NUCKS1 Acts As A Promising Novel Bio-Marker in The Prognosis of Patients with Hepatocellular Carcinoma

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Abstract

Background: Nuclear casein kinase and cyclin-dependent kinase substrate 1 (*NUCKS1*) was over-expressed in some tumors, including hepatocellular carcinoma (HCC). However, the clinical significance of *NUCKS1* in HCC was still unclear. The aim of this study was to explore the expression and prognostic value of *NUCKS1* in HCC.

Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the relative mRNA expression of *NUCKS1* in HCC tissues and corresponding adjacent normal tissues. The relationship between *NUCKS1* expression and clinical characteristics of patients was analyzed by χ^2 test. Kaplan-Meier method and cox regression analysis were applied to estimate the prognostic value of *NUCKS1* in HCC.

Results: Compared with normal tissues, the relative mRNA expression level of *NUCKS1* was significantly up-regulated in HCC tissues ($P < 0.001$). And high *NUCKS1* expression was closely associated with tumor differentiation, TNM stage, vascular invasion and metastasis ($P < 0.05$). Kaplan-Meier analysis revealed that the overall survival of HCC patients with low expression of *NUCKS1* was obviously longer than those with high *NUCKS1* expression (log rank test, $P = 0.001$). *NUCKS1* was an independent prognostic factor of HCC patients via univariate and multivariate cox regression analyses.

Conclusions: *NUCKS1* may be correlated with the progression of HCC and may serve as a potential factor for the prognosis of this disease.

Background

Hepatocellular carcinoma (HCC) is one of the most aggressive fatal malignant tumors worldwide [1]. The incidence and mortality rates of HCC are high in the world [2]. The tumorigenesis and development of HCC include multifaceted causes, such as liver cirrhosis, hepatitis B virus (HBV) and C virus (HCV) infection [3]. Surgical resection is a major therapeutic method for HCC patients, but it is not effective for patients with late stages [4]. Although the treatments and perioperative management had been improved, the prognosis of HCC was still poor with a very low 5-year overall survival rate due to advanced stages, recurrence and metastasis [5, 6]. Therefore, finding novel and effective bio-markers for the diagnosis, therapy and prognosis is an urgent need.

Nuclear casein kinase and cyclin-dependent kinase substrate 1 (*NUCKS1*, also named *NUCKS*) gene, located on human chromosome 1q32.1, encodes a highly phosphorylated and DNA binding protein that also is methylated, ubiquitylated and acetylated [7]. The expression of *NUCKS1* is observed in almost all mammalian tissues and *NUCKS1* protein is a substrate of some protein kinases *in vitro* [8, 9]. *NUCKS1* had been proven to be involved in several cellular processes, including cell proliferation, cell cycle regulation and the activation of various genes during rapid cell growth [10, 11]. A large number of studies had demonstrated that the aberrant expression of *NUCKS1* was confirmed in a variety types of human cancers and its over-expression was associated with tumor growth and metastasis [12, 13]. A study of

Cheong et al. mentioned that the expression of *NUCKS1* was increased in HCC tissues compared with that in adjacent non-cancerous tissues at protein level [14]. However, little is known about the prognostic value of *NUCKS1* in HCC.

In this study, we investigated the clinical significance of *NUCKS1* in HCC patients through detecting *NUCKS1* expression levels in HCC tissues as well as corresponding adjacent normal tissues, and analyzing the correlation between its expression and the progression of HCC patients. Moreover, we also explored whether the elevated *NUCKS1* expression could be associated with poor prognosis of HCC patients.

Materials And Methods

Patients and samples

In this study, we investigated 126 HCC patients who underwent surgery in Harrison international Peace Hospital. The diagnosis of HCC was confirmed by pathological examinations. None of patients had received any therapies before surgery. This study was approved by the Ethical Committee of this hospital and the written informed consents were obtained from all participators in advance.

HCC tissues and corresponding adjacent normal tissues were collected from 126 patients enrolled in this study. The tissue samples were immediately frozen in liquid nitrogen and store at – 80 °C for use. The detail clinicopathological characteristics of HCC patients, including age, gender, liver cirrhosis, tumor size, AFP level, tumor differentiation, TNM stage, vascular invasion and metastasis, were shown in Table 1. The follow-up was performed with 5 years by a telephone or questionnaires. Patients who died from other diseases or unexpected events should be excluded from our study.

Table 1
Relationship between *NUCKS1* expression and clinical features of HCC patients

Clinical Features	Cases (n = 126)	<i>NUCKS1</i> expression		χ^2	<i>P</i>
		High (n = 57)	Low (n = 69)		
Age (years)				0.094	0.759
≤ 57	66	29	37		
> 57	60	28	32		
Gender				0.115	0.734
Male	82	38	44		
Female	44	19	25		
Liver cirrhosis				0.062	0.803
No	39	17	22		
Yes	87	40	47		
Tumor size (cm)				3.413	0.065
≤ 5	71	27	44		
> 5	55	30	25		
AFP (ng/mL)				0.218	0.640
≤ 20	47	20	27		
> 20	79	37	42		
Tumor differentiation				4.281	0.039
Well + Moderate	68	25	43		
Poor	58	32	26		
TNM stage				6.039	0.014
I-II	66	23	43		
III-IV	60	34	26		
Vascular invasion				3.964	0.046
Absence	74	28	46		
Presence	52	29	23		

Clinical Features	Cases (n = 126)	<i>NUCKS1</i> expression		χ^2	<i>P</i>
		High (n = 57)	Low (n = 69)		
Metastasis				4.443	0.035
Negative	81	31	50		
Positive	45	26	19		

RNA extraction and qRT-PCR

Total RNA was severally isolated from tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed with PrimeScript™ RT reagent kit (Takara, Dalian, China), while SYBR Premix Ex Taq (Takara, Dalian, China) was used to detect the relative mRNA expression of *NUCKS1* in specimens on Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). *β-actin* served as internal reference and $2^{-\Delta\Delta Ct}$ method was applied to calculate the relative quantification of *NUCKS1* expression. Each sample was examined in triplicate.

Statistical analysis

All statistical analyses were carried out by SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). The data were expressed as mean ± standard deviation (SD). The difference between two groups was evaluated via Student's t-test. χ^2 test was applied to analyze the relationship between *NUCKS1* expression and clinical features of patients. The survival curve was established through Kaplan-Meier analysis with log rank test. Univariate and multivariate cox regression analyses were utilized to estimate the prognostic value of *NUCKS1* in HCC patients. $P < 0.05$ was considered as statistically significant.

Results

The clinical characteristics of HCC patients

As shown in **Table 1**, there were 44 female and 82 male patients with a mean age of 57.26 ± 15.25 years (range, 34-77 years). Of 126 patients, 87 cases had liver cirrhosis. The sizes of 71 tumors were less than 5 cm and 55 were more than 5 cm. 47 patients had AFP levels less than 20 ng/mL and 79 patients had AFP levels more than 20 ng/mL. Tumor differentiation of 68 cases was well or moderate and 58 cases had poor tumor differentiation. Among these patients, 66 cases were at stage I-II and 60 cases were at stage III-IV. There were 52 patients of vascular invasion and 45 with metastasis.

The relative expression of NUCKS1 was up-regulated in HCC patients

QRT-PCR was used to detect the relative mRNA expression levels of *NUCKS1* in HCC tissues and adjacent normal tissues. The result showed that *NUCKS1* expression was significantly increased in HCC tissues compared to that in corresponding adjacent normal tissues at mRNA level ($P < 0.001$, **Figure 1**).

Association between NUCKS1 expression and clinical characteristics of HCC patients

To explore the role of *NUCKS1* in HCC, 126 patients were divided into two groups (high *NUCKS1* expression group and low *NUCKS1* expression group) according to mean expression of *NUCKS1*. Then the relationship between *NUCKS1* expression and clinical features of patients was analyzed. Elevated *NUCKS1* expression was closely associated with tumor differentiation ($P = 0.039$), TNM stage ($P = 0.014$), vascular invasion ($P = 0.046$) and metastasis ($P = 0.035$) (**Table 1**). However, there was no obvious correlation between *NUCKS1* expression and age, gender, liver cirrhosis, tumor size or AFP level ($P > 0.05$, **Table 1**).

Increased NUCKS1 expression was correlated with poor prognosis of HCC patients

To estimate the prognostic value of *NUCKS1* in HCC patients, the follow-up was conducted with 5 years. According to Kaplan-Meier analysis, patients with high expression of *NUCKS1* had obviously shorter overall survival than those with low *NUCKS1* expression (log rank test, $P = 0.001$, **Figure 2**). Univariate cox regression analysis demonstrated that *NUCKS1* expression ($P = 0.001$), tumor size ($P = 0.022$), AFP level ($P = 0.024$), tumor differentiation ($P = 0.045$), TNM stage ($P = 0.008$), vascular invasion ($P = 0.014$) and metastasis ($P = 0.005$) were all associated with the overall survival of HCC patients (**Table 2**). Besides, *NUCKS1* expression (HR = 1.905, 95% CI = 1.106-3.283, $P = 0.020$), TNM stage (HR = 1.845, 95% CI = 1.074-3.170, $P = 0.027$) and metastasis (HR = 1.867, 95% CI = 1.100-3.167, $P = 0.021$) all were independent prognostic factors for HCC patients (**Table 2**).

Table 2

Univariate and multivariate cox regression analyses of prognostic factors in patients with HCC patients

Factors	Univariate analysis			Multivariate analysis		
	HR	(95% CI)	<i>P</i>	HR	(95% CI)	<i>P</i>
<i>NUCKS1</i> (High vs Low)	2.424	1.433-4.100	0.001	1.905	1.106-3.283	0.020
Age (years) (> 57 vs ≤ 57)	1.218	0.727-2.041	0.453	-	-	-
Gender (Male vs Female)	1.119	0.646-1.937	0.688	-	-	-
Liver cirrhosis (Yes vs No)	1.441	0.777-2.672	0.246	-	-	-
Tumor size (cm) (> 5 vs ≤ 5)	1.835	1.091-3.086	0.022	-	-	-
AFP (ng/mL) (> 20 vs ≤ 20)	2.004	1.097-3.663	0.024	-	-	-
Tumor differentiation (Poor vs Well + Moderate)	1.704	1.012-2.867	0.045	-	-	-
TNM stage (Ⅲ-Ⅳ vs Ⅰ-Ⅱ)	2.056	1.209-3.496	0.008	1.845	1.074-3.170	0.027
Vascular invasion (Presence vs Absence)	1.914	1.139-3.218	0.014	-	-	-
Metastasis (Positive vs Negative)	2.101	1.253-3.521	0.005	1.867	1.100-3.167	0.021
Note: - indicated no data.						

Discussion

In the present study, we detected the relative mRNA expression levels of *NUCKS1* in HCC tissues and adjacent normal tissue samples. The result of qRT-PCR showed that *NUCKS1* expression was significantly higher in HCC tissues than that in normal tissues. In addition, the increased *NUCKS1*

expression was tightly associated with some clinical factors, including well/moderate tumor differentiation, advanced TNM stage, presence of vascular invasion and positive metastasis of patients. This results revealed that *NUCKS1* functioned as an oncogene in HCC, and was involved in carcinogenesis and tumor progression.

NUCKS1 protein is identified as one of the cell cycle-related proteins and participates in the response of DNA damage induction [8, 15]. Previous studies had reported that *NUCKS1* played a crucial role in the regulation of glucose homeostasis and insulin signalling [16, 17]. Besides, *NUCKS1* had been shown to be a key transcriptional regulator and chromatin modifier and it could be a potential bio-marker for human diseases, including cancers and metabolic diseases [18]. Similar to the high-mobility group (HMG) proteins, *NUCKS1* belonged to the most modified proteins in the proteome of mammals and was associated with the progression and diagnosis of human cancer [19]. A study carried out by Kikuchi et al. demonstrated that the mRNA and protein expression levels of *NUCKS1* were significantly up-regulated in colorectal cancer (CRC) tissues compared to that in normal tissues [20]. A notable correlation was found between *NUCKS1* over-expression and invasion, TNM stage and metastasis of CRC patients. In addition, Liu et al. revealed that increased *NUCKS1* expression was strongly correlated with stage, histological grade and metastasis of endometrial cancer [21]. Our results were consistent with all the previous studies.

Due to frequent recurrence and metastasis, the prognosis of HCC remains undesirable. Commonly, serum AFP level is considered as a diagnostic and prognostic factor of HCC patients [22]. Moreover, TNM stage and metastasis are both associated with the prognosis of HCC patients. However, they lack accuracy [23, 24]. Accumulated evidences had supported that increased *NUCKS1* was linked with poor prognosis of human cancers [20, 25]. However, its prognostic value in HCC remains poorly known. In current study, Kaplan-Meier survival analysis result showed a negative relationship between *NUCKS1* expression and overall survival of HCC patients. Moreover, cox regression analysis further demonstrated that the *NUCKS1* expression level was an independent prognostic factor for the survival of HCC patients. Besides, TNM stage and metastasis both were independent factors.

There were still some limitations in our study. Firstly, high expression of *NUCKS1* was indicated to be associated with tumor size of patients with cervical squamous cell carcinoma [26], which was inconsistent with our findings. The functions of *NUCKS1* in different tumors were varied and the sample size as well as its source might also cause differences. Next, the precise molecular mechanism of *NUCKS1* in the regulation of HCC is still unclear. Thus, given these constraints, further studies are needed to gain insight into the functions of *NUCKS1* in the progression of HCC.

Conclusions

In conclusion, the mRNA expression of *NUCKS1* is up-regulated in HCC and associated with the progression of this tumor. Besides, *NUCKS1* may be a potential bio-marker for the prognosis of HCC.

List Of Abbreviations

Nuclear casein kinase and cyclin-dependent kinase substrate 1 (*NUCKS1*)

hepatocellular carcinoma (HCC)

Quantitative real-time polymerase chain reaction (qRT-PCR)

hepatitis B virus (HBV)

C virus (HCV)

colorectal cancer (CRC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Harrison international Peace Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

X.Z., X.L. design of the work; X.Z., H.B. the acquisition, analysis, G.L., N.L. interpretation of data; X.Z., H.B. the creation of new software used in the work; H.L., J.D. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

Figure 1

The relative mRNA expression of NUCKS1 in HCC tissues and corresponding adjacent normal tissues. Compared with normal tissues, NUCKS1 expression was obviously up-regulated in HCC tissues at mRNA level ($P < 0.001$). *** indicated P less than 0.001.

Figure 2

Kaplan-Meier curve analysis for the overall survival of HCC patients. The overall survival of patients with low expression of NUCKS1 was significantly longer than those with high NUCKS1 expression (log rank test, $P = 0.001$).