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Original Article

Expression of long non-coding RNA LOC285194 and its prognostic significance in human pancreatic ductal adenocarcinoma

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Abstract: Introduction: Dysregulation of long non-coding RNAs (lncRNAs) plays critical roles in tumor progression. lncRNA LOC285194 was previously shown to be correlated with aggressive clinicopathological features and poor prognosis in several cancers. The aim of this study was to investigate relationship between LOC285194 expression and clinical outcomes in human pancreatic ductal adenocarcinoma (PDAC). Methods: Quantitative real-time PCR (qRT-PCR) assay was performed to detect the expression of lncRNA LOC285194 in human PDAC cells and tissue samples. The association of LOC285194 expression with clinicopathologic features was analyzed. Kaplan-Meier analyses were used to assess survival of patients. Univariate and multivariate analyses were performed using the Cox proportional hazards model to analyze the prognostic significance of LOC285194 expression. Results: Our data showed that the relative level of LOC285194 in PDAC cells was significantly lower than that in normal human pancreatic duct epithelial cell line. Also, the expression of LOC285194 in PDAC tissues was significantly lower than that in adjacent non-tumor tissues. By statistical analyses, low LOC285194 expression was observed to be closely correlated with clinical stage, lymphnode metastasis and liver metastasis. Kaplan-Meier survival analysis revealed that patients with low LOC285194 expression had a poor overall survival compared with the high LOC285194 group ($P < 0.05$). Univariate and multivariate analyses showed that low LOC285194 expression was an independent poor prognostic factor for PDAC patients. Conclusions: Our data provided the first evidence that reduced LOC285194 in PDAC tissues was correlated with tumor progression, and lncRNA LOC285194 might be a potential molecular biomarker for predicting the prognosis of patients.

Keywords: Long non-coding RNA, LOC285194, overall survival, pancreatic ductal adenocarcinoma

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in Western countries and the sixth in China, is characterized by aggressive invasion, early metastasis, and lack of specific symptoms [1, 2]. Despite recent advances in the diagnosis and treatment of this malignancy, the mortality rate of PDAC remains high and the 5-year overall survival rate is only 3%-5% [3]. Pancreatic carcinogenesis is known to be a multi-step process involving multiple genetic and epigenetic alterations [4]. Hence, a better understanding the molecular mechanisms involved in pancreatic carcinogenesis will be helpful for identification of new and effective biomarkers for early diagnosis and prognosis of PDAC.

Non-coding RNAs (ncRNAs), once regarded as "transcriptional noise", have recently been demonstrated to be functional molecules. These protein-non-coding sequences account for the majority of human genome while protein-coding genes only about 2% [5]. The ncRNAs include not only well-characterized microRNAs and other non-coding transcripts less than 200 nucleotides (nt) but also a large class of long (> 200 nt) ncRNAs (lncRNAs), which have emerged as a new layer of cell biology [6]. A growing volume of literature has indicated the vital roles of lncRNAs in cancer biology. Alterations in lncRNAs have been shown to exhibit pro-oncogenic or tumor-suppressive activities [7, 8].

LOC285194, also called LSAMP antisense RNA3, is an lncRNA consisting of 4 exons with more than 2 kb in length and is located at

osteo3q13.31. It was first reported to be within a tumor suppressor unit in osteosarcoma and depletion of this lncRNA promoted proliferation of normal osteoblasts through regulation of apoptotic and cell cycle transcripts as well as VEGF receptor 1 [9]. Recent studies showed that lncRNA LOC285194 was down-regulated in several cancers such as esophageal squamous cell carcinoma, and colorectal cancer, which play key roles in tumor development and progression [10, 11]. However, the relationship between expression of LOC285194 and the prognosis of patients with PDAC remains unclear.

In our study, qRT-PCR assay was performed to detect the expression of lncRNA LOC285194 in PDAC and adjacent non-tumor tissues. Moreover, the correlations of LOC285194 expression with clinicopathologic features of PDAC patients were statistically analyzed. Finally, we determined the potential role of LOC285194 in PDAC prognostic prediction. Our data showed that lncRNA LOC285194 was significantly downregulated in PDAC tissues and could be served as a potential molecular biomarker for the prediction of poor prognosis.

Materials and methods

Patients and specimens

A total of 85 primary PDAC and paired adjacent non-tumor tissues (located > 2 cm from the tumors) were collected from patients who undergone pancreatic surgical resection with informed consent at the Affiliated Tumor Hospital of Zhengzhou University, China, from 2004 to 2009. Both tumor and non-tumor tissues were confirmed by two experienced pathologists. None of these patients received neoadjuvant or adjuvant treatment before operation. Median follow-up time of surviving patients was 10.2 months. The study was approved by the Medical Ethical Committee of Zhengzhou University. Informed consent had been obtained from all of the patients for use of the clinical specimens.

Cell culture

A normal human pancreatic duct epithelial cell line (HPDE6-C7) and four PDAC cell lines (PANC-1, BxPC-3, AsPC-1, and PL45) were purchased from American Type Culture Collection (Manassas, VA). All cells were grown and maintained in DMEM (Gibico) medium at 37°C, 5% CO_2. Medium was supplemented with 10% fetal bovine serum (FBS, Gibico), 100 U/ml penicillin and 100 U/ml streptomycin.

Quantitative real-time PCR

Total RNA was extracted from cells or tissues with Trizol regent (Invitrogen), and the reverse transcription reactions were performed with random primers and a Moloney murine leukemia virus reverse transcriptase kit (Invitrogen) following the manufacturer's protocol. Real-time PCR was performed by using a standard SYBR Green PCR kit (Takara) protocol on Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems) according to the instructions. GAPDH was used as references for LOC285194. Each sample was analyzed in triplicate. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative quantitation of gene expression levels.

The primers of LOC285194 and GAPDH used for qRT-PCR are listed as follows: LOC285194 Forward 5'-TGTGCCTGTTTGACCTCTGA-3', LOC285194 Reverse: 5'-AGGAAGGATAAAAGACCGACCA-3'; GAPDH Forward: 5'-GTCAACGGATTTGGTCTGTATT-3', GAPDH Reverse: 5'-AGTCTTCTGGGTGGCAGTGAT-3'.

Statistical analysis

All statistical analyses were performed by using the SPSS 18.0 statistical software package. The data were presented as the mean ± SD. The association between LOC285194 expression level and clinicopathologic factors of the patients was analyzed using the chi-square test. Survival curves were plotted by the Kaplan-Meier method and compared by the log-rank test. The significance of different variables with respect to survival was analyzed using the multivariate Cox proportional hazards model. Differences were considered statistically significant when $P < 0.05$.

Results

Expression of lncRNA LOC285194 in human PDAC cells and tissue samples

To detect the expression of lncRNA LOC285194 expression in PDAC, qRT-PCR assay was performed to firstly detected the expression level

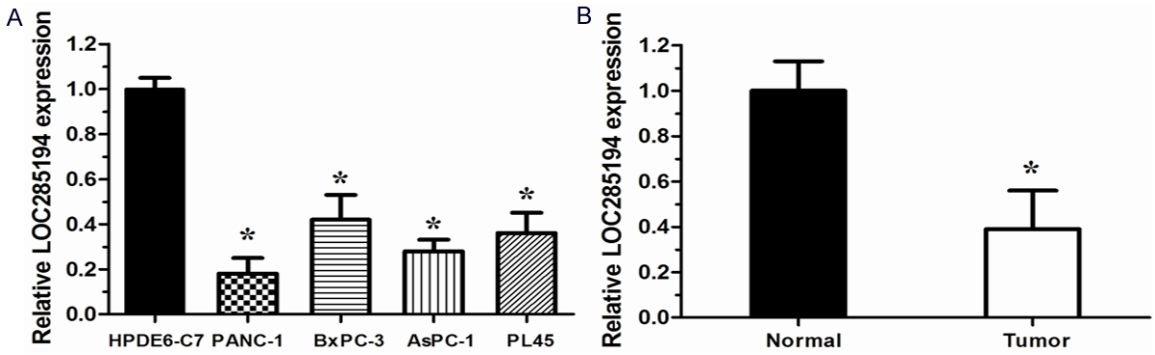

Figure 1. qRT-PCR analysis of lncRNA LOC285194 expression in PDAC cell lines and tissue samples. A. The expression level of LOC285194 in a normal human pancreatic duct epithelial cell line (HPDE6-C7) and PDAC cell lines (PANC-1, BxPC-3, AsPC-1, and PL45). B. The relative level of LOC285194 expression in PDAC tissues and non-tumor tissues. GAPDH was used as an internal control. Results are expressed as mean ± SD for three replicate determination $^{*}P < 0.05$.

Table 1. Association between lncRNA LOC285194 expression and clinicopathologic features of PDAC patients

Clinicopathological feature	Total	LOC285194 expression		*P* value
		Low	High	
Age (years)				0.536
< 60	37	16	21	
≥ 60	48	24	24	
Gender				0.124
Male	56	23	33	
Female	29	17	12	
Tumor size (cm)				0.976
< 2	11	6	5	
≥ 2	74	34	40	
Differentiation				0.306
Well	7	2	5	
Moderate + Poor	78	38	40	
Clinical stage				0.000
I + II	32	6	26	
III + IV	53	34	19	
T classification				0.625
T1 + T2	47	21	26	
T3 + T4	38	19	19	
Lymphnode metastasis				0.000
Absent	44	11	33	
Present	41	29	12	
Liver metastasis				0.001
Absent	70	27	43	
Present	15	13	2	

of LOC285194 in normal human pancreatic duct epithelial cell line (HPDE6-C7) and PDAC cell lines (PANC-1, BxPC-3, AsPC-1, and PL45). According to the results of qRT-PCR, the relative level of LOC285194 expression in PDAC cell lines was significantly lower than that in normal human pancreatic duct epithelial cell line ($P < 0.05$, **Figure 1A**). We next determined the expression of LOC285194 in 85 cases of PDAC tissues and adjacent non-tumor tissues, and results indicated that the mean level of LOC285194 expression in PDAC tissues was significantly lower than that in adjacent non-tumor tissues ($P < 0.05$, **Figure 1B**).

Correlations of lncRNA LOC285194 expression with clinicopathologic features of PDAC patients

To further assess the correlations of LOC285194 expression with clinicopathologic features of PDAC patients. The relative expression of LOC285194 were divided into two groups based on the mean value (0.37): High LOC285194 expression group had LOC285194 expression levels more than mean value and low LOC285194 expression group had LOC285194 expression levels less than mean value. Then, the correlations of LOC285194 expression with clinicopathologic features of patients were statistically analyzed. As shown in **Table 1**, low LOC285194 expression was observed to be closely correlated with advanced clinical stage, higher incidence of lymphnode metastasis and liver metastasis ($P < 0.05$). However, there were no significant correlations between LOC285194 expres-

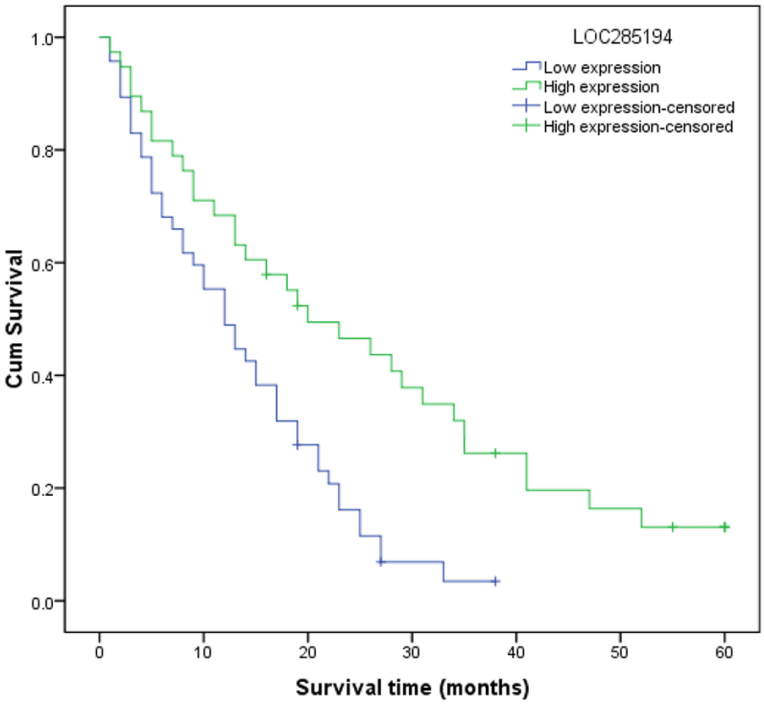

Figure 2. LncRNA LOC285194 expression is correlated with overall survival in patients with PDAC. Kaplan-Meier curve for overall survival in patient tissues with low versus high LOC285194 expression. Corresponding *P* values analyzed by log-rank test.

sion and other clinicopathologic factors including age, gender, tumor size, differentiation and T classification ($P > 0.05$).

Correlations of lncRNA LOC285194 expression with overall survival of PDAC patients

To further investigate the correlation of LOC285194 expression with survival of PDAC patients, Kaplan-Meier analyses were performed. As shown in **Figure 2**, the 5-year overall survival of low LOC285194 expression group was significantly shorter than that of high LOC285194 expression group ($P < 0.05$). Our results demonstrated that downregulation of LOC285194 might be correlated with poor survival of PDAC patients.

Univariate and multivariate analyses of prognostic variables in PDAC patients

To further determine the prognostic significance of LOC285194 expression for PDAC patients, survival data were obtained for each patient and univariate and multivariate analyses were performed (**Table 2**). Univariate Cox regression analysis showed that clinicopathological variables including clinical stage, lymphnode metastasis, liver metastasis, and LOC285194 expression were significantly associated with overall survival ($P < 0.05$). Also, to evaluate whether low LOC285194 expression (low vs. high) might be as an independent predictor for overall survival of PDAC patients, multivariate Cox regression analyses were performed. Along clinical stage, lymphnode metastasis, and liver metastasis ($P < 0.05$), low LOC285194 expression was an independent molecular biomarker for predicting of the poor overall survival of PDAC patients (RR: 2.415, 95% CI: 1.208-7.073, $P = 0.009$).

Discussion

Accurate prediction of the prognosis for the individual PDAC patient is of great importance, and molecular biomarkers that could be served as prognostic factors would be useful in determining an individualized treatment plan for a PDAC patient [12]. However, the biomarkers used in this tumor group today are not satisfactory [13], and it is needed to exploit additional markers to fine-tune this process.

LncRNAs are a new class of transcripts recently discovered to be pervasively transcribed in human genome and play a critical role in epigenetic regulation [14]. For example, Gupta's study showed HOTAIR expression was low in normal breast epithelia but high in primary breast cancer as well as metastatic lesions, HOTAIR expression level was a powerful predictor of patient outcomes [15]. Zhang's result indicated that lncRNA ANRIL upregulated in human gastric cancer tissues and correlated with a higher TNM stage and tumor size. What's more their data indicated that ANRIL expression served as an independent predictor for overall survival of gastric cancer patient [16]. Xie's data showed SPRY4-IT1 levels were significantly higher in esophageal squamous cell carcinoma (ESCC) tissues and cells than in adjacent non-tumor tissues and non tumorigenic

Table 2. Univariate analyses of different prognostic factors in PDAC patients

Clinicopathological feature	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	*P*	Risk ratio	95% CI	*P*
Age (years)	1.189	0.351-1.916	0.274			
≥ 60 vs. < 60						
Gender	1.302	0.571-2.026	0.339			
Male vs. Female						
Tumor size	1.973	0.574-2.936	0.203			
≥ 2 cm vs. < 2 cm						
Differentiation	1.475	0.261-2.847	0.537			
Moderate + Poor vs. Well						
T classification	2.644	0.735-4.286	0.161			
T3 + T4 vs. T1 + T2						
Liver metastasis	3.102	0.461-6.019	0.015	2.531	0.744-5.196	0.013
Present vs. Absent						
N classification	3.187	1.649-7.247	0.019	2.706	1.584-6.216	0.008
Present vs. Absent						
Clinical stage	2.588	1.391-5.711	0.008	2.217	1.298-4.879	0.005
III + IV vs. I + II						
LOC285194	2.773	1.394-6.917	0.011	2.415	1.208-6.073	0.009
Low vs. High						

esophageal epithelial cells, and the ESCC patients with higher SPRY4-IT expression had an advanced clinical stage and poorer prognosis than those with lower SPRY4-IT1 expression [17]. These data demonstrated the potential oncogenic or tumor suppressor role of lncRNAs. However, the relationship between lncRNA and cancer patient prognosis remains largely unknown.

Here we reported lncRNA LOC285194, which was previously shown to function as a tumor suppressor in osteosarcoma and was significantly down-regulated in 43 osteosarcoma tumor samples and 5 osteosarcoma cell lines [9]. Tong et al. found that LOC285194 expression was significantly down-regulated in ESCC tumor tissues, the patients with low expression of LOC285194 had a decreased disease free survival and overall survival. Which indicated that LOC285194 could serve as a molecular marker to predict the clinical outcome of ESCC patients after surgery [10]. Qi et al. showed LOC285194 was significantly lower in colorectal cancer tissues and correlated with tumor size, tumor stage, distant metastasis. Patients with low LOC285194 expression had a poor disease free survival. Which indicate that LOC285194 might be a novel prognostic indicator in colorectal cancer and may be a potential target for diagnosis and gene therapy [11]. Liu et al. demonstrated that LOC285194 is a p53 transcription target, ectopic expression of LOC285194 inhibits tumor cell growth both in vitro and in vivo. And LOC285194-mediated growth inhibition is in part due to specific suppression of miR-211 [18]. However, the association between LOC285194 expression and the clinicopathologic features and patient prognosis in PDAC is unknown.

In this study, we suggested for the first time that the relative level of LOC285194 in PDAC cell lines was significantly lower than that in normal human pancreatic duct epithelial cell line. At the same time, we compared the expression of LOC285194 in PDAC tissues and adjacent non-tumor tissues, and showed that the expression of LOC285194 in PDAC tissues was also significantly lower than that in adjacent non-tumor tissues. Then, we investigated the clinicopathologic significance of LOC285194 expresssion in PDAC. Statistical analyses demonstrated that low LOC285194 expression was significant correlated with advanced clinical stage, higher incidence of lymphnode metastasis and liver metastasis. These data implied that downregulation of LOC285194 might play critical roles in PDAC progression and development. Furthermore, we found LOC285194 ex-

pression was observed to be significantly associated with overall survival of PDAC patients. Kaplan-Meier analysis of overall survival showed that patients with low LOC285194 expression tended to have a significantly shorter overall survival than did those with high LOC285194 expression. Cox proportional hazards model proved that low LOC285194 was an independent prognostic marker for predicting the poor prognosis of PDAC patients. Thus, lncRNA LOC285194 expression could be used as a molecular prognostic factor to identify patients who are more likely to have higher risk of death.

Taken together, the current study showed that downregulation lncRNA LOC285194 was associated with tumor progression and poor prognosis in PDAC and was identified for the first time as an independent poor prognostic factor for PDAC patients, suggesting that LOC285194 expression may serve as a novel prognostic biomarker and a potential molecular therapeutic target in PDAC.

Disclosure of conflict of interest

None.

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