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LncRNALUADT1 is overexpressed in colorectal cancer and its expression level is related to clinicopathology

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Abstract. – OBJECTIVE: LncRNAs participate in the proliferation, apoptosis, and invasion of colorectal cancer. We aimed at investigating the uncovered effect of lncRNALUADT1 on colorectal cancer.

PATIENTS AND METHODS: The relative expression level of lncRNALUADT1 in tumor specimen was tested by Real-time quantitative PCR. The association of lncRNALUADT1 with clinical pathological data was analyzed by univariate, multivariate Cox and Kaplan-Meier curve.

RESULTS: LncRNALUADT1 expression was up-regulated in colorectal cancer, and correlated with tumor size, metastasis, and TNM staging. Both univariate analysis and multivariate test indicated that lncRNALUADT1 high expression, TNM staging, and lymph node metastasis were closely related. Moreover, high expression of lncRNALUADT1 suggested the poor overall survival of patients.

CONCLUSIONS: LncRNA LUADT1 might contribute to the development of colorectal cancer.

Key Words:
Colorectal cancer, lncRNALUADT1, Overall survival, Kaplan-Meier.

Introduction

Colorectal cancer is one of the most common malignancies worldwide. In the developed countries, nearly 1 million people suffer from colorectal cancer each year, with a disease-specific death rate of nearly 33%[1,2]. In China, with the improvement of people's living standard and the change of eating habits, the incidence rate of colorectal cancer is also on the rise[3]. Seriously, the trend shows that the incidence of colorectal cancer is getting younger. Long-chain non-coding RNAs (lncRNAs) are a class of RNA molecules longer than 200 nt, and participate in the biological influence of the epigenetic, transcriptional, post-transcriptional, translation and other aspects[4,5]. Besides, their abnormal expressions are associated with the occurrence and progression of many major diseases including malignant tumors[6,7]. Down-regulated lncRNA-MEG3 is able to be implicated for poor prognosis and upregulated gastric cancer cell proliferative ability[8]. Another study[9] reported that overexpression of lncRNA PVT1 can enhance the multidrug resistance processes. As a competing endogenous RNA, lncRNA FER1L4 can inhibit cell growth via targeting PTEN[10]. In esophageal adenocarcinoma, lncRNA HNF1A-AS1 has a role in cell proliferation and migration[11]. Low expression of long noncoding XLOC_010588 indicates a poor prognosis and promotes proliferation through upregulation of c-Myc in cervical cancer[12]. LncRNA LUAD transcript 1 (LUADT1) was first found in female non-smokers by Qiu et al[13]. Previous evidence also demonstrated that lncRNALUADT1 was able to inhibit p27 expression via bounding to SUZ12, a core component of the polycomb repressive complex 2 (PRC2). Both *in vivo* and *in vitro* results showed that LUADT1 knockdown can induce cell cycle arrest and inhibit cell growth. In our study, a preliminary study of lncRNALUADT1 was carried out to provide reference for the early diagnosis and prognosis evaluation of colorectal cancer.

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Table I. lncRNA LUADT1 expression and clinical features of patients with colorectal cancer.

Features	No.	lncRNA LUADT1		p
		High	Low	
No.	142	71	71	
Gender				0.614
Male	69	33	36	
Female	73	38	35	
Age (years)				0.313
< 60	66	30	36	
≥ 60	76	41	35	
Tumor size (cm)				0.001
< 5	82	31	51	
≥ 5	60	40	20	
Lymph nodes metastasis				0.000
Negative	87	30	57	
Positive	55	41	14	
TNM stage				0.000
I-II	85	24	61	
III-IV	57	47	10	

Patients and Methods

Tissue Specimens

A total of 142 patients with colorectal cancer with complete clinical data were enrolled in our hospital. The entire specimens were collected with the consent of patients that signed informed consent. This study was approved by the Ethics Committee of The Hangzhou Third Hospital. Meanwhile, the para-cancerous tissues > 2 cm from the edge of the cancer tissue were collected as control. Patients were discharged by telephone and outpatient follow-up including general information and clinical symptoms. The follow-up stated from the date of surgery or pathological biopsy to August 31, 2017.

qRT-PCR Assay

According to TaKaRa kit instructions (TaKaRa, Otsu, Shiga, Japan), total RNA was obtained by TRIzol reagent and was reversely transcribed to cDNA. Through using the synthesized cDNA as a template, lncRNALUADT1 and GAPDH (as an internal control) were amplified. qRT-PCR reaction system was 20 μL, the reaction conditions (35 cycles) were 94°C for 2 min, 94°C for 30 s, 58°C for 30 s, 72°C for 30 s. Each experiment was repeated 3 times. The primers used in this study were shown below: LUADT1, forward: TTTCCGTTCAGCAAATCCACAC, reverse: TTAGGTCCAGCACTGTTATCCA; GAPDH , forward, 5'-GTCAACGGATTTGGTCTGTATT-3', reverse, 5'-AGTCTTCTGGGTGGCAGTGAT-3'.

Statistical Analysis

The Statistical Product and Service Solutions 17.0 (SPSS Inc., Chicago, IL, USA) software was used for statistical analysis. The association of lncRNALUADT1 with clinical pathological data was analyzed by x^2-test, and Kaplan-Meier curve was plotted in survival analysis. *p<0.05.

Results

High lncRNA LUADT1 Was Expressed in Colorectal Cancer Tissues

The qRT-PCR technique was used to detect the expression of lncRNALUADT1 in tumor tissues and paracancerous tissues of the patients with colorectal cancer. As shown in Figure 1, the expression of lncRNALUADT1 was over-expressed in colorectal cancer tissues compared with that in the adjacent normal tissues. Thus, based on the abnormal expression of lncRNALUADT1, 142 colorectal cancer tissues were divided into high expression group (n=71) and low expression group (n=71). The median was regarded as cut off value. Furthermore, the analysis of clinical information suggested that aberrant expression of lncRNA LUADT1 was correlated with tumor size, TNM staging and lymph node metastasis. However, there was no association between lncRNA LUADT1 expression and other clinicopathological features (Table I).

Table II. Univariate analyses of lncRNA LUADT1 expression in 142 colorectal cancer patients.

Variables	Hazard ratio	CI (95%)	*p*
Gender	1.099	0.609-1.984	0.754
Age (years)	1.327	0.906-2.214	0.245
Tumor size (cm)	1.925	1.214-3.124	0.004
Lymph nodes metastasis	1.613	1.004-2.337	0.024
TNM stage	1.864	1.041-3.481	0.046

Table III. Multivariate analyses of lncRNA LUADT1 expression in 142 colorectal cancer patients.

Variables	Hazard ratio	CI (95%)	*p*
Gender	1.757	0.819-2.011	0.899
Age (years)	1.551	1.023-2.987	0.608
Tumor size (cm)	1.661	1.002-2.540	0.019
Lymph nodes metastasis	1.698	1.074-2.417	0.015
TNM stage	1.981	1.141-3.361	0.039

Univariate Analysis of lncRNA LUADT1 Expression and Clinicopathological Data

In addition, the univariate analysis showed that the over-expression of lncRNALUADT1 was closely associated with TNM staging, lymph node metastasis and tumor size in colorectal cancer ($p<0.05$), but not correlated with age and sex ($p>0.05$, Table II).

Multivariate Analysis of lncRNA LUADT1 Expression and Clinicopathological Data

As shown in Table III, multivariate analysis showed that the high expression of lncRNALUADT1, TNM staging and lymph node metastasis were closely related ($p<0.05$), but no significant correlation was found in age and gender ($p>0.05$). TNM staging, lymph node metastasis and lncRNA LUADT1 expression were independent risk factors.

Effect of lncRNA LUADT1 on the Prognosis of Patients with Colorectal Cancer

Finally, 142 cases of colorectal cancer were followed up for 5 years. The Kaplan-Meier curve survival analysis was used to examine the effect of lncRNA LUADT1 on the prognosis of patients with colorectal cancer. The Kaplan-Meier analysis showed that the higher expression of lncRNALUADT1 indicated the poorer survival of patients as relative to lower expression of lncRNA LUADT1 (Figure 2).

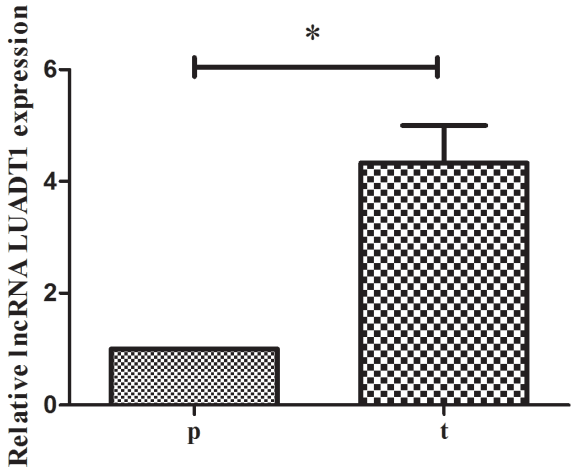

Figure 1. The expression of lncRNA LUADT1 is highly expressed in colorectal cancer. P: para-cancerous tissues; t: tumor tissues; *$p<0.05$.

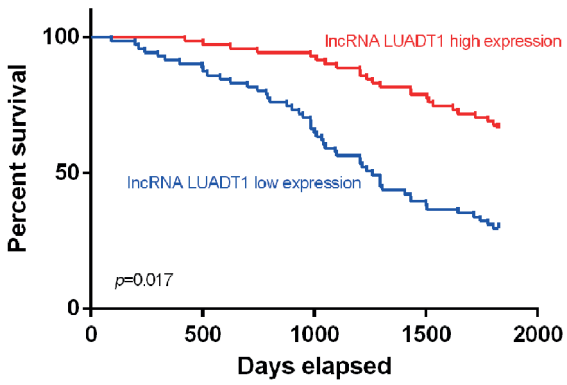

Figure 2. Kaplan-Meier analyses show that the higher expression of lncRNALUADT1 indicates the poorer survival of patients as relative to lower expression of lncRNA LUADT1; $p=0.017$.

Discussion

The incidence rate of colorectal cancer is the third most common tumor in the world, and the fourth leading cause of malignant tumor in China[14]. The invasion and metastasis of colorectal cancer are the key factors that lead to the reduction of clinical efficacy and short survival of patients. In recent years, researchers have used lncRNA chips, lncRNA sequencing and Real-time quantitative PCR methods to screen and identify lncRNAs that are abnormally expressed in tumor tissues. We also found that lncRNALUADT1 was highly expressed in colorectal cancer and closely related to the malignant phenotype of the tumor. LncRNA acts as an oncogene or tumor suppressor gene in the development and progression of tumors[15-17]. LncRNAs is able to be divided into antisense lncRNAs, intronic transcripts, large intergenic noncoding RNAs, promoter-associated lncRNAs, and UTR associated lncRNAs[18,19]. Downregulation of lncRNA MEG3 can regulate colorectal cancer cell proliferation ability and may be regarded as a marker for indicating poor prognosis in patients[20]. In ovarian cancer, lncRNA AB073614 contributes to tumorigenesis and also indicates poor prognosis[21]. Overexpression of lncRNA TUG1 promotes colon cancer progression[22]. LncRNA AK027294 abnormal expression level is related to colorectal cancer cell proliferation, migration, and apoptosis[23]. LncRNA AOC4P can inhibit hepatocellular carcinoma metastasis via promoting vimentin expression and suppressing epithelial-mesenchymal transition[24]. In gastric cancer, upregulation of lncRNA ZFAS1 is involved in the epithelial-mesenchymal transition process[25].

The expression of lncRNA is directly associated with the occurrence and development of tumors and plays an essential regulatory role in the invasion and metastasis of colorectal cancer[23,26,27]. LOC100287225 expression in colorectal cancer tissues is significantly lower compared with that in the corresponding adjacent tissues[28]. We aimed at exploring lncRNALUADT1 expression in colorectal cancer and found that lncRNALUADT1 was closely related to malignant phenotype of colorectal cancer. Moreover, high expression of lncRNALUADT1 was implicated in the prognosis of patients. This suggests that lncRNALUADT1 acts as an oncogene promoting the progression of colorectal cancer.

Conclusions

We showed that LncRNALUADT1 was over-expressed in colorectal cancer and its expression level was closely related to TNM staging, lymph node metastasis and tumor size. Our present study might provide new insights in the diagnosis and therapy of colorectal cancer.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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