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Downregulation of long non-coding RNA LINC01133 is predictive of poor prognosis in colorectal cancer patients

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Abstract. – OBJECTIVE: This study aims to investigate long non-coding RNA LINC01133 (LINC01133) expressions in colorectal cancer (CRC) patients, and discuss its correlation with CRC clinicopathological features and prognosis.

PATIENTS AND METHODS: qRT-PCR was performed to measure expression levels of LINC01133 in CRC tissues. The chi-square test was used to assess LINC01133 expression with respect to clinicopathological parameters. Kaplan-Meier analysis and the log-rank test were performed to identify survival differences in CRC patients. Univariate and multivariate analysis were performed using the Cox proportional hazard analysis.

RESULTS: LINC01133 was significantly down-regulated in CRC tissues compared to normal tissue samples ($p < 0.001$), and a low expression of LINC01133 was found to be significantly associated with lymph node metastasis ($p = 0.004$), distant metastasis ($p = 0.043$), N classification ($p = 0.022$) and TNM stage ($p = 0.011$). Moreover, Kaplan-Meier survival analysis revealed that high LINC01133 expression predicted significantly better overall survival ($p = 0.0093$). Finally, multivariate analysis results indicated that LINC01133 was an independent prognostic factor in CRC.

CONCLUSIONS: Our results indicated that reduced LINC01133 expression contributed to CRC metastasis and poor prognosis. Thus, LINC01133 might serve as a promising biomarker for prognosis of CRC.

Key Words:
Long non-coding RNA LINC01133, Colorectal cancer, Prognosis.

Introduction

Cancer is a major age-related disease worldwide. Each year, more than 1.5 million people are diagnosed[1]. Colorectal cancer (CRC) is one of the leading causes of cancer death worldwide, with an estimated over 1 million new cases and more than half million deaths in 2012[2,3]. At present, surgical resection remains the main treatments for patients with CRC[4]. Although the encouraging advances have been achieved in population screen and therapeutic techniques, the 5-year survival rate for metastatic CRC is only 10-15%[5]. Therefore, many researchers devote themselves to explore new biomarkers for the diagnosis, prognosis, and treatment of CRC.

Long noncoding RNA (lncRNA), > 200 nucleotides in length, is a type of noncoding RNA molecule that is often deregulated in a wide variety of diseases, including cancer[6]. Accumulating evidence has demonstrated that lncRNAs participate in a wide range of biological processes such as proliferation, differentiation, apoptosis[7,8]. Indeed, several studies reported that lncRNAs related to CRC development and progression by regulating cell growth and metastasis[9,10]. However, there are still many lncRNAs waiting for the investigation and elucidating about their potential biological or clinical functions in CRC.

Recently, LINC01133 was identified as a novel lncRNA by Zhang et al[11]. They found that LINC01133 was upregulated in lung squamous cell cancer. On the contrary, Kong et al[12] showed that LINC01133 was downregulated in CRC. These findings suggested that LINC01133 played a different role in the different type of tumors. In the present study, we aimed to explore the clinical significance of LINC01133 expression in CRC.

Patients and Methods

Patients and Tissue Samples

Tumor tissues with paired adjacent normal tissues were obtained from 187 CRC patients at

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the Department of Clinical Laboratory, Linyi People's Hospital from 2004 to 2005. The diagnosis was confirmed by two pathologists, and the cancer staging was determined based on pathological findings according to the American Joint Committee on Cancer (AJCC). Ethics Committee approved all protocols, according to the Declaration of Helsinki. Written informed consent was obtained from all patients. The specimens were handled anonymously according to ethical and legal standards. Additional patient characteristics are listed in Table I. No patients received chemotherapy or radiotherapy before surgery.

RNA Extraction and Quantitative Real-Time PCR

Total RNA from cancerous and noncancerous specimens was extracted with the Trizol reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China). QRT-PCR reactions were performed by using an ABI7500 system (Applied Biosystems, Foster City, CA, USA) and SYBR Green PCR Master Mix (TaKaRa). All qRT-PCR reactions were performed in duplicate The LINC01133 primers were:5'-GGAGCGAGATCCCTCCAAAAT-3' (sense) and 5'-GGCTGTTGTCATACTTCTCATGG-3' (antisense). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control, and the relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Statistical analysis was performed using the SPSS statistical software package (standard version 18.0, SPSS Inc., Chicago, IL, USA). All values were expressed as mean ± SD. The χ^2-test was used to analyze the correlation between the expression of LINC01133 and clinicopathologic parameters. Kaplan-Meier analysis was adopted to evaluate the effects of LINC01133 expression on the overall survival of patients with colorectal cancer. Cox regression (proportional hazard model) was adopted for multivariate analysis of prognostic factors. In all cases, *p*-value less than 0.05 was considered as statistical significant.

Table I. Associations between the expression of LINC01133 with clinicopathologic features in patients with colorectal cancer.

Clinicopathological fea-tures	Total	LINC01133 expression		*p*-value
		Low	high	
Age (years)				0.538
> 60	82	42	40	
≤ 60	105	50	55	
Sex				0.241
Female	73	32	41	
Male	114	60	54	
Tumor size (cm)				0.198
> 4	80	35	45	
≤ 4	107	57	50	
Lymph node metastasis				0.004
Absent	130	55	75	
Present	57	57	20	
Vascular invasion				0.518
Absent	146	70	76	
Present	41	22	19	
Distant metastasis				0.043
Absent	162	75	87	
Present	25	17	8	
Depth of invasion				0.137
T1 + T2	71	30	41	
T3 + T4	116	62	54	
N classification				0.022
Negative	53	19	34	
Positive	134	73	61	
TNM stage				0.011
I-II	97	39	58	
III-IV	90	53	37	

Results

Low Expression Level of LINC01133 in CRC Tissues

RT-qPCR was performed to detect the expression levels of LINC01133 in CRC tissues and matched normal tissues. It was observed that LINC01133 expression was significantly lower in 187 CRC tissues than that in adjacent colorectal tissues ($p < 0.001$, Figure 1).

Low LINC01133 Expression Correlates with Clinicopathological Parameters in CRC Patients

Next, we compared LINC01133 expressions in different clinical parameters. The relationships between clinical pathological characteristics and LINC01133 expression was shown in Table I. Chi-square analysis revealed that low expression of miR-203 was found to significantly correlate with lymph node metastasis ($p = 0.004$), distant metastasis ($p = 0.043$), N classification ($p = 0.022$) and TNM stage ($p = 0.011$). However, there were no significant associations between LINC01133 levels and pathological type or age (Table I). These data indicated that downregulation of LINC01133 might play a critical role in CRC progression.

LINC01133 Expression is Associated with Poor Survival of CRC Patients

During follow-up, we analyzed the clinical information from the CRC patients. In total, 102 of the 187 CRC patients died (59 from the low LINC01133 expression group and 43 from the high LINC01133 expression group). Next, we performed the log-rank test and Kaplan-Meier analysis to analyze the association between LINC01133 expression level and clinical information in these CRC patients. The results showed that patients with low LINC01133 expression had shorter mean months of overall survival than did patients with high LINC01133 expression ($p = 0.0093$, Figure 2). Moreover, we performed univariate Cox proportional hazards regression model analysis, and the results indicated that relative LINC01133 expression level, lymph node metastasis, N classification and TNM stage were correlated with an overall survival rate in ovarian cancer patients (all $p < 0.05$, Table II). Finally, decreased LINC01133 expression was an independent poor prognostic factor for CRC patients through multivariate analysis ($p = 0.007$, Table II).

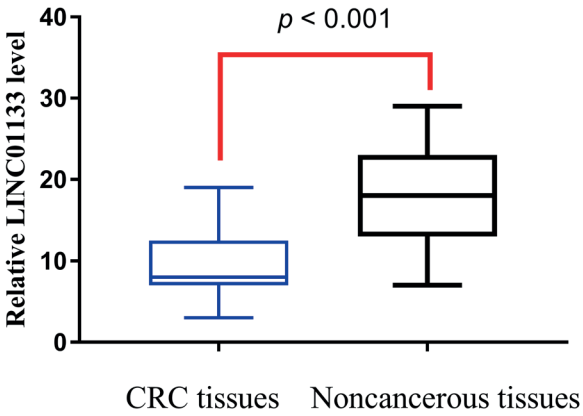

Figure 1. Relative expression levels of LINC01133 in 187 paired human CRC and normal colorectal tissues were measured by qRT-PCR. The TUSC7 expression was decreased in CRC tissues compared with colorectal tissues ($p < 0.001$).

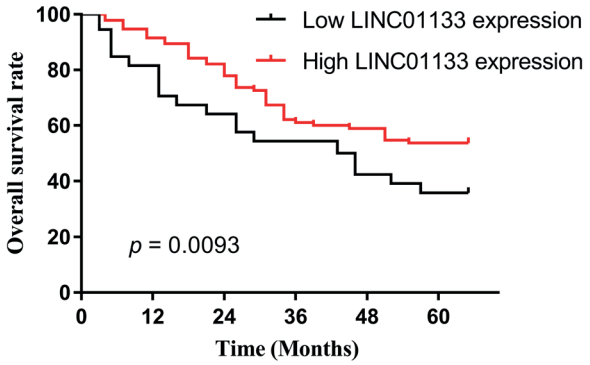

Figure 2. Kaplan-Meier analysis for the overall survival of patients with CRC and different levels of LINC01133 ($p < 0.05$, log-rank test).

Discussion

CRC is one of the most frequent causes of cancer-related deaths worldwide[13]. Exploring new prognosis biomarker and pivotal molecular-associated with progression of CRC is very important. Many researchers focused on lncRNAs. Some lncRNAs have been investigated and found to be associated with the behaviours and prognosis of different tumors[14,15]. For instance, previous reports showed that high tumor lncRNA MALAT1 expression conferred a poor outcome in several cancer patients including osteosarcoma[16], ovarian cancer[17] and clear cell renal cell carcinoma[18]. LncRNA HOTTIP overexpression was found to serves as an unfavorable prognosis predictor for

Table II. Univariate and multivariate analyses of prognostic factors in colorectal cancer.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age (years)	1.331	0.671-2.893	0.341	–	–	–
Sex	1.512	0.831-2.238	0.571	–	–	–
Tumor size	1.993	0.568-3.315	0.236	–	–	–
Lymph node metastasis	3.371	1.365-5.566	0.003	2.783	1.158-4.778	0.001
Vascular invasion	1.671	0.862-2.773	0.341	–	–	–
Distant metastasis	1.783	0.732-3.554	0.117	–	–	–
Depth of invasion	2.663	0.617-4.225	0.096	2.251	0.477-3.889	0.067
N classification	3.115	1.256-6.655	0.011	3.224	1.673-8.114	0.006
TNM stage	1.983	1.114-4.422	0.008	1.677	1.016-3.776	0.012
LINC01133	3.663	1.562-6.411	0.004	3.311	1.334-5.616	0.007

hepatocellular carcinoma and CRC patients[19,20]. These results revealed that lncRNAs have the potential to become new prognosis biomarker for CRC.

The lncRNA LINC01133, 1154nt in length, is located in chromosome1q23.2.As a newly identified lncRNA, only a few study reported the effect of LINC01133 in tumors. Zhang et al[11] found that the expression of lncRNA was upregulated in lung squamous cell cancer tissues compared to normal lung tissue and ectopic expression of LINC01133 in lung squamous cell cancer cells increased cell proliferation and clonal formation. Zang et al[21] showed that LINC0113 was increased in non-small cell lung cancer tissues and cell lines. Inhibition of LINC0113 led to a reduction of cell growth and motility. They further identified that LINC01133 serve as a tumor promoter through regulating KLF2. Recently, Kong et al[12] indicated that LINC01133 was down-regulated in CRC specimens as well as in cell lines. They further found that LINC01133 downregulation inhibited whereas LINC01133 upregulation promoted CRC migration and invasion in vitro. The results indicated that LINC01133 functioned as a tumor suppressor in CRC. However, whether LINC01133 could be recognized as reliable markers to predict the survival in patients with CRC has not been reported.

In the present investigation, we observed that LINC01133 exerted lower expression in CRC tissues comparing with adjacent normal tissues. Low LINC01133 expression level was observed to be closely correlated with lymph node metastasis, distant metastasis, N classification and TNM stage. To identify the prognostic value of the LINC01133 expression in CRC patients, we performed the Kaplan-Meier analyses to explore the association between the levels of LINC01133 expression and overall survival. Our results showed that the overall survival was significantly poor in low LINC01133 expression CRC patients. The results were consistent with these findings by Kong et al[12]. However, they didn't perform univariate and multivariate Cox regression analyses to determine whether LINC01133 expression level was independent factors for prognostic prediction in CRC patients. In our study, the results of univariate and multivariate analyses confirmed that low LINC01133 expression was an independent factor for the prediction of the 5-year overall survival of patients with CRC.

Conclusions

We observed that LINC01133 was associated with the progression of CRC, and patients with low LINC01133 level have a worse prognosis. The findings revealed that LINC01133 could be a promising biomarker and a therapeutic target for the treatment of CRC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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