European Review for Medical and Pharmacological Sciences 2016; 20: 2230-2234

Association of LncRNA HMlincRNA717 with prognosis in pancreatic cancer

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Abstract. – OBJECTIVE: The aim of this study was to investigate the expression of LncRNA HMlincRNA717 in human pancreatic cancer and its correlation with clinicopathological features

PATIENTS AND METHODS: Using reverse transcription quantitative polymerase chain reaction, HMlincRNA717 expression was detected in primary pancreatic cancer tissues. The correlation of HMlincRNA717 with clinicopathological features and prognosis were also analyzed. Survival analysis was performed using the Kaplan-Meier method and Cox's proportional hazards model.

RESULTS: The expression of HMlincRNA717 was significantly decreased in pancreatic cancer tissues compared with paired adjacent normal tissues ($p < 0.01$). It was also proved that HMlincRNA717 expression was to be associated with clinical stage ($p = 0.001$), tumor size ($p < 0.001$), lymph node metastasis ($p = 0.003$), and distant metastasis ($p < 0.001$) in pancreatic cancer patients. Significantly shorter 5-year overall survival (OS) were observed in patients with lower expression of the HMlincRNA717 ($p < 0.01$). Multivariate analysis showed that decreased HMlincRNA717 expression was a poor independent prognostic factor for pancreatic cancer patients.

CONCLUSIONS: Our findings showed that the expression of lncRNA HMlincRNA717 was downregulated in pancreatic cancer and associated with overall survival, suggesting that HMlincRNA717 could be a potential prognostic biomarker for pancreatic cancer progression.

Key Words:
Long non-coding RNA, HMlincRNA717, Pancreatic cancer, Prognosis.

Introduction

Pancreatic cancer is one of the most aggressive human malignant tumors and a leading cause of cancer-related mortality around the world[1,2]. Despite a large number of efforts and improvements in surgery and perioperative management, the 5-year survival rate is still < 5%[3,4]. Extensive invasion and distant metastasis are the most reason of the high mortality rate of pancreatic cancer. Unfortunately, most pancreatic cancer patients were diagnosed at an advanced stage because of lack of early symptoms. Therefore, clinical indicators that accurately predict pancreatic cancer progression and prognosis are essential for improving patient survival.

The long non-coding RNAs (lncRNAs) are defined as endogenous cellular RNAs with length longer than 200 nucleotides[5]. Accumulation data suggests that lncRNAs may play important roles in cellular development, differentiation, and many other biological processes[6,7]. The aberrant expressions of lncRNAs have been linked with many types of cancer[8-10]. Furthermore, more and more lncRNAs have been found to play a critical role in pancreatic cancer[11,12]. Those results pointed out that lncRNAs plays a significant role in the tumorigenesis of pancreatic cancer.

LncRNA HMlincRNA717 is a long intergenic non-coding RNA (lincRNA) with 818 nucleotides in length and the gene which is located at 18p11.228. As a newly found LncRNA, little studies were reported about the effect of HMlincRNA717 in cancer. Shao et al[13] found that low HMlincRNA717 expression levels were correlated with distal cancer metastasis, venous invasion, and nervous invasion. However, the clinical and prognostic significance of lncRNA HMlincRNA717 expression in pancreatic cancer has not been reported yet. Here, we focus on the pathological role of lncRNA HMlincRNA717 in patients with pancreatic cancer.

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Patients and Methods

Patients and Tissue Samples

The study was approved by the Research Ethics Committee of The First Affiliated Hospital of Zhejiang University. Informed consent was obtained from all patients in writing. All specimens were made anonymous and handled according to the ethical and legal standards.

A total of 150 prostate cancer patients who underwent radical prostatectomy were included from Department of Radiotherapy, The First Affiliated Hospital of Zhejiang University ranged from 2005 to 2011. None of the patients received any kinds of therapy prior to the surgery. The mean age of this study population was 48.5 years (range: 30-76 years). There are 98 males and 52 females. Confirmed histopathologically, all samples were immediately frozen and stored in liquid nitrogen. The clinicopathological characteristics of the pancreatic cancer patients are summarized in Table I.

Quantitative Teal-Time RT-PCR.

RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration of RNA was determined using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was synthesized from 5 ng of total RNA using the Taqman miRNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Then real-time PCR was performed with the Applied Biosystems prism 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). U6 small nuclear RNA was used as an endogenous control. Each sample was measured in triplicate, and the relative amount of HMlincRNA717 to U6 was calculated using the equation $2^{-\Delta Ct}$, where $CT = (CT^{miR\text{-}124}\text{-}CT^{U6})$.

Statistical Analysis

All statistical analyses were carried out using the software of SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SD. The correlations were analyzed using the Student's *t*-test, the Chi-square test and analysis of variance (ANOVA). Survival curves were estimated based on the Kaplan-Meier method. The levels of statistical significance were set at least at $p < 0.05$ (two-sided).

Results

Expression of HMlincRNA717 in Pancreatic Tissues

To test the effect of lncRNA HMlincRNA717 on tumor progression, the level of HMlincR-

Table I. The relationship between HMlincRNA717 expression and the clinicopathological characteristics.

Characteristics	n	High expression	Low expression	p
Age (year)				0.500
< 60	67	31	36	
≥ 60	83	43	40	
Gender				0.210
Female	52	22	30	
Male	98	52	46	
Clinical stage				0.001
Early stages (≤ IIa)	92	55	37	
Advanced stages (> IIa)	58	19	39	
Tumor size (cm)				< 0.001
< 2	81	51	30	
≥ 2	69	23	46	
Lymph node metastasis				0.003
Negative	81	49	32	
Positive	69	25	44	
Distant metastasis				< 0.001
Absent	124	68	56	
Present	26	6	20	
Differentiation				0.325
Well	16	5	11	
Moderate	74	36	38	
Poor	60	33	27	

NA717 was detected in 150 paired pancreatic cancer tissues and adjacent normal tissues by qRT-PCR. Compared with non-cancerous prostate tissues, tissue levels of HMlincRNA717 expression were significantly decreased in pancreatic cancer patients ($p < 0.01$, Figure 1).

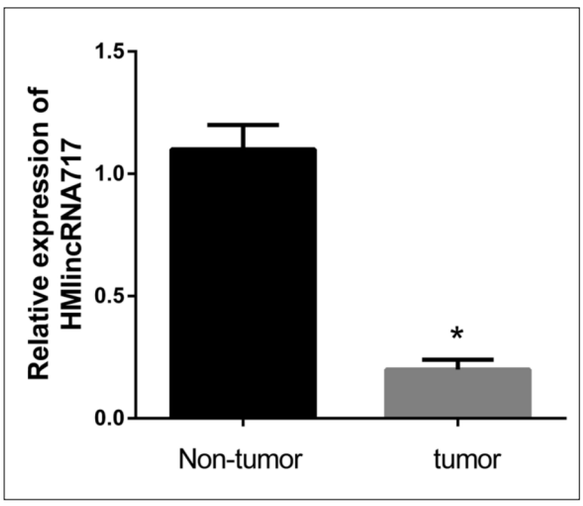

Figure 1. Expression of HMlincRNA717 in Pancreatic tissues and matched non-tumor tissues was examined by qRT-PCR. *$p < 0.05$.

Association of HMlincRNA717 Expression With Clinicopathological Parameters of Pancreatic Cancer Patients

We further explored the association between the expression of lncRNA MALAT1 and clinicopathological characteristics of pancreatic cancer. Pancreatic cancer tissue samples were classified into two groups based on the median value of relative HMlincRNA717. The clinicopathological factors were compared between the two groups (Table I). Low lncRNA HMlincRNA717 expression was observed to be closely correlated with clinical stage ($p = 0.001$), tumor size ($p < 0.001$), lymph node metastasis ($p < 0.003$), and distant metastasis ($p < 0.001$) in pancreatic cancer patients. However, there were no significant correlations between HMlincRNA717 expression and other clinicopathologic features, such as age, or grade.

Relationship Between Clinicopathologic Features, HMlincRNA717 Expression, and pancreatic Cancer Patients' Survival: Univariate survival Analysis

We next performed survival analysis to evaluate whether HMlincRNA717 expression had the prognostic potential for overall survival (OS) of pancreatic cancer patients with clinical follow-up information. Survival curves were constructed by Kaplan-Meier method and compared by the log-rank test. The overall survival was significantly lower in patients with lower HMlincRNA717 expression than in patients with higher HMlincRNA717 expression ($p = 0.004$, Figure 2). Furthermore, we also found that down-expression of lncRNA HMlincRNA717 showed unfavorable prognosis in pancreatic cancer patients, regardless of clinical stage, tumor size, lymph node metastasis, and distant metastasis. Finally. HMlincRNA717 expression level could develop as a powerful independent prognostic factor in pancreatic cancer patients.

Table II. Cox proportional regression analysis for assessing the correlation of HMlincRNA717 expression with the prognosis of pancreatic cancer.

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Age (year) < 60 *vs.* ≥ 60	0.772	0.512-1.657	0.469			
Gender female *vs.* male	1.325	0.616-1.559	0.581			
Clinical stage						
Early stage (≤ IIa) vs. advanced stage (> IIa)	2.236	1.448-3.471	0.003	1.329	0.512-2.771	0.325
Tumor size (cm) < 2 *vs.* ≥ 2	2.452	1.617-3.192	< 0.001	2.558	1.417-3.261	0.001
Lymph node metastasis negative vs. positive	2.261	1.514-3.369	0.002	1.771	0.448-2.237	0.148
Distant metastasis absent vs. present	3.359	1.551-6.692	0.003	1.517	0.581-3.239	0.212
Differentiation well *vs.* moderate *vs.* poor	1.223	0.652-1.458	0.331			
HMlincRNA717 low *vs.* high	2.791	1.238-3.228	< 0.001	1.349	1.236-3.027	0.013

HR hazard ratio, 95% CI 95% confidence interval.

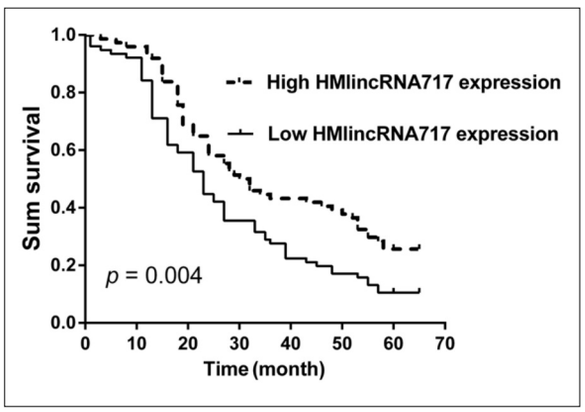

Figure 2. Decreased lncRNA HMlincRNA717 expression predicts a poor prognosis in pancreatic cancer patients.

Discussion

Pancreatic cancer is a highly malignant tumor with a high mortality because of late clinical presentation and lack of effective early detection measures[14]. Although a growing number of novel treatment strategies have been developed for pancreatic cancer, to our disappointment, therapeutic outcomes remained poor[15]. Therefore, it is urgent to find new biomarkers, which are able to identify specific patients who may benefit from aggressive therapies and predict the prognosis of pancreatic cancer LncRNAs have been implicated as master regulators of many important biological processes ranging from cell growth and apoptosis, to cancer development[16]. Numerous studies have demonstrated that many different tumors involve the dysregulation of lncRNAs expression, including pancreatic cancer. For example, Yang et al[17] reported that lncRNA PVT1 was not only over-expression but could promote the progress of NSCLC and impact the prognosis of the cancer patients. Choudhry et al[18] showed that high tumor NEAT1 expression confered a poor outcome in breast cancer patients. Tuo et al[19] revealed that UCA1 was a novel oncogenic lncRNA, which negatively regulated by miR-143 in breast cancer. Kim et al[20] showed that lncRNA Hox transcript antisense intergenic RNA (HOTAIR) expression was elevated in pancreatic cancer tissues in comparison to non-cancerous tissue and was correlated with the more aggressive status of pancreatic cancer. Moreover, Pang et al[12] reported that overexpression of long non-coding RNA MALAT1 is correlated with clinical progression and unfavorable prognosis in pancreatic cancer. Xie et al[21] found that LncRNA HMlincRNA717 is down-regulated in non-small cell lung cancer and associated with poor prognosis[21], suggesting that LncRNA HMlincRNA717 may serve as a tumor suppressor in tumors. However, to our knowledge, the roles of lncRNA HMlincRNA717 in pancreatic cancer are still unclear.

We firstly explored the expression of lncRNA HMlincRNA717 in pancreatic cancer tissues. Our result showed that the expression of HMlincRNA717 was reduced significantly, compared with adjacent normal pancreatic tissues. Moreover, lncRNA HMlincRNA717 was positively associated with clinical stage, tumor size, lymph node metastasis, and distant metastasis in pancreatic cancer patients. Kaplan-Meier analysis of clinical survival showed that low lncRNA HMlincRNA717 expression was associated with poor clinical survival of patients with pancreatic cancer. More importantly, according to multivariate analysis, lncRNA HMlincRNA717 was an independent poor prognostic factor for pancreatic cancer patients.

Conclusions

Our data suggested that lncRNA HMlincRNA717 down-regulation was associated with poor prognosis in pancreatic cancer. lncRNA HMlincRNA717 may be a promising biomarker and a therapeutic target for pancreatic cancer. The underlying mechanisms of the heterogeneous expression levels need to be extensively investigated in the future.

Acknowledgements

This study was supported by The program of Department of Education of Zhejiang province (grant no. Y201329085). The authors kindly acknowledge and thank this institutions for their support throughout this study.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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