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High expression of long non-coding RNA ATB is associated with poor prognosis in patients with renal cell carcinoma

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Abstract. – OBJECTIVE: To compare the expression of lncRNA-ATB in renal cell carcinoma (RCC) tissues and adjacent non-tumor tissues to determine whether lncRNA-ATB could be used as a potential prognostic biomarker for RCC.

PATIENTS AND METHODS: qRT-PCR was performed to determine the expression level of lncRNA-ATB in RCC tissues and corresponding non-tumor tissues. The relationship between lncRNA-ATB expression and clinicopathologic features was analyzed. Patient survival analysis was determined according to the Kaplan-Meier method using the log-rank test. A Cox's regression model was used for univariate and multivariate analysis.

RESULTS: The expression level of lncRNA-ATB was significantly upregulated in RCC tissues vs. corresponding non-tumor tissues ($p<0.01$) and the high expression of lncRNA-ATB was significantly associated with histological grade ($p=0.008$), lymph nodes metastasis ($p=0.015$), and distant metastasis ($p=0.008$). Also, Kaplan-Meier analysis indicated patients with high lncRNA-ATB expression that had a significantly shorter overall survival than those with low lncRNA-ATB expression ($p<0.001$). Univariate and multivariate analysis showed that lncRNA-ATB expression was an independent risk factor for overall survival in RCC patients.

CONCLUSIONS: lncRNA-ATB was a potential prognostic marker and a therapeutic target for patients with RCC.

Key Words:
lncRNA-ATB, Renal cell carcinoma, Prognosis.

Introduction

Renal cell carcinoma (RCC) accounts for almost 90% of all renal malignancies. Clear cell renal cell carcinoma (ccRCC) represents approximately 75% of all RCCs[1]. Up to date, RCC responds poorly to chemotherapy and radiotherapy[2]. Although complete surgical resection can achieve a cure for localized RCC, the prognosis of patients with distant metastasis remains very poor[3]. It is reported that 5-year overall survival rate of these patients with distant metastasis were still only 10%[4]. Therefore, there is a need for new therapeutic approaches, as well as prognostic and predictive markers.

In recent years a newly discovered class of long noncoding RNAs (lncRNAs) was reported to be associated with human diseases, including tumors[5]. Long noncoding RNAs (lncRNAs) are transcribed RNA molecules longer than 200 nucleotides in length[6]. Although lncRNAs don't code protein, growing data indicates that the dysregulation of lncRNAs may contribute to tumor cell proliferation, migration and invasion, as well as participate in metastasis[7,8]. Several lncRNAs have been reported to be correlated with cancer development and prognosis in various cancers, including RCC. For instance, Zhang et al[9] reported that the expression level of lncRNA MALAT1 was up-regulated in ccRCC tissues and knockdown expression of MALAT1 decreased renal cancer cell proliferation, migration, and invasion. Ellinger et al[10] lnc-ZNF180-2 expression levels were an independent predictor of progression-free survival, cancer-specific survival and overall survival in ccRCC patients. Li et al[11] reported that lncRNA UCA1 expression levels were significantly increased in RCC tissues and cells and over-expression of UCA1 significantly promoted the RCC cells proliferation. Those results indicated that lncRNAs were frequently involved in the progression of RCC.

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Table I. RT-PCR primers for amplification of expression of lncRNA-ATB.

Primer	Primer sequence (5'-3')
LncRNA-ATB F	CTTCACCAGCACCCAGAGA
LncRNA-ATB R	AAGACAGAAAAACAGTTCCGAGTC
GAPDH F	CAGGAGGCATTGCTGATGAT
GAPDH R	GAAGGCTGGGGCTCATTT

Recently, a new identified lncRNA, which was named lncRNA activated by transforming growth factor-b (lncRNA-ATB), was observed to be dysregulated in several tumors, such as non-small cell lung cancer[12], hepatocellular carcinoma[13], and prostate carcinoma[14]. Notably, a previous study by Xiong et al[15] showed that the expression level of lncRNA-ATB was significantly up-regulated in RCC tissues and cells, suggesting that lncRNA-ATB may be involved in the development of RCC. However, the effect of lncRNA-ATB in RCC remains largely unknown. In the present work, we collected the information of patients with RCC from our hospital and explored whether lncRNA-ATB was associated with the survival rate of RCC patients.

Patients and Methods

Human Tissues

From January 2010 to January 2012, surgical specimens (paired normal and cancerous tissues) were obtained from 198 consecutive patients in Changyi City People' Hospital. Patients selected were aged from 26 to 65 years (median age 42.9 years). Samples were immediately stored in liquid nitrogen until RNA extraction. All the patients did not receive any pre-operative chemotherapy or radiotherapy before the surgery. Information of age, gender, histopathological type, tumor size, tumor stage, lymph nodes metastasis and distant metastasis were retrieved from the medical records. Additional patient characteristics are listed in Table II. The research Ethics Committee of Changyi City People' Hospital provided Ethical Approval for this study, and all patients provided written informed consent.

RNA and miRNA Extraction and Quantitative RT-PCR

Total RNA was extracted from the tissue samples with Trizol reagent (TaKaRa, Dalian, China), and first-strand cDNA was synthesized from 2 μg of total RNA random primers under standard conditions for the PrimeScript RT reagent Kit (TaKaRa, Dalian, China). lncRNA-ATB expression levels were measured with quantitative Real-time PCR (qRT-PCR) using an ABI7500 system and the SYBR Green PCR Master Mix (TaKaRa, Dalian, China). For the normalization of tissue data, gene expression was normalized to the respective GAPDH expression level. The primer sequences of lncRNA-ATB and GAPDH were shown in Table I. All reactions were carried out in triplicate, and the $2^{-\Delta\Delta Ct}$ Ct method was used to quantify the relative amount of lncRNA-ATB.

Statistical Analysis

The SPSS statistical software program version 22 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The comparison of the expression level of lncRNA-ATB between RCC tissue

Table II. Correlations between lncRNA-ATB expression and clinical characteristic in RCC.

Parameters	Group	Total	lncRNA-ATB expression		*p*
			High	Low	
Gender	Male	88	45	43	0.924
	Female	110	57	53	
Age (years)	< 65	72	34	38	0.361
	≥ 65	126	68	58	
Histological grade	Low or undiffer	45	31	14	0.008
	Middle or high	153	71	82	
Tumor size (cm)	<4	95	50	45	0.763
	≥4	103	52	51	
Tumor stage	T1-T2	138	68	70	0.339
	T3-T4	60	34	26	
Lymph nodes metastasis	Absence	55	36	19	0.015
	Presence	143	66	77	
Distant metastasis	Absence	52	35	17	0.008
	Presence	146	67	79	

and adjacent normal tissue was performed using the two-sample Student's *t*-test. The X^2-test was used to assess lncRNA-ATB expression with respect to clinicopathological parameters. Overall survival was analyzed by Cox proportional hazards regression and Kaplan-Meier curves; $p<0.05$ was considered statistically significant.

Results

lncRNA-ATB is Upregulated in RCC Tissues

The expression levels of lncRNA-ATB in 198 RCC and adjacent non-cancerous tissues were analyzed by Real-time quantitative RT-PCR. As shown in Figure 1, it was observed that the expression level of lncRNA-ATB was significantly upregulated in RCC tissues *vs.* corresponding non-tumor tissues ($p<0.01$).

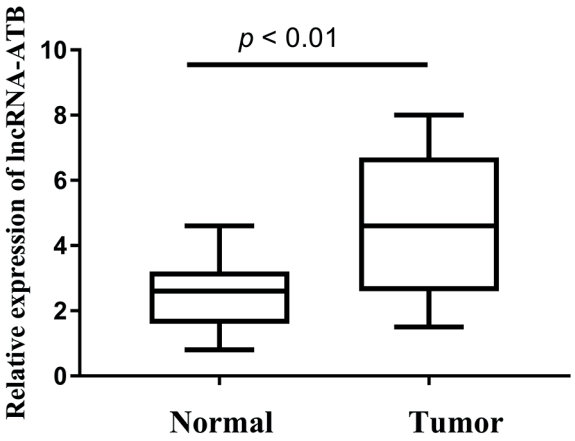

Figure 1. The relative expression of lncRNA-ATB in RCC tissues and normal matched tissues. Bars represent the means of the relative expression of lncRNA-ATB.

Correlation of Clinicopathological Features of RCC with lncRNA-ATB

To explore the association between lncRNA-ATB expression and clinicopathological characteristics, we divided the 198 patients with RCC into two groups (high group and low group) according to the median value of lncRNA-ATB expression level. As shown in Table II, we observed that the high expression of lncRNA-ATB was significantly associated with histological grade ($p=0.008$), lymph nodes metastasis ($p=0.015$), and distant metastasis ($p=0.008$). However, no correlation was observed between lncRNA-ATB expression and other clinicopathological characteristics.

Upregulation of lncRNA-ATB Associates with Poor Prognosis in Patients with RCC

To determine the role of lncRNA-ATB in predicting the prognosis of patients, Kaplan-Meier survival analyses were performed. The Kaplan-Meier survival curves show that the high expression of the lncRNA-ATB was correlated with shorter survival time ($p<0.01$, log-rank test, Figure 2). Furthermore, in univariate Cox model, our results revealed that histological grade ($p=0.008$), lymph nodes metastasis ($p=0.003$), distant metastasis ($p=0.007$) and lncRNA-ATB expression ($p=0.004$) were an unfavorable prognostic factor in RCC patients (Table III). Finally, multivariate analysis confirmed that lncRNA-ATB expression level was independent prognostic factors for overall survival of RCC patients (RR=2.783, 95% CI, 1.778-5.874; $p=0.009$, Table III).

Discussion

It is known to us that exploring biomarkers, which can accurately predict prognosis for RCC patients and guide treatment, was very important to

Table III. Univariate and multivariate Cox proportional regression analysis on overall survival of RCC patients.

	Univariate analysis			Multivariate analysis		
Variable	Risk ratio	95% CI	p	Risk ratio	95% CI	p
Gender	1.341	0.782-1.753	NS			
Age (years)	1.416	0.619-1.673	NS			
Histological grade	3.776	1.842-6.452	0.008	3.155	1.351-4.894	0.015
Tumor size (cm)	1.743	0.486-2.131	NS			
Tumor stage	1.455	0.781-1.783	NS			
Lymph nodes metastasis	5.331	2.783-8.652	0.003	4.137	2.213-6.637	0.008
Distant metastasis	4.563	2.447-7.226	0.007	3.779	2.037-5.569	0.011
lncRNA-ATB expression	3.226	2.145-7.732	0.004	2.783	1.778-5.874	0.009

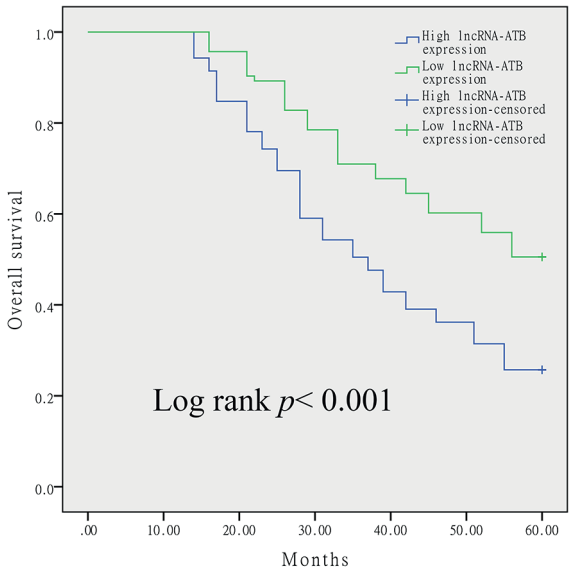

Figure 2. Kaplan-Meier analyses for the association between lncRNA-ATB expression and overall survival of RCC patients.

improve the prognosis of patients with tumors[16]. Recently, more and more lncRNAs were reported to be valuable prognostic marker and promising treatment target for tumor, such as lncRNA SBF2-AS1 for non-small cell lung cancer[17], lncRNA HULC for osteosarcoma[18], and lncRNA SPRY4-IT1 for cervical cancer[19]. However, to our best knowledge, very little study reported the prognostic value of lncRNAs in RCC. In the present study, our attention focused on the association between lncRNA-ATB with RCC

LncRNA-ATB was located on chromosome 14[20]. Several studies have reported the role of lncRNA-ATB in various cancers. For instance, Yuan et al[13] reported that lncRNA-ATB promoted hepatocellular carcinoma cell invasion through the TGF-b/miR-200 s/ZEB signaling pathway. Shi et al[21] found that LncRNA-ATB expression was significantly up-regulated in breast cancer and could promote trastuzumab resistance and invasion-metastasis cascade in breast cancer by competitively biding miR-200c. Xu et al[14] showed that overexpression of lncRNA-ATB promoted, and knockdown of lncRNA-ATB inhibited the growth of prostate cancer cells via regulations of cell cycle regulatory protein expression levels. They also showed that high lncRNA-ATB expression indicated poorer prognosis in patients with prostate carcinoma. In addition, the similar prognostic value of lncRNA-ATB in colon cancer was also reported by Yue et al[22]. Those findings showed a similar result that lncRNA-ATB served as a tumor promoter in above tumors. Notably, Xiong et al[15] showed that the expression levels of lncRNA-ATB in RCC was up-regulated and over-expression of lncRNA-ATB promoted cell migration and invasion in renal cell carcinoma. It indicated that lncRNA-ATB may play a positive effect in tumorigenesis of RCC. Thus, we suggest that lncRNA-ATB may be associated with the prognosis of patients with RCC.

We found that lncRNA-ATB was highly expressed in RCC tissues compared with non-malignant tissues. Moreover, we examined correlation between lncRNA-ATB levels with clinicopathological factors. Our data showed that elevated lncRNA-ATB expression was associated with high histological grade, lymph nodes metastasis, and distant metastasis, suggesting that higher lncRNA-ATB expression predicted a more malignant RCC phenotype. Then, Kaplan-Meier analysis revealed that RCC patients with high lncRNA-ATB expression had poorer overall survival. In addition, lncRNA-ATB expression correlated negatively with overall survival in RCC patients according to both univariate and multivariate analysis. Thus, the above results revealed that lncRNA-ATB could be an independent prognostic marker in RCC.

Conclusions

To our knowledge, we show for the first time that elevated lncRNA-ATB expression is significantly correlated with the aggressive phenotype and poor prognosis of CRC. Further study should be focused on how lncRNA-ATB regulated the progression of RCC.

Conflict of interest

The authors declare no conflicts of interest.

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