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Long non-coding RNA Loc554202 expression as a prognostic factor in patients with colorectal cancer

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Abstract. – OBJECTIVE: To investigate the expression and clinical significance of long non-coding RNA Loc554202 (lncRNA Loc554202) in human colorectal cancer (CRC).

PATIENTS AND METHODS: The expression of Loc554202 was detected in 178 CRC tissues and matched normal colorectal tissue samples by qRT-PCR. The potential relationship between Loc554202 levels and clinicopathological features of CRC Kaplan-Meier curves and multivariate Cox proportional models were used to study the impact on clinical outcome.

RESULTS: Our findings showed that Loc554202 appeared to have lower expression in the CRC tissues, compared with the adjacent non-cancerous colorectal tissues. Down-regulated expression of Loc554202 was significantly associated with TNM stage, histologic grade, and lymph node metastasis ($p < 0.05$), but not other clinical parameters. Kaplan-Meier analysis clearly illustrated that the patients with the lower expression of Loc554202 had a worse outcome compared to patients with higher Loc554202 expression ($p < 0.001$). Furthermore, in a multivariate Cox model, we found that Loc554202 expression was an independent poor prognostic factor for both 5-year OS ($p = 0.007$) and 5-year DFS ($p = 0.004$) in CRC.

CONCLUSIONS: Our data indicated that Loc554202 may serve as a promising biomarker for predicting the prognosis of CRC.

Key Words:
Colorectal cancer, lncRNA Loc554202, Prognosis, Quantitative Real-time PCR.

Introduction

As one of the most common malignant cancers worldwide, colorectal cancer (CRC) has become the fifth leading cause of cancer death for men and women in China[1]. CRC patients with early stage can be treated successfully with surgical resection, and are most likely to be completely curative[2,3], but many CRC remain undiagnosed until the disease has progressed to a late stage[4]. An urgent needing to search for specific, sensitive biomarkers for the early diagnosis and prognosis prediction of CRC exists.

The long non-coding RNAs (lncRNAs) are a class of non-coding RNA over 200 nucleotides with no protein-coding potential[5,6]. Recent studies demonstrated that lncRNAs have an important role in numerous biological processes, including transcriptional regulation, cell growth and tumorigenesis[7,8]. lncRNA Loc554202 was also reported to play an important role in tumor. Shi et al[9] showed that the expression level of Loc554202 was up-regulated in breast cancer and knockdown of Loc554202 decreased breast cancer cell proliferation, induced apoptosis. Ding et al[10] found that Loc554202 was significantly downregulated in CRC tissues and the overexpression of Loc554202 decreased the cell proliferation and induced apoptosis *in vitro* and hindered tumorigenesis *in vivo*. However, the clinical significance of Loc554202 in CRC is still unknown.

In the present study, we explored the expression of Loc554202 in CRC tissues. Then, we analyzed its clinicopathologic characteristics and prognostic significance.

Patients and Methods

Patients and Tissue Samples

The use of tissues for this study has been approved by the Ethics Committee of Xianyang Central Hospital and Affiliated Hospital of the Shaanxi University of Traditional Chinese Medicine. A total of 178 CRC tissues and corresponding noncancerous tissues were collected through

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the Department of Hepatobiliary Surgery, Xianyang Central Hospital and Department of Oncology, Affiliated Hospital of the Shaanxi University of Traditional Chinese Medicine. All patients did not receive anticancer treatment, including chemotherapy, radiotherapy and biotherapy, prior to surgery resection. After collection, all tissue samples were immediately frozen in liquid nitrogen and stored at -80 °C until use. The overall survival was defined as the time from surgery to death. Table I summarized the clinicopathologic characteristics of all 178 CRC patients enrolled in this study. Written informed consent was obtained from all participants.

Quantitative Real-Time PCR

Total RNA was extracted from tissue samples using TRIzol ((Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The RNA concentration was measured with a NanoDrop 2000 spectro-photometer (Thermo Fisher Scientific, Waltham, MA, USA). The PCR amplification were performed for 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, on a Applied Biosystems 7900HT (Applied Biosystems, Foster City, CA, USA) with 1.0 □l of cDNA and SYBR Green Real-time PCR Master Mix (Takara, Dalian, Liaoning, China). □-Actin was used as an endogenous control to normalize the data. The relative quantitative value was expressed by the $2^{-\Delta\Delta Ct}$ methods. Each experiment was performed in triplicates and repeated three times.

Statistical Analysis

Statistical analysis was performed using SPSS software, version 19.0 (SPSS, Chicago, IL, USA). Values are expressed as means ± SD. Comparison of continuous data was analyzed using an independent *t*-test between the two groups, whereas categorical data was analyzed by the chi-square test. The univariate analysis was performed with the Kaplan-Meier survival curve method, and statistical differences were compared with a log-rank test. Multivariate survival analysis was performed using the Cox proportional hazard model. Statistical significance was considered at a value of $p < 0.05$.

Results

Expression of Loc554202 in CRC

To investigate the functional role of Loc554202 in CRC tissues. qRT-PCR was used to detect the expression of Loc554202 in CRC

Table I. Relationship between Loc554202 expression and clinicopathological characteristics.

Characteristic	n	Loc554202 expression		*p*-value
		Low	High	
Total	178	93	85	
Gender				0.755
Male	111	59	52	
Female	67	34	33	
Age (years)				0.405
< 60	78	38	40	
≥ 60	100	55	45	
Tumor size (cm)				0.180
< 5 cm	72	42	30	
≥ 5 cm	106	51	55	
Histological grade				< 0.000
Well and moderately	97	36	61	
Poorly	81	57	24	
Local invasion				0.172
T1-T2	91	43	48	
T3-T4	87	50	37	
Lymph nodes metastasis				0.006
Negative	127	58	69	
Positive	51	35	16	
TNM stage				0.004
I-II	95	40	55	
III-IV	83	53	30	

tissues. As shown in Figure 1, Loc554202 was significantly downregulated in CRC tissues compared with adjacent normal tissues ($p < 0.01$). The results indicated that Loc554202 might play a tumor suppressor role in CRC.

Relationship between Loc554202 Expression and the Clinicopathologic Features of CRC

In order to explore the relationship between lncRNA Loc554202 expression and clinicopathological features in CRC. We divided the 178 CRC patients into a high expression group (n=85) and a low expression group (n=93) according to the median value of Loc554202 expression levels in CRC tissues. Our results showed that Loc554202 expression was significantly associated with TNM stage ($p = 0.004$), histologic grade ($p < 0.000$), and lymph node metastasis ($p = 0.006$). However, there was no association between Loc554202 expression and gender, age, tumor size, and local invasion.

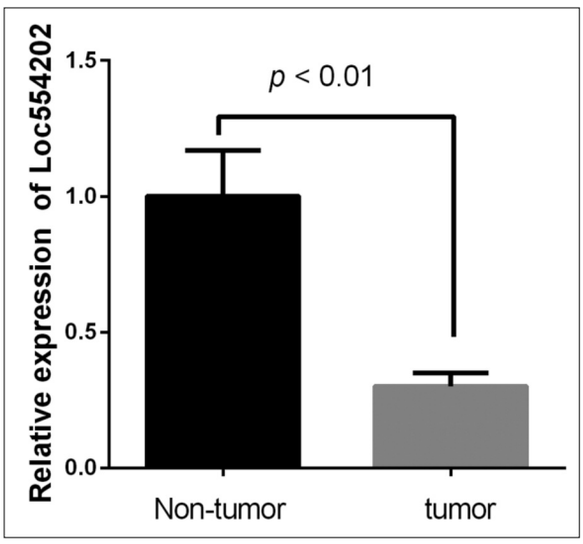

Figure 1. The relative expression of Loc554202 in colorectal cancer tissues and adjacent noncancerous tissues.

Correlations of Loc554202 Expression with Patient Survival

Kaplan-Meier method and log-rank test were used to evaluate the differences of overall survival between low expression group and high expression group. We found that the low expression of Loc554202 was associated with short OS ($p < 0.001$) and DFS ($p < 0.001$). A univariate Cox proportional hazard regression analysis showed that Loc554202 expression ($p = 0.005$) and the well-known clinicopathological prognostic parameters, such as Histological grade ($p = 0.016$), Lymph nodes metastasis ($p < 0.013$), and TNM stage ($p < 0.018$), were significantly associated with OS and DFS (Table II). In addition, Multivariate analysis showed that Loc554202 expression was an independent prognostic factor for the OS ($p = 0.007$) and DFS ($p = 0.004$) of CRC patients (Table II).

Discussion

Despite the clinical implementation of numerous therapeutic strategies, However, be-

Table II. Univariate and multivariate analyses of prognostic parameters in patients with CRC by Cox regression analysis.

	Overall survival			Disease-free survival		
	Hazard ratio	95% CI	p	Hazard ratio	95% CI	p
Univariate analyses						
Gender	1.348	0.656-3.692	0.438	1.149	0.454-3.338	0.365
Age (years)	1.936	0.742-5.658	0.325	1.673	0.514-4.923	0.284
Tumor size (cm)	1.339	0.538-3.682	0.385	1.219	0.437-3.143	0.345
Histological grade	3.249	1.348-7.348	0.016	3.055	1.538-7.893	0.006
Local invasion	2.994	0.936-7.852	0.128	2.653	1.214-6.549	0.095
Lymph nodes metastasis	3.548	1.214-8.225	0.013	3.884	1.659-8.953	0.009
TNM stage	2.563	1.147-7.632	0.018	2.239	1.048-6.883	0.011
Loc554202 expression	2.653	1.139-7.023	0.005	2.837	1.338-8.149	0.002
Multivariate analyses						
Histological grade	2.733	1.646-6.818	0.013	2.261	1.732-7.026	0.007
Lymph nodes metastasis	3.149	1.552-7.835	0.018	2.883	1.841-8.125	0.012
TNM stage	2.823	0.841-6.348	0.013	2.236	1.143-7.248	0.009
Loc554202 expression	2.451	1.338-7.742	0.007	2.752	1.553-7.931	0.004

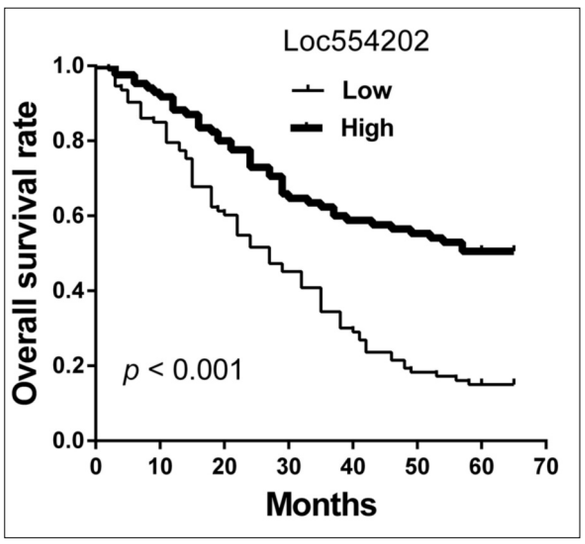

Figure 2. Kaplan-Meier curves for overall survival and Loc554202 expression in group of 178 colorectal cancer patients.

cause of the absence of typical symptoms or signs, and the lack of a sufficiently sensitive and specific biomarker, CRC continues to have an extremely poor prognosis[11,12]. CRC progression-specific targets have a significant impact on the clinical management of CRC patients[13,14]. More and more studies informed that understanding of LncRNAs function will provide us broad prospects to find proper tumor progression-specific targets[15].

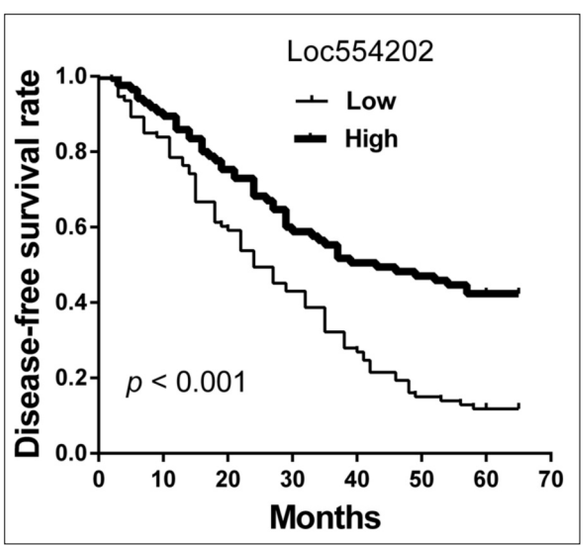

Figure 3. Kaplan-Meier curves for disease-free survival and Loc554202 expression in group of 178 colorectal cancer patients.

lncRNAs are implicated in a number of important events, such as various cellular processes, development, and human diseases[16,17]. Abnormal lncRNAs expression is found in various human tumours and acts as prognostic biomarkers in human tumours. For example, Tuo et al[18] showed that UCA1 was significantly upregulated in breast cancer tissues and over-expression of UCA1 could promote breast cancer cell growth and apoptosis. Gupta et al[19] reported that HOTAIR is highly expressed in metastatic breast cancer and its high expression in primary breast tumors is a significant predictor of subsequent metastasis and mortality. Yang et al[20] found that High MALAT1 expression predicts a poor prognosis of cervical cancer and promotes cancer cell growth and invasion. Wang et al[21] showed that AFAP1-AS1 expression was markedly upregulated in colorectal cancer tissues and associated with poor prognosis, ectopic expression of AFAP1-AS1 was demonstrated to promotes tumorigenesis in colorectal cancer *in vitro* and *in vivo*. However, the prognostic role of Loc554202 in CRC are still unknown.

In the present work, we investigated the expression pattern of Loc554202 and its biological significance in CRC for the first time. We found that the levels of Loc554202 in CRC patients were significantly lower than those in normal subjects. Moreover, we further explored the role of Loc554202 in the development and progression of CRC. We found that Loc554202 was positively associated with TNM stage, histologic grade, and lymph node metastasis. In addition, survival analysis showed that patients with low expression of Loc554202 implicated poor OS and DFS. The multivariate survival analysis indicated that the over-expression of Loc554202 was independently associated with OS and DFS in patients with CRC. Taken together, these results indicated that Loc554202 could function as an antioncogene.

Conclusions

We demonstrated that Loc554202 is downregulated in CRC tissues and its low expression indicates poor prognosis of CRC patients. Our findings reveal that Loc554202 expression might be an independent prognostic factor and a therapeutic target for CRC.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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