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Long noncoding RNA LINC01510 is highly expressed in colorectal cancer and predicts favorable prognosis

J.-F. ZHOU, H.-G. WANG, T.-H. MA, W. YAN, S.-N. WU, Y.-T. SHI, X.-Z. YANG

Department of Gastroenterology, The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University, Huai'an, China

Abstract. – OBJECTIVE: Long noncoding RNAs (lncRNAs) have recently emerged as important regulators in governing fundamental biological processes, as well as in tumorigenesis. LncRNA LINC01510 (LINC01510) was recently shown to be involved in colorectal cancer (CRC); however, its role in CRC remains unknown. The objective of this study was to evaluate LINC01510 expression and its relevance to the prognosis of CRC.

PATIENTS AND METHODS: LINC01510 expression was detected in CRC tissues and cell lines by using quantitative real-time PCR (qRT-PCR). The correction between LINC01510 expression and clinical characteristics was evaluated with x^2-test. Survival curves and log-rank test were used to analyze patients' survival. A Cox proportional hazard model was constructed to evaluate the association of LINC01510 expression with overall survival and disease-free survival, respectively.

RESULTS: Here, we found that the levels of LINC01510 in CRC tissues were significantly higher than those in matched tumor-adjacent tissues. Moreover, high LINC01510 expression was observed to be closely correlated with histology/differentiation (p = 0.001), depth of invasion (p = 0.004) and TNM stage (p = 0.003). From the Kaplan-Meier survival curves, it was observed that patients with high expression of LINC01510 had shorter overall survival (p = 0.004) and disease-free survival (p = 0.000) as compared with the LINC01510-low group. In the multivariate analysis, high LINC01510 expression was an independent prognostic factor for both overall survival (p = 0.001) and disease-free survival (p = 0.001).

CONCLUSIONS: We demonstrated that low LINC01510 expression was associated with the progression of CRC and could serve as a potential independent prognostic biomarker for patients with CRC.

Key Words:
LINC01510, Long non-coding RNA, Colorectal cancer, Prognosis.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies and causes large mortalities around the world each year[1]. In China, the risk of CRC has significantly increased in the last 50 years due to inherited factors and lifestyle changes[2]. Although substantial progress has been made in the past decades, including surgical treatment, radiotherapy and chemotherapy, the long term survival of CRC patients is still poor[3,4]. Distant metastasis is the leading cause of cancer-related death among CRC patients[5]. The precise molecular mechanisms underlying the migration and invasion of CRC remain largely unknown. Thus, to find the biomarkers of CRC metastasis for its targeted therapy and prognosis is urgently required for clinical medicine.

Long noncoding RNA (lncRNA) belongs to a class of RNA molecular that is longer than 200 nucleotides without the function of encoding proteins, but it regulates gene expression at the chromatin modification, transcriptional, or post-transcriptional level[6,7]. The rapid development of human genomics has highlighted the important effect of lncRNAs in diverse biological processes of disease[8]. Previous studies[9,10] have showed that lncRNAs have important roles in a wide range of cellular processes, such as development, differentiation and determination of cell fate. Moreover, emerging evidence indicates that lncRNAs may play complex and extensive roles in promoting the development and progression of various cancer[11-13]. Up to date, more and more lncRNAs were identified as functional lncRNAs in various tumors, such as lncRNA AFAP1-AS1 in pancreatic ductal adenocarcinoma, lncRNA NEAT1 in breast cancer and lncRNA-CTD903 in CRC[14-16]. However, there are still a variety of lncRNAs waiting for investigation and elucidating about their potential biolog-

Corresponding Author: Xiao-Zhong Yang, MD; e-mail: yxz3176@163.com

ical or clinical functions in CRC. Long intergenic non-protein coding RNA 1510, an enhancer lncRNA (LINC01510), was recently reported to be a functional lncRNA which was demonstrated to be highly expressed in CRC by microarray analysis. Functionally, forced LINC01510 expression could promote CRC progression *in vitro*, indicating that it served as a tumor promoter in CRC[17]. However, there are no other studies reporting the function of LINC01510 in other tumors. In addition, the clinical significance of LINC01510 in CRC has not been reported. Thus, we firstly tried to explore the prognostic value of LINC01510 in CRC patients.

Patients and Methods

Patients and Tissue Specimens

A total of 153 clinical samples were obtained from patients with CRC and paired adjacent non-tumor bone tissue at Huai'an First People's Hospital, Nanjing Medical University. All the tumors were confirmed pathologically from the specimens obtained from surgery. None of the patients had received chemotherapy or radiation therapy prior to the surgery. Tumor classification and staging were according to the 2002 TNM system and the 2004 World Health Organization Classification of Tumours. All the CRC patients were followed for 60 months and a set of complete clinical data was recorded properly. Detailed information is listed in Table II. Following surgical removal, the tissue samples were immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction. The study was approved by the Research Ethics Committee of Huai'an First People's Hospital, Nanjing Medical University. Written informed consent was obtained from all patients.

Total RNA Extraction and qRT-PCR Analysis

Total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacture's protocol. The cDNA was synthesized from 2 ng of total RNAs with a Reverse Transcription Toolkit (Promega Corp., Madison, WI, USA). The expressions of LINC01510 were determined using astandard SYBR-Green method on ABI7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). 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. β-Actin was used as an internal control, and lncRNA ANRIL values were normalized to β-Actin. The relative expression fold change of mRNAs was calculated by the $2^{-\Delta\Delta CT}$ method. The primers used were shown in Table I.

Statistical Analysis

All data were analyzed SPSS 17.0 using the software package (SPSS Inc., Chicago, IL, USA). The differential expression of LINC01510 between glioma tissues and normal brain tissues was evaluated by independent sample *t*-test. The x^2 test was used to assess LINC01510 expression with respect to clinicopathological factors. The Kaplan-Meier curves were constructed, and the survival differences between groups were assessed using the log rank test. The significance of different variables with respect to survival was analyzed using the univariate and multivariate Cox proportional hazards model. Differences were considered statistically significant when p was less than 0.05.

Results

LINC01510 Expression Increases in CRC Tissues

To explore the clinical significance of LINC01510 in CRC, qRT-PCR assays were used to evaluate the expression of LINC01510 in CRC tissues and paired adjacent normal tissues. As shown in Figure 1, we found that the expression of LINC01510 was significantly higher in the CRC tissues compared with matched normal tissues ($p < 0.01$). Our findings indicated that upregulation of LINC01510 may play a role in the pathogenesis and development of CRC.

LINC01510 Upregulation Associates with Aggressive Clinical Parameters of Human CRC

We further analyzed the association between the expression of LINC01510 and clinicopathological characteristics of CRC patients. CRC

Table I. Primer sequences used for RT-qPCR.

Gene	Primer 5′-3′
LINC01510 (Forward)	CTGTGGAAGTTTGAGTGAC
LINC01510 (Reverse)	TTCATCTATCCTCCTGCT
β-Actin (Forward)	CTCTTCCAGCCTTCCTTCCT
β-Actin (Reverse)	GACAGCACTGTGTTGGCGTA

Table II. Association of miR-1256 expression with clinicopathological features of CRC.

Clinicopathological features	No. of cases	LINC01510 expression		*p*-value
		High	Low	
Age				NS
≤ 60	84	40	44	
> 60	79	35	34	
Gender				NS
Male	94	44	50	
Female	59	31	28	
Tumor size				NS
≤ 5 cm	93	40	53	
> 5 cm	60	35	25	
Tumor site				NS
Colon	78	38	40	
Rectum	75	37	38	
Histology/differentiation				0.001
Well + Moderate	90	34	46	
Poor	63	41	22	
Depth of invasion				0.004
T1 + T2	95	38	57	
T3 + T4	58	37	21	
TNM stage				0.003
I-II	84	32	52	
III	69	43	26	

tissue samples were classified into the low-expression group (n = 78) and the high-expression group (n = 75) according to the median expression level of all retinoblastoma samples (median expression value 4.15). As shown in Table II, our results indicated that high LINC01510 expression was closely correlated with histology/differentiation (p = 0.001), depth of invasion (p = 0.004) and TNM stage (p = 0.003). However, there were no significant correlations of LINC01510 expression with other clinical features such as age, gender, tumor size, and tumor site ($p > 0.05$).

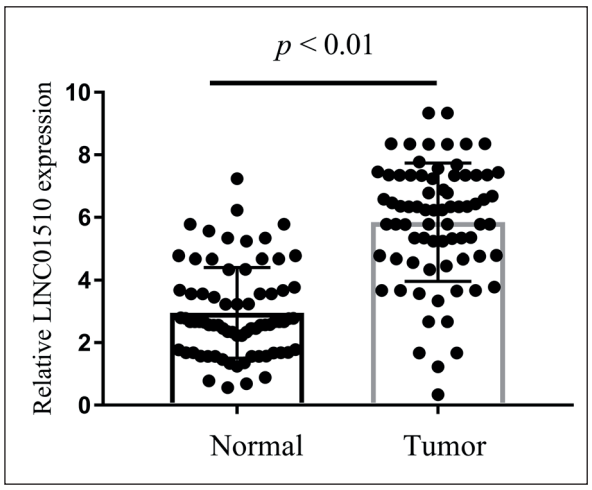

Figure 1. Relative expression levels of LINC01510 in 153 pairs of CRC specimens and adjacent noncancerous bone tissues were examined using qRT-PCR assay. β-Actin was served as internal controls and all experiments were performed in triplicate.

LINC01510 Expression was a Potential Independent Prognostic Marker for CRC Patients

The median follow-up period for the patients studied was 34 months, with a range of 6-60 months. To assess the correlation between LINC01510 expression and CRC patients' prognosis, Kaplan-Meier analysis was performed. We found that the patients with high expression of LINC01510 presented a poor overall survival (p = 0.004; Figure 2) and poor disease-free survival ($p < 0.05$; Figure 3) than those with low LINC01510 expression, indicating upregulation of LINC01510 predicted a poor prognosis in patients with CRC. Moreover, in a multivariate Cox model, our results revealed that LINC01510 expression was an independent poor prognostic factor for both 5-year overall survival (HR = 3.677, CI = 1.483-6.215, p = 0.001, Table III) and 5-year disease-free survival (HR = 4.144, CI = 1.739-5.893, p = 0.001, Table III) in CRC patients.

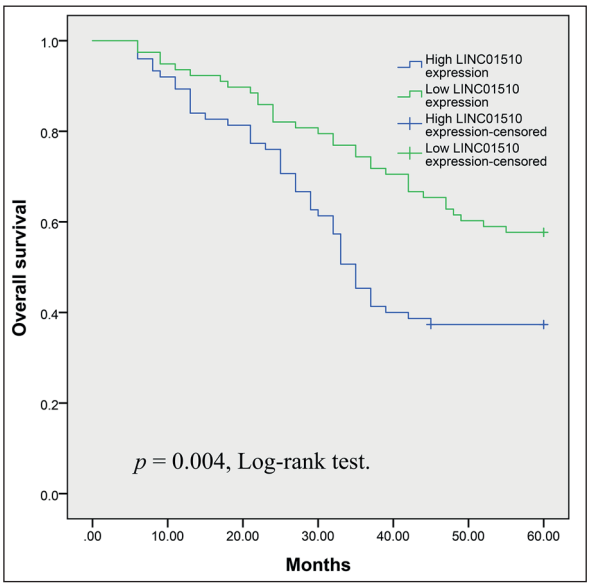

Figure 2. CRC patients with high LINC01510 expression had a significantly shorter overall survival than those with low LINC01510 expression ($p = 0.004$, log-rank test).

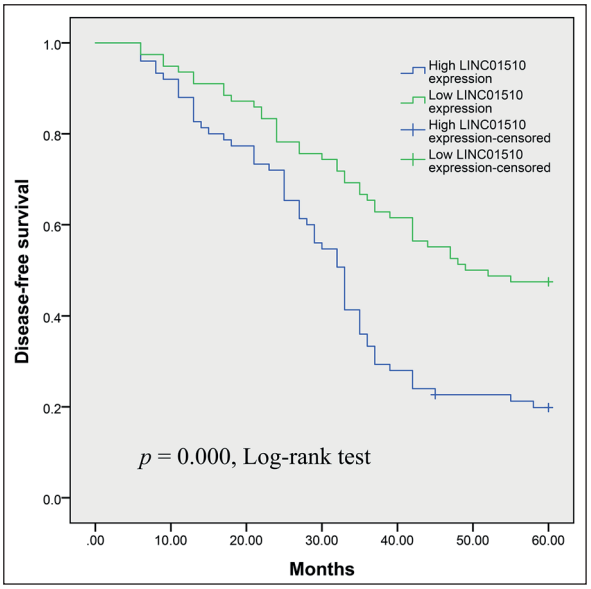

Figure 3. CRC patients with high LINC01510 expression had a significantly shorter disease-free survival than those with low LINC01510 expression ($p = 0.004$, log-rank test).

Discussion

Encouraging progress in diagnosis and cancer therapy has been achieved in the past decade. However, satisfactory therapeutic outcomes have not been achieved, and CRC remains a major public health concern[18]. In clinical practice, in order to achieve better treatment effect, it is urgently for us to find predictive and prognostic biomarkers to supply practical information[19,20]. Up to date, several clinicopathologic factors, such as TNM stage or depth of invasion, are important for the prognosis of CRC, However, these factors may not accurately estimate outcome because of heterogeneity in the CRC patient population[21]. In addition, several novel molecular biomarkers were proved to act as potential diagnostic and prognostic targeting[22,23]. However, they have insufficient sensitivity and specificity at early stage. Thus, developing novel reliable biomarkers is critical. Recent advances have implicated lncRNAs in the development and progression of CRC. For instance, Tang et al[24] reported that lncRNA AFAP1-AS1 expression was significantly up-regulated in CRC and its overexpression was associated with colorectal cancer patient survival. Functional assay indicated that knockdown of AFAP1-AS1 inhibited CRC cell proliferation, cell cycle and tumorigenesis *in vitro* and *in vivo*. Shan et al[25] found that in CRC, there is a correlation between lncRNA Linc00675 expression and metastatic stage. *In vitro* experiments revealed that overexpression of Linc00675 inhibited the proliferation, inva-

Table III. Multivariate analyses for progression-free survival and overall survival by Cox regression model.

Variable	Overall survival			Disease-free survival		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age (years)	1.213	0.665-1.932	0.315	-	-	-
Gender	1.442	0.715-2.231	0.163	-	-	-
Tumor size	0.933	0.453-2.428	0.188	-	-	-
Tumor site	1.344	0.933-2.218	0.113	-	-	-
Histology/differentiation	3.783	1.342-5.557	0.003	4.137	1.455-6.589	0.001
Depth of invasion	2.894	1.421-4.457	0.005	3.136	1.556-5.328	0.003
TNM stage	2.655	1.356-3.674	0.013	2.933	1.134-4.324	0.007
LINC01510 expression	3.677	1.538-5.239	0.001	4.144	1.739-5.893	0.001

sion and migration of CRC cells via acting on miR-942 and Wnt/β-catenin signaling. Zhu et al[26] reported that lncRNA FOXD2-AS1 was highly expressed in both CRC tissues and cell lines, and its suppressed CRC cell, proliferation, invasion and migration by regulating EMT and Notch signaling pathway. Cen et al[17] found that LINC01510 expression was significantly upregulated in CRC and associated with the advanced grade and stage. Functionally, overexpression of LINC01510 promoted the growth of colorectal cancer cells by modulating MET expression, indicating that LINC01510 may be a target for new therapies in CRC patients. However, the expression of LINC01510 needed to be further confirmed. In addition, the prognostic value of LINC01510 had not been investigated.

In this study, we initially detected the expression levels of LINC01510 in 153 pairs of primary CRC tissues as well as adjacent non-tumor tissues. We found that LINC01510 expression levels in tumors were higher than those in the corresponding normal tissues on the whole. In accordance with the clinical information of patients with pancreatic cancer, it was discovered that high LINC01510 expression was closely correlated with histology/differentiation, depth of invasion and TNM stage. In addition, Kaplan-Meier analysis with the log-rank test indicated that high LINC01510 expression had a significant impact on overall survival and disease-free survival. More importantly, according to multivariate analyses, LINC01510 overexpression was an independent unfavorable prognostic biomarker for CRC patients. These results highlight the clinical significance of LINC01510 in CRC patients and imply a potentially critical role for LINC01510 in predicting the progression of CRC patients.

Conclusions

We showed that LINC01510 may be a tumor promoter gene and may be associated with biological aggressiveness and poor prognosis in CRC. Due to the limited sample size in our study, more investigations would be needed to further verify the clinical significance of LINC01510 in CRC patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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