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Evaluating the diagnostic and prognostic value of long non-coding RNA SNHG15 in pancreatic ductal adenocarcinoma

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Abstract. – OBJECTIVE: Long non-coding RNA SNHG15 (SNHG15) has been reported to play very important roles in the malignancy behaviors of various tumors, including pancreatic ductal adenocarcinoma (PDAC). However, its clinical significance in PDAC remains largely unclear. The aim of this study was to investigate whether the aberrant expression of SNHG15 can be used as potential prognostic and diagnostic markers of human PDAC.

MATERIALS AND METHODS: TaqMan Real Time-PCR was performed to investigate the expression of SNHG15 in PDAC tissues and serum samples. Receiver operator characteristic (ROC) analysis was applied to obtain the diagnostic utility of SNHG15. Association between SNHG15 levels and clinicopathological factors was analyzed. Kaplan-Meier curves and multivariate Cox proportional models were used to study the impact on clinical outcome.

RESULTS: SNHG15 levels were significantly up-regulated in both sera and tumors tissues from PDAC patients. ROC curve analysis revealed that SNHG15 may be a potential biomarker for differentiating PDAC tissues from normal pancreatic tissues, and the plasma levels of SNHG15 may be a potential biomarker for differentiating PDAC patients from healthy controls. Clinicopathologic analysis revealed that high SNHG15 expression was associated with tumor differentiation ($p = 0.000$), lymph node metastasis ($p = 0.001$) and tumor stage ($p = 0.005$). Furthermore, patients with high SNHG15 expression had a shorter overall survival compared with the low SNHG15 expression group ($p = 0.003$). Also, Cox multivariate analyses confirmed that SNHG15 expression was an independent prognostic factor in PDAC ($p < 0.004$).

CONCLUSIONS: Our study firstly indicated the potential value of SNHG15 as an important biomarker for the diagnosis and prognosis prediction of PDAC.

Key Words:
Long non-coding RNA, SNHG15, Pancreatic ductal adenocarcinoma, Prognosis, Diagnosis.

Introduction

Pancreatic cancer is currently one of the leading causes of cancer deaths in China, and its incidence appears to be increasing[1]. Of all pulmonary carcinomas, pancreatic ductal adenocarcinoma (PDAC) represents around 90%[2]. This highly malignant tumor is rarely diagnosed at an early stage and difficult to treat due to its resistance to traditional therapies[3]. Great effort has been spent in the research of pancreatic cancer. However, the survival rate of advanced pancreatic cancer has not improved over the past decades[4]. Therefore, it is of great clinical significance to develop new strategies for the early diagnosis of PDAC to improve treatment strategies and the prognostic outcomes in these patients. Long noncoding RNAs (lncRNAs) are a group of transcribed RNA consisting of more than 200 nucleotides and generally poorly conserved[5]. Growing evidence indicates that lncRNAs are regulators of gene expression through multiple mechanisms such as transcriptional regulation and post-translational protein modification[6,7]. In recent years, several lncRNAs have been shown to be involved in the cancer progression[8]. For instance, Zhang et al[9] reported that upregulation of lncRNA CCAT-1 promotes cell proliferation via modulating the Wnt signaling pathway and correlates with tumor size, advanced FIGO stage and prognosis in cervical cancer. Yan et

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al[10] reported that lncRNA MNX1-AS1 up-regulation was involved in ovarian cancer tumorigenesis and progression and its knockdown could dramatically suppress cell proliferation and migration *in vitro*. Of note, in pancreatic cancer, Qin et al[11] found that Long non-coding RNA TUG1 was highly expressed in pancreatic cancer and its down-regulation could induce proliferation and migration of pancreatic cancer via EMT pathway. Dysregulation of serum lncRNAs has attracted the attention of many researchers. Growing studies[12,13] revealed that serum lncRNAs may serve as an effective biomarker for cancer detection. A recently identified long non-coding RNA, named small nucleolar RNA host gene 15 (SNHG15), is located on chromosome 7p13. Although SNHG15 does not encode protein, emerging evidence shows that the dysregulation of SNHG15 is associated with the progression and metastasis of various tumors including pancreatic cancer[14-16]. Ma et al[16] found that SNHG15 expression was significantly up-regulated in pancreatic cancer and exhibited carcinogenic functions. However, the clinical significance and biological function of SNHG15 in PDAC are not fully elucidated.

Patients and Methods

Tissue Samples

PDAC tumor tissues and adjacent normal tissues were collected from 171 patients with PDAC during surgery at Xi'an Central Hospital (Shaanxi, China). Freezing and storage of the samples collected from resection surgery were performed at a temperature of -80°C. All patients were diagnosed with PDAC based on the histopathological evaluation. All samples were derived from patients who had not received adjuvant treatment including radiotherapy. The clinical characteristics of all the patients were summarized in Table I. Written informed consent was obtained from all subjects. The Human Research Ethics Committee of Xi'an Central Hospital approved all aspects of this study.

Serum Sample Collection

Venous blood samples were collected from 171 PDAC patients and 59 healthy volunteers. Whole blood was collected in regular tubes and immediately processed to prevent contamination by cellular nucleic acids. All samples were processed within 1 h after collection and

Table I. Correlation between SNHG15 expression and clinicopathological features of PDAC.

Variables	N = 171	SNHG15 expression		*p*-value
		High	Low	
Gender				0.425
Female	78	40	38	
Male	93	42	51	
Age (year)				0.563
≤ 50	79	36	43	
> 50	92	46	46	
Tumor size (cm)				0.127
≤ 2.0	104	45	59	
> 2.0	67	37	30	
Neural invasion				0.321
Negative	86	38	48	
Positive	85	44	41	
Tumor margin				0.339
Negative	79	41	38	
Positive	92	41	51	
Tumor differentiation				0.000
Well or Moderate	96	33	63	
Poor	75	49	26	
Lymph node metastasis				0.001
Absent	106	40	66	
Present	65	42	23	
Tumor stage				0.005
I/II	108	43	65	
III/IV	63	39	24	

separated by centrifugation (3,000 rpm for 20 min). Then, the blood was carefully stored into 1.5 ml non-RNAase centrifugal tube at -80°C for further use.

Reverse Transcription and Real-Time RT-PCR

Total RNA was extracted from plasma and tumor tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. First-strand cDNAs were generated from 4 μg of total RNA using commercially available kits (Biosystems, Foster City, CA, USA). Quantitative PCR (qPCR) was performed on a StepOne Plus (Biosystems, Foster City, CA, USA) thermocycler using SYBR Green PCR Master Mix (Biosystems, Foster City, CA, USA) reagents. The conditions of real-time PCR were as follows: 94°C for 10 s, 94°C for 5 s, 52°C for 30 s to anneal, 72°C for 15 s followed by 40 cycles. Results of SNHG15 were normalized to a constitutive expression gene, GAPDH. Relative quantification of gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. The primer sequences used in the present study were as the followings: SNHG15: 5'-GCTGAGGTGACGGTCTCAAA-3' (Forward); 5'-GCCTCCCAGTTTCATGGACA-3'(Reverse). GADPH: 5'-GTCAACGGATTTGGTCTGTATT-3' (Forward), 5'-AGTCTTCTGGGTGGCAGTGAT-3' (Reverse).

Statistical Analysis

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Measurement data were analyzed using the Student's *t*-test, whereas categorical data were examined using the x^2-test. The feasibility of SNHG15 as a diagnostic tool for detecting PDAC was calculated by the receiver operating characteristic (ROC) curve. Survival curve was constructed by the Kaplan-Meier method and examined with the log-rank test. A Cox proportional hazards modeling of the factors potentially related to survival was performed to identify those related factors. The differences were considered to be statistically significant at a p-value of < 0.05.

Results

SNHG15 Expression is Increased in PDAC Tissues and Exerts a Diagnostic Value

To explore the role of SNHG15 in PDAC progression, we performed RT-PCR to detected the expression of SNHG15 in PDAC tissues and matched normal tissues. The results were shown in Figure 1A, the expression level of SNHG15 in PDAC tissues was higher than that in adjacent normal tissues ($p < 0.01$). We also evaluated the diagnostic capabilities of SNHG15 using ROC curve analysis. As shown in Figure 1B, we found that SNHG15 expression levels could distinguish PDAC tissues from normal pancreatic tissues

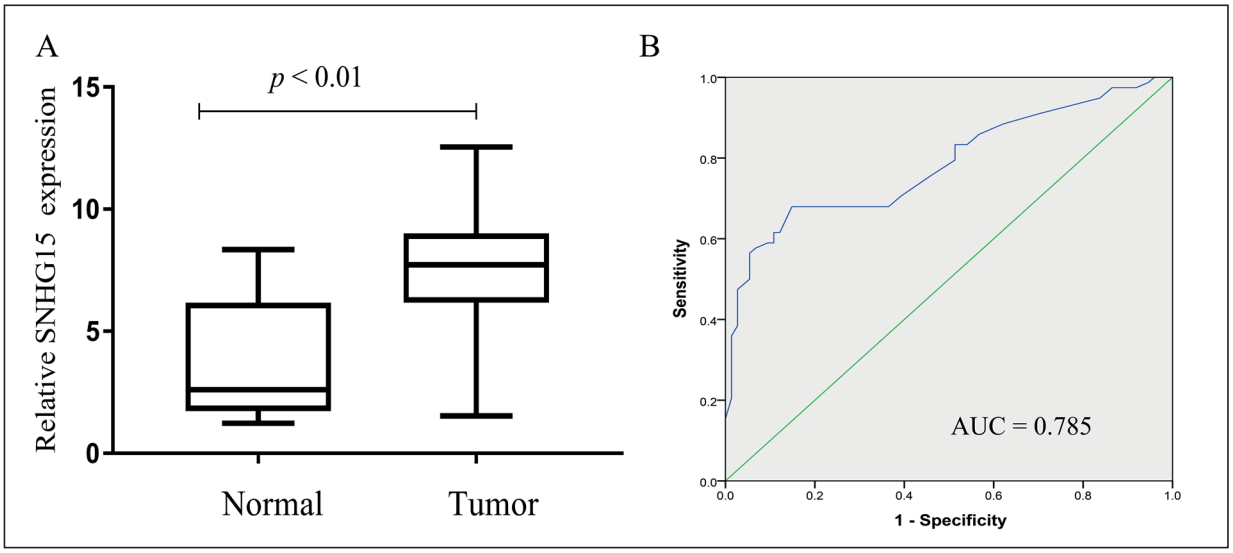

Figure 1. The expression of SNHG15 in PDAC tissues and its diagnostic value. **A,** The expressions of SNHG15 in 171 paired PDAC tissues and non-cancer tissues were measured by qRT-PCR. **B,** ROC curve analysis of the diagnostic potential of SNHG15 in PDAC tissues.

(AUC = 0.785). The optimal cutoff value for SNHG15 in PDAC tissues was 5.38 with a sensitivity of 71.7% and a specificity of 92.6%.

Serum SNHG15 Expression is Increased in PDAC Patients and Exerts a Diagnostic Value

Then, we detected the expression levels of serum SNHG15 in sera from 171 PDAC patients and healthy controls by RT-PCR. As shown in Figure 2A, our data showed that SNHG15 levels in sera from PDAC patients were significantly higher than those in healthy controls ($p < 0.001$). Similarly, ROC curve analysis revealed that the serum SNHG15 level may be a potential biomarker for screening PDAC patients from healthy controls with AUC of 0.727. The optimal cutoff value for SNHG15 in sera of PDAC patients was 6.82 with a sensitivity of 68.3% and a specificity of 89.6%.

Correlation of SNHG15 Expression and Clinical Parameters in PDAC

To explore the clinical significance of SNHG15, all patients were divided into two groups high-SNHG15 expression group (n = 82) and low-SNHG15 expression group (n = 89) according to the median value of SNHG15. Table I summarized the association between SNHG15 expression and clinicopathological parameters in PDAC. Higher expression of SNHG15 was positively associated with poor tumor differentiation ($p = 0$ .000), positive lymph node metastasis ($p = 0.001$), and advanced tumor stage ($p = 0.005$). However, there were no significant associations between SNHG15 expression and other clinical features including age, gender, tumor size, neural invasion and tumor margin (All $p > 0.05$).

Prognostic Values of SNHG15 Expression in PDAC

To study the association between the expression levels of miR-133a and patients' survival, we performed Kaplan-Meier analyses with the log-rank test. As shown in Figure 3, PDAC patients with higher SNHG15 expression have shown significantly poorer overall survival than those with lower SNHG15 expression ($p = 0.003$). Furthermore, univariate analysis was performed, and the results showed that the overall survival of patients with PDAC was associated with tumor differentiation, lymph node metastasis, tumor stage and SNHG15 expression. Then, multivariate analysis was performed using the Cox model for all of the observed variables. The results confirmed that high expression of SNHG15 was a poor independent prognostic factor for patients with PDAC (RR: 3.251, 95% CI 1.177-6.362, $p = 0.004$).

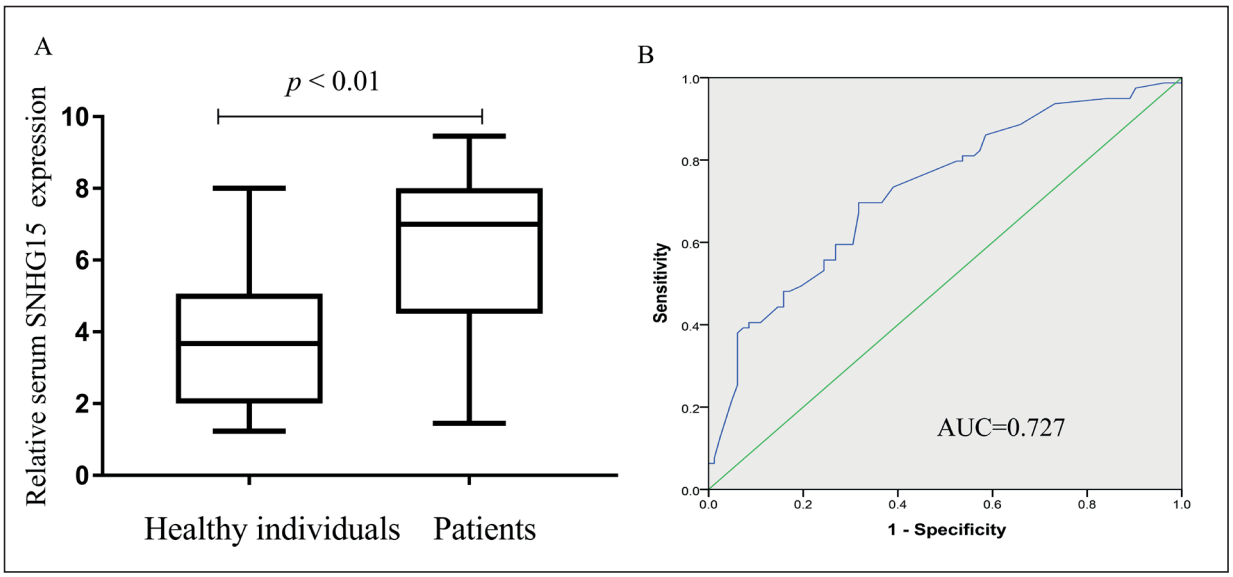

Figure 2. The expression of serum SNHG15 expression in PDAC patients and healthy individuals. **A,** The expressions of serum SNHG15 in 171 PDAC patients and 59 healthy individuals were measured by qRT-PCR. **B,** ROC curve analysis of the diagnostic performance of serum SNHG15.

Table II. Univariate and multivariate analysis of overall survival in PDAC patients.

Variable	Univariate analysis			Multivariate analysis		
	RR	95% CI	p	RR	95% CI	p
Gender	1.321	0.673-1.889	0.324	–	–	–
Age	1.456	0.783-2.778	0.173	–	–	–
Tumor size	2.323	0.482-3.467	0.197	–	–	–
Neural invasion	1.664	0.682-2.261	0.115	–	–	–
Tumor margin	1.293	0.773-1.884	0.163	–	–	–
Tumor differentiation	4.673	1.564-7.632	0.001	3.163	1.263-5.773	0.006
Lymph node metastasis	3.563	1.338-6.774	0.003	3.253	1.037-5.348	0.009
Tumor stage	3.265	0.893-6.362	0.005	2.893	1.213-5.253	0.009
SNHG15 expression	3.774	1.472-7.328	0.001	3.251	1.177-6.362	0.004

Discussion

PDAC exhibits with a 5-year survival rate of 8% one of the worst survival rates; thus, it is important to identify novel biomarkers for the diagnosis, prognosis, and treatment of PDCA[17]. Up to date, although several serum tumor markers, such as carcinoembryonic antigen and carbohydrate antigen 19-9 (CA19-9), have been used as convenient diagnostic assays for early detection, lacking sufficient sensitivity and specificity limits their clinical application[18,19]. With the development of high throughput sequencing, dysregulation of lncRNAs in tissues becomes easy to detect[20]. However, they cannot be used for clinical diagnosis because of difficulty in getting tumor tissues from PDCA patients. Several studies[21,22] characterized the possibility of circulating lncRNAs as biomarkers. The prognostic and diagnostic values of several lncRNAs have been reported in various tumors, such as lncRNA UCA1 for osteosarcoma[23], lncRNA miR210HG for glioma[24], and lncRNA CCDC26 for pancreatic cancer[25]. These findings encouraged us to find more effective and less invasive biomarkers for early diagnosis of PDAC.

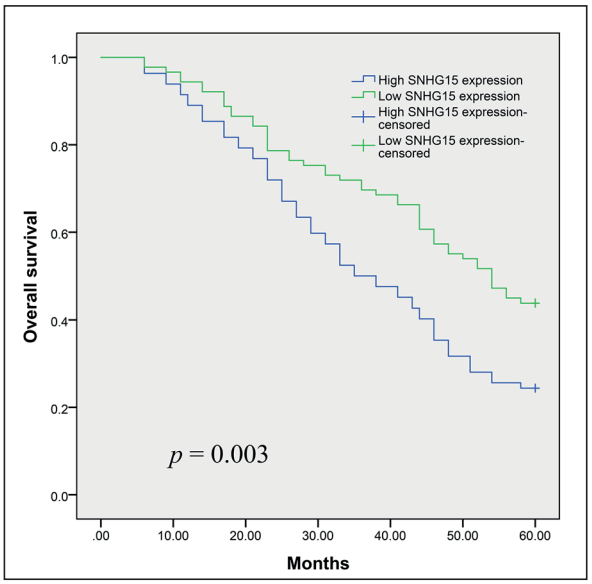

Figure 3. Overall survival curves for two groups defined by low and high expression of SNHG15 in PDAC patients. The patients with high SNHG15 expression had a significantly shorter overall survival than those with low SNHG15 expression ($p = 0.003$).

SNHG15, a novel lncRNA, was initially identified because it was found to promote cell proliferation and invasion in patients with gastric cancer[26]. Subsequently, dysregulation and biological function of SNHG15 were reported in various tumors. For instance, Liu et al[15] found that SNHG15 expression was highly expressed in osteosarcoma and its knockdown significantly suppressed osteosarcoma cell proliferation, invasion, and migration by sponging miR-141. Kong et al[27] showed that up-regulation of SNHG15 was correlated with TNM stage, lymph node metastasis and survival in breast cancer patients, and its down-regulation inhibited human breast cancer proliferation, migration and invasion by sponging miR-211-3p. Besides these findings, SNHG15 was also involved in hepatocellular carcinoma[28], glioma[29], and colon cancer[30]. Of note, a recent study by Ma et al[16] reported that the expression levels of SNHG15 were significantly up-regulated in pancreatic cancer, and its overexpression can promote pancreatic cancer proliferation through EZH2-mediated H3K27me3. However, it is not clear whether SNHG15 in plasma had a strong association with PDAC. In addition, the prognostic value of SNHG15 has not been investigated in PDAC.

In this work, we detected the expression levels of SNHG15 in PDAC patients and the results showed SNHG15 was upregulated in PDAC tissue and serum samples compared with paracancerous tissue and healthy controls. Then, we estimated the diagnostic value of SNHG15 in PDAC via ROC curve. In tissues, we found SNHG15 could efficiently screen PDAC tissues from the paracancerous tissue. Then, we showed serum SNHG15 had a moderate diagnostic value for PDAC. The AUC value is an indicator of the efficacy of the assessment system. Those findings revealed that SNHG15 might serve as a novel biomarker panel in the diagnosis of PDAC. Further investigation indicated that high expression of SNHG15 was significantly associated with tumor differentiation, lymph node metastasis, and tumor stage. Because metastasis and tumor stage were associated with the prognosis of tumor patients, we further supposed that SNHG15 might affect the prognosis of PDAC patients. The results of Kaplan-Meier analysis indicated that patients with high SNHG15 expression lived shorter than those with low expression. Further investigation by multivariate analysis demonstrates that SNHG15 was an independent predictor of PDAC. Taken together, our findings revealed that the level of SNHG15 is not only a potential diagnostic biomarker but also a predictor of overall survival in human PDAC.

Conclusions

We firstly showed that serum SNHG15 is a useful non-invasive tumor marker for the molecular diagnosis of PDAC. The expression of SNHG15 in PDAC tissues could act as an independent predictor of unfavorable clinical outcomes. Prospective studies with larger sample size should be carried out to confirm our findings in future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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