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Increased expression of long noncoding RNA AK021443 predicts worse clinical outcome in hepatocellular carcinoma

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Abstract. – OBJECTIVE: Dysregulation of long non-coding RNAs (lncRNAs) plays critical roles in the process of carcinogenesis and tumor progression. LncRNA AK021443 (AK021443) has been reported to serve as a tumor promoter in hepatocellular carcinoma (HCC). The purpose of this study was to investigate the expression of AK021443 and its prognostic value in HCC..

PATIENTS AND METHODS: Quantitative Real-Time-PCR was used to examine the expression of AK021443 in 193 HCC tissues and adjacent non-tumor tissues. The correction between AK021443 expression and clinicopathological characteristics was evaluated with Chi-square test. To determine its prognostic value, overall survival was evaluated using the Kaplan-Meier method. Univariate and multivariate analyses were performed to analyze the prognostic significance of AK02144 expression.

RESULTS: The results revealed that AK021443 expression levels were significantly higher in HCC tissues than in the corresponding noncancerous tissues ($p<0.01$). Besides, high AK021443 expression was correlated with poor tumor differentiation ($p = 0.002$), advanced clinical stage ($p=0.001$) and positive lymph node metastasis ($p=0.005$). In addition, the Kaplan-Meier survival curves revealed that HCC patients with high AK021443 expression level had shorter overall survival than those with low AK021443 expression level ($p=0.0005$). Finally, the multivariable analysis suggested that increased AK021443 expression was an independent prognostic factor of overall survival in HCC patients.

CONCLUSIONS: Our findings indicated that AK021443 may play an important role in tumorigenesis and progression and would be a powerful marker to predict the prognosis of HCC patients.

Key Words
Long noncoding RNA, Prognosis, Hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequently diagnosed malignancies, especially in Asia, and both incidence and mortality rates of HCC have increased in recent years[1,2]. The mechanisms involved in the development and progression of HCC remain poorly understood[3]. Although current therapeutic strategies have improved over the past decades, the overall survival of patients with HCC following resection remains unsatisfactory, and high metastasis and recurrence rates are the main factors affecting the prognosis of HCC patients[4,5]. Therefore, it is of critical importance to identify effective prognostic biomarkers for understanding the molecular mechanism of metastasis involved in HCC, and for improving clinical outcome of patients suffering HCC. Long non-coding RNAs (LncRNAs) are a subgroup of non-coding RNAs of > 200 nucleotides in length and lack an open reading frame of significant length and the capability of coding protein[6]. Growing evidence indicates that lncRNAs play a critical role in regulating various cellular processes, including cell growth, proliferation, differentiation and cell death, through gene regulation, including genomic imprinting, chromatin modification, and posttranscriptional processing[7,8]. Importantly, more and more studies indicated that the dysregulation of lncRNAs occurs in numerous diseases, including cancers, and affects tumor development and progression[9-11]. The important role of lncRNA in tumor progression revealed that dysregulated lncRNAs probably could function as new biomarkers for early diagnosis, effective therapeutic targets, and prognosis prediction of malignant tumors, including HCC[12-14]. Recently, a newly found lncRNA,

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Table I. Sequences of the primers used for RT-qPCR.

Name	Sequence (5′→3′)
AK021443-F	CTTGAACCCAGAAGACAGG
AK021443-R	ATGGAACATTAGAGGTAGCAC
β-actin-F	GGGAAATCGTGCGTGACATTAAG
β-actin-R	TGTGTTGGCGTACAGGTCTTTG

AK021443, was found to be up-regulated in HCC by microarray data assay[15]. Then, a subsequent study by Yang et al[16] reported that knockdown of AK021443 could suppress the proliferation, colony formation, invasion and migration of HCC cells, indicating that AK021443 served as a tumor promoter in HCC progression. To our best knowledge, there are no other studies about the effect of AK021443 in other tumors. More importantly, until now, it remains unclear whether AK0214431 was related to the prognosis of HCC patients.

Patients and Methods

Patients and Clinical Samples

A total of 193 primary HCC and corresponding noncancerous tissue samples were collected from the Department of Hepatobiliary Surgery, Jining No. 1 People's Hospital for RT-qPCR analysis between March 2008 and February 2012. All tissues specimens were confirmed by pathological examination. The samples were snap frozen in liquid nitrogen and stored at -80°C for later RNA extraction. No patients received preoperative chemotherapy or embolization. Related important clinical information was collected from each patient's medical records. The clinicopathological characteristics are described in detail in Table II. The histological grade of each tumor was determined based on the Edmondson-Steiner grading system. Informed consent was obtained from the patients enrolled in this study. The investigation was approved by the Ethics Committee of Shanghai Jiao Tong University.

RNA Extraction and Quantitative Real-Time PCR

Total RNA from tumor tissues and matched normal tissues was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was reversely transcribed using a First Strand Synthesis kit (Invitrogen, Carlsbad, CA, USA). The qRT-PCR reactions were performed using an ABI7500 System (Applied Biosystems, Foster City, CA, USA) and SYBR Green PCR Master Mix (TaKaRa, Otsu, Shiga, Japan). The cycling conditions were 40 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s. Moreover, the relative amount of AK021443 was normalized with respect to β-actin. Fold-change in expression was calculated by the relative quantification ($2^{-\Delta\Delta Ct}$) method. The PCR primer sequences used were shown in Table I.

Table II. Association of AK021443 with clinicopathological characteristics of HCC patients.

Parameters	Group	Total	AK021443 expression		*p*-value
			High	Low	
Gender	Male	119	61	58	NS
	Female	74	37	37	
Age (years)	< 60	113	53	60	NS
	≥ 60	80	45	35	
Tumor size (cm)	< 5	122	57	65	NS
	≥ 5	71	41	30	
Tumour number	Solitary	44	24	20	NS
	Multiple	149	74	75	
HBsAg	Postive	75	34	41	NS
	Negative	118	64	54	
AFP	< 20	104	50	54	NS
	> 20	89	48	41	
Tumor differentiation	Well/Moderate	128	55	73	0.002
	Poor	65	43	22	
Lymph node metastasis	Absence	120	50	70	0.001
	Presence	73	48	25	
Clinical stage	I-II	132	58	74	0.005
	III-IV	61	40	21	

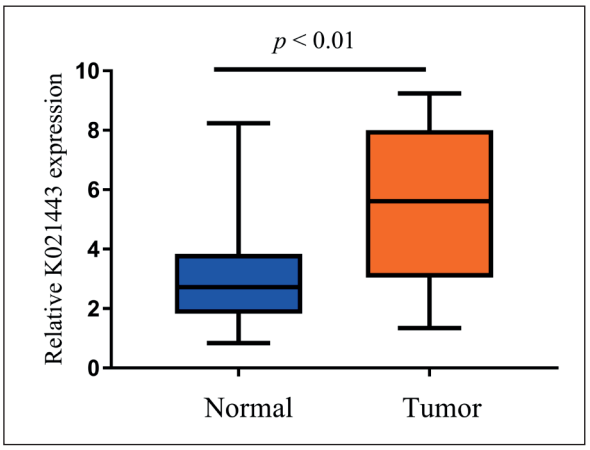

Figure 1. AK021443 expression in 193 pairs of clinical HCC and adjacent non-tumor tissues were detected by qRT-PCR. The expression level of AK021443 in HCC tissues was significantly higher than that in non-tumor tissues ($p<0.01$).

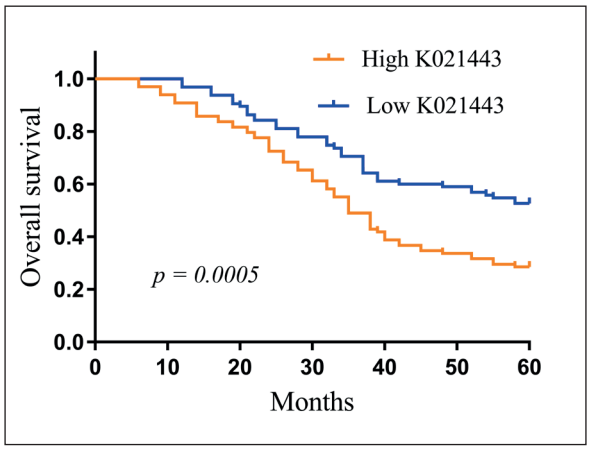

Figure 2. Overall survival curves for two groups defined by low and high expression of AK021443 in HCC patients. The patients with high AK021443 expression had a significantly shorter overall survival than those with low AK021443 expression ($p=0.0005$).

Statistical Analysis

Statistical analysis was performed using the SPSS 19.0 program (IBM, Armonk, NY, USA). The paired-samples *t*-test was used in the analysis of differential AK021443 expression between tumor and normal tissues. Association of AK021443 expression with clinical parameters was analyzed by Chi-square test. Survival curves were presented using Kaplan-Meier analyses and compared using the log-rank test. A Cox proportional hazards model was used for univariate and multivariate analysis. Results were considered statistically significant at $p<0.05$.

Results

AK021443 Is Significantly Up-Regulated in HCC Tissues

In order to verify whether AK021443 was differentially expressed in HCC tissues, 193 paired HCC lung tissues and matched normal tissues were tested for AK021443 expression. As shown in Figure 1, the results of RT-PCR showed that expression levels of AK021443 in HCC tissues were significantly higher than those in adjacent non-tumor tissues ($p<0.01$), implying that deregulated expression of AK021443 may play a role in the development of HCC.

Association Between AK021443 Upregulation and Clinicopathological Parameters of Patients With HCC

To identify the clinical relevance of AK021443 expression in HCC, the expression levels of AK021443 in tumor tissues were categorized as low or high in relation to the mean value. Then, the correlation of AK021443 expression level and clinicopathological characteristics was listed in Table II. We found that high AK021443 expression was correlated with poor tumor differentiation ($p=0.002$), advanced clinical stage ($p=0.001$) and positive lymph node metastasis ($p=0.005$). However, there was no association between AK021443 expression and age or gender ($p>0.05$). Our clinical assay indicated that AK021443 was implicated in the progress and development of HCC.

High Level of AK021443 Was Predictive of Poor Prognosis of HCC Patients

To further evaluate the association between AK021443 expression and HCC patient prognosis, Kaplan-Meier survival analysis and log-rank tests using patient post-operative survival were performed. As shown in Figure 2, patients with high AK021443 expression had a significantly poorer prognosis than those with low AK021443 expression ($p=0.0005$). Furthermore, Cox regression analyses were conducted to explore the prognostic factors in 193 HCC patients. Univariate analysis showed that tumor differentiation ($p=0.005$), lymph node metastasis ($p=0.001$), clinical stage ($p=0.007$), and AK021443 ($p=0.001$) were associated with overall mortality (Table III). When applying multivariate analysis using the Cox proportional hazards model, we found that AK021443 expression was an independent poor prognostic factor for HCC patients ($p=0.001$, Table III).

Table III. Univariate and multivariate analyses of overall survival of HCC patients.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p	HR	95% CI	p
Gender Male vs Female	1.423	0.677-2.231	0.338	–	–	–
Age >60 vs. ≤60	1.566	0.831-1.773	0.215	–	–	–
Tumor size < 5 vs. ≥ 5	1.677	0.713-2.446	0.188	–	–	–
Tumour number Solitary vs. Multiple	2.215	0.811-2.674	0.145	–	–	–
HBsAg Postive vs. Negative	1.778	0.563-2.451	0.217	–	–	–
AFP < 20 vs. > 20	2.316	0.859-2.893	0.137	–	–	–
Tumor differentiation Well/Moderate vs. Poor	3.452	1.523-6.578	0.005	2.782	1.216-4.776	0.008
Lymphnode metastasis Absence vs. Presence	4.337	1.672-8.349	0.001	3.231	1.233-6.241	0.006
Clinical stage I-II vs. III-IV	3.132	1.468-5.662	0.007	2.763	1.183-4.223	0.010
AK021443 expression High vs. Low	4.232	1.773-8.832	0.001	3.332	1.438-6.675	0.001

Discussion

HCC, the fifth most common cancer worldwide, is confirmed to be a severe threat to public health[17]. Although current therapeutic strategies have improved over the past decades, the long-term survival remains unsatisfactory[18]. Up to date, most decisions throughout the clinical management of HCC are dictated by TNM staging[19]. However, for some patients, this system had not been proven to be sufficiently effective. The novel molecular biomarkers for early diagnosis and predictor of prognosis are desperately required now. However, identification of molecular biomarkers with clinical value is an enormous challenge.

Recently, more and more lncRNAs were reported to be used as molecular tumor markers. These markers, together with clinical data, showed potential prognostic and diagnostic value for HCC patients[20,21]. For instance, Zhang et al[22] found that lncRNA AFAP1-AS1 was upregulated in HCC tissues and predicted the poor prognosis of patients with OS. Moreover, its overexpression promoted cell proliferation and invasion via upregulation of the RhoA/Rac2 signaling. Wang et al[23] reported that PCAT-14 was highly expressed in HCC, and its forced expression promotes proliferation, invasion, and cell cycle arrest in HCC cells by inducing methylation of miR-372. Yang et al[24] indicated that HCC patients with lncRNA SNHG15 higher expression have a shorter overall survival than those with lower lncRNA SNHG15 expression, indicating SNHG15 as a potential prognostic biomarker for HCC. Recently, Yang et al[16] reported that AK021443 expression was significantly up-regulated in HCC tissues. Further *in vitro* and *in vivo* assay suggested AK021443 as a tumor promoter because its inhibition suppressed proliferation, colony formation, invasion, and migration of HCC cells by modulating Epithelial-Mesenchymal Transition. However, the associations between the AK021443 expression, clinicopathological characteristics and the prognostic value of AK021443 in HCC have not yet been reported.

In the present work, we performed RT-PCR to determine the levels of AK021443 in HCC tissues and matched normal tissues and found that AK021443 expression levels were higher in HCC tissues than in adjacent non-tumor tissues. This result was in line with previous internet data. Then, we evaluated the correlation between AK021443 expression and clinicopathological factors and found that high AK021443 expression was correlated with poor tumor differentiation, advanced clinical stage and positive lymph node metastasis, indicating that AK021443 may be a metastatic-related lncRNA. Furthermore, the results of Kaplan-Meier method showed that overall patient survival for those with low AK021443 expression was significantly longer than those patients with

high AK021443 expression. More importantly, the univariate and multivariate analysis results indicated that AK021443 might be as a valuable prognostic factor for HCC patients. These findings demonstrated for the first time the clinical significance of AK021443 in HCC. Nevertheless, the limitation of this study was that the number of tissue samples was relatively small.

Conclusions

We firstly indicated that AK021443 expression may be an independent marker for predicting the clinical outcome of patients with HCC. It is necessary to illuminate the function of AK021443 in HCC then support its potential as a prognostic and therapeutic target.

Conflict of Interest:

The Authors declare that they have no conflict of interests.

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