis was conducted adopting SPSS version 13.0 for Windows (SPSS, Inc, IL, USA). All data are presented as mean ±SD. Differences between 2 groups were analyzed by *t* test, while those among 3 groups were assessed via one-way ANOVA. The relationship between *lncRNA-D16366* expression and clinicopathological characteristics of patients with HCC was evaluated via chi-square test. Diagnostic value of *lncRNA-D16366* was estimated through building ROC curves. Kaplan-Meier and Cox regression analyses were used to estimate the prognostic value of *lncRNA-D16366* in HCC. $P<0.05$ was considered to be statistically significant.

Results

Expression of lncRNA-D16366 in patients with HCC

The expression level of *lncRNA-D16366* in tumor tissues and adjacent ones was detected through qRT-PCR assay. As shown in Figure 1, *lncRNA-D16366* expression in tumor tissues (0.212±0.001) was significantly lower than that in adjacent ones (1.772±0.076) ($P<0.05$).

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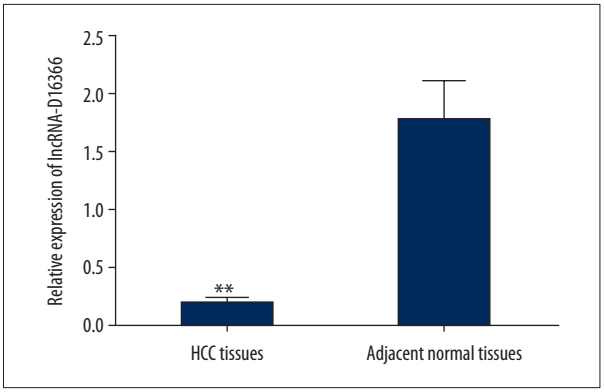

Figure 1. The expression of *lncRNA-D16366* in tissue samples. *lncRNA-D163663* was downregulated in tumor tissues compared with adjacent ones. ** $P<0.01$ represents significant difference between 2 groups.

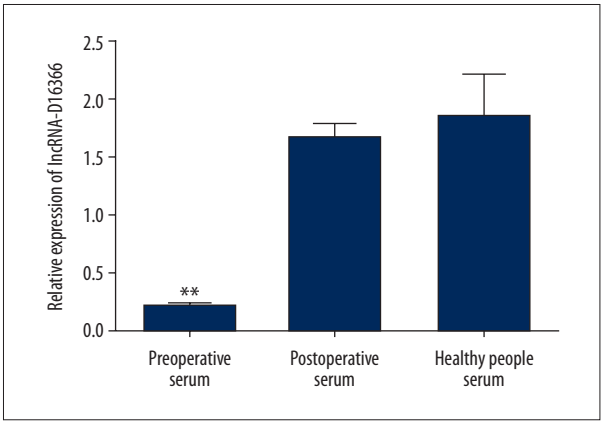

Figure 2. Serum expression of *lncRNA-D16366* before and after surgery. *lncRNA-D16366* expression in preoperative serum was significantly lower than that in postoperative serum and that in serum from healthy people. ** $P<0.01$ or * $P<0.05$ represents significant difference between 2 groups.

Serum lncRNA-D16366 expression in patients with HCC, in benign controls, and in healthy controls

QRT-PCR analysis was also used to detect serum *lncRNA-D16366* expression in each group. The results demonstrated that *lncRNA-D16366* expression in preoperative serum was lower than that in postoperative serum among 107 patients with HCC (Figure 2, $P<0.01$). Serum *lncRNA-D16366* expression was decreased in patients with HCC compared with benign controls and healthy controls (Figure 3, $P<0.01$). We also found that serum *lncRNA-D16366* level in benign controls was lower than that in healthy controls ($P<0.05$). The results indicated that serum *lncRNA-D16366* expression could distinguish HCC patients from other cases with benign liver diseases.

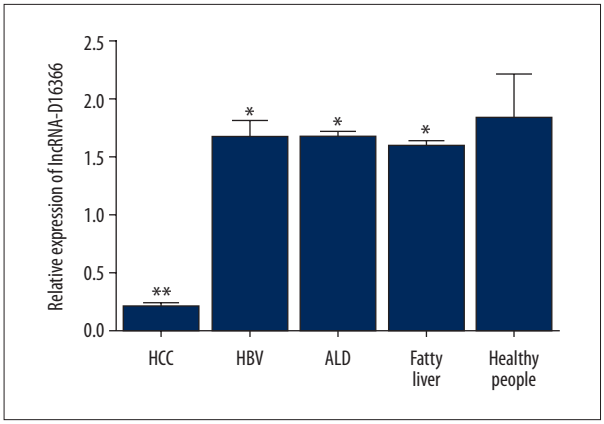

Figure 3. Serum *lncRNA-D16366* expression in patients with HCC, patients with benign liver diseases, and healthy people. It was obviously lower in patients with HCC than in the other 2 groups ($P<0.05$). ** $P<0.01$ or * $P<0.05$ represents significant difference between 2 groups.

The relationship between lncRNA-D16366 expression and clinicopathological characteristics

In this study, 107 patients with HCC were divided into high- and low-expression groups according to their mean expression levels of lncRNA-d16366 (0.212) in HCC tissues. Fifty-one patient were in the high *lncRNA D16366* group, while the others were in the low *lncRNA-D16366* group. Then, we analyzed the relationship between *lncRNA-D16366* and clinicopathological characteristics. The results showed that tumor size ($P<0.001$), HbsAg ($P=0.005$), portal vein tumor thrombus ($P=0.001$), Child-Pugh score ($P=0.033$), therapies ($P=0.003$), and neoplasm metastasis ($P<0.001$) significantly influenced the expression of *lncRNA-D16366* (Table 1).

Diagnostic value of lncRNA-D16366 in HCC

To explore diagnostic value of *lncRNA-D16366,* an ROC curve was established. The outcome showed an AUC of 0.752 with a sensitivity of 65.5% and a specificity of 84.6%. The ideal cut-off value was 0.196 (Figure 4).

Association between the expression of lncRNA-D16366 and overall survival

To analyze prognostic value of *lncRNA-D16366* in HCC, Kaplan-Meier and Cox regression analyses were performed. Kaplan-Meier analysis demonstrated that overall survival of patients in the low *lncRNA-D16366* expression group was shorter than in the high *lncRNA-D16366* expression group (Figure 5). Log-rank testing revealed the result possessed statistical significance ($P<0.001$). Then, multivariate analysis was conducted through Cox regression analysis. Portal vein tumor thrombus

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Table 1. The relationship between expression of *lncRNA-D16366* and clinicopathological characteristics.

Clinicopathological characteristics	n	lncRNA-D16366 expression		*P*
		High	Low	
Age				0.762
40–50	25	12	13	
51–60	47	24	23	
61–70	35	15	20	
Sex				0.164
Female	45	25	20	
Male	62	26	36	
Tumor size				0.000
<3 cm	43	34	9	
3-5 cm	33	13	20	
5–10 cm	24	2	22	
>10 cm	7	2	5	
Tumor site				0.282
Left	49	27	22	
Right	43	19	24	
Both	15	5	10	
HbsAg				0.005
No	50	31	19	
Yes	57	20	37	
AFP				0.783
<20 ng/mL	41	20	21	
20-400 ng/mL	34	17	17	
>400–1000 ng/mL	21	8	13	
>1000 ng/mL	11	6	5	
Portal vein tumor thrombus				0.001
No	42	12	30	
Yes	65	39	26	
Child-Pugh				0.033
A	9	8	1	
B	62	28	34	
C	36	15	21	
Therapies				0.003
TACE	35	9	26	
Conservative treatment	32	16	16	
Combined therapy	40	26	14	
Neoplasm metastasis				0.000
No	40	28	12	
Yes	67	23	44	

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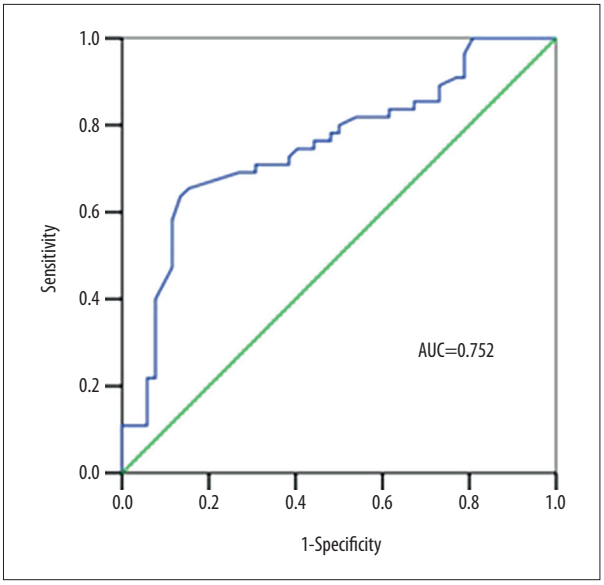

Figure 4. ROC curve for patients with HCC based on the expression of *lncRNA-D16366*.

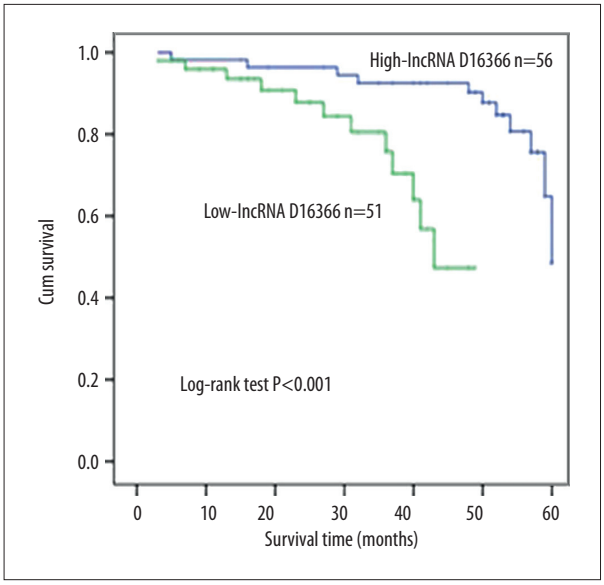

Figure 5. Kaplan-Meier analysis for patients with HCC based on the expression of *lncRNA-D16366*.

Table 2. Cox regression analysis for the prognostic value of *lncRNA-D16366* in HCC.

Parameter	Risk ratio	95% CI	*P*
Portal vein tumor thrombus	3.104	1.197–8.048	0.020
High lncRNA-D16366 expression	–	–	–
Low lncRNA-D16366 expression	6.472	2.046–20.470	0.001

(HR=3.104, 95CI%=1.197–8.048, P=0.020) and low expression of *lncRNA-D16366* (HR=6.472, 95CI%=2.046–20.470, *P*=0.001) appear to be related to the prognosis of HCC (Table 2). Low expression level of *lncRNA-D16366* was an unfavorable factor for the prognosis in HCC.

Discussion

HCC is the sixth most common cancer and the third leading cause of cancer-related deaths, and accounts for 70% to 85% of total liver cancers worldwide [1,17,18]. Rapid infiltrating growth, early metastasis, high-grade malignancy, and poor prognosis are important characteristics of HCC [19]. Accurate bio-markers are useful in predicting the prognosis of different diseases, including HCC. Studies have proposed many biomarkers for HCC prognosis, such as miRNAs, specific expression gene, and lncRNAs. Among them, lncRNAs offer a new direction and have attracted much attention.

In previous studies, many virtual lncRNAs have been studied in HCC. For instance, H19 was the first non-coding RNA reported to show abnormal expression in HCC [20]. Li et al. found that in HCC, maternally expressed gene 3(MEG3) was downregulated and played an inhibitory role in the malignancy growth [21]. Wu et al. found that the over-expression of lncRNA HOTAIR enhanced carcinogenic activity of HCC cells, and predicted unfavorable clinical outcomes [22]. Metastasis-associated lung and adenocarcinoma transcript 1(MALAT1) was reported to promote the viability, mobilization force, and invasiveness of HCC cells through sponging miR-204 and releasing SIRT1 [23]. lncRNA-HULC and lncRNA-HANR were both upregulated in patients with HCC [24,25]. Zhang et al. found that the dysregulation of lncRNAs influences the pathogenesis of HBV-HCC [26]. These results show that lncRNAs play important roles in the development and progression of HCC.

In our study, we detected the expression of *lncRNA-D16366* in both tissue and serum samples, showing its downregulation in patients with HCC, which was consistent with the view of Liu et al. [16]. To further explore whether it is involved in the development of HCC, we explored the association of *lncRNA-D16366* with clinicopathological characteristics, and found that tumor size, HbsAg, portal vein tumor thrombus, Child-Pugh scores, therapies received, and neoplasm metastasis were significantly associated with the expression of *lncRNA-D16366* in HCC.

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We also investigated the clinical significance of *lncRNA-D16366*. Our results suggest that its abnormal expression is related to the diagnosis or prognosis of HCC. The high diagnostic value of lncRNA-*D16366* was demonstrated by an ROC curve with an AUC of 0.752, a sensitivity of 65.5%, and a specificity of 84.6%. In addition, Kaplan-Meier analysis indicated *lncRNA-D16366* expression is correlated with the prognosis of HCC. Therefore, we conducted a multivariate analysis with Cox regression analysis. The outcome verified that *lncRNA-D16366* predicted prognosis of HCC.

Conclusions

lncRNA-D16366 is downregulated in patients with HCC. Its abnormal expression could be used as an independent diagnostic and prognostic marker for this lethal cancer. However, its mechanisms and other effects remain unknown. Moreover, this study is limited by its small sample. Hence, further studies are needed.

Conflict of interests

None.

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