Int J Clin Exp Pathol 2017;10(2):2048-2052
www.ijcep.com /ISSN:1936-2625/IJCEP0043417

Original Article

The expression level of lncRNA-ATB associates with clinicopathological parameters and prognosis of cervical cancer

Xiao-Liang Mo, Dian-Zhong Luo, Xiao-Li Jiang, Gui-Rong Qin, Yan Sun

Department of Gynecology and Obstetrics, The First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guang Xi, PR China

Received November 2, 2016; Accepted December 20, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: Background: Long noncoding RNAs (lncRNAs) play important roles in tumor development and progression. Previous studies have found that dysregulation of lncRNA-ATB was significantly associated with tumor progression and poor survival outcome in various cancers. However, whether the expression level of lncRNA-ATB is associated with the clinicopathological parameters and prognosis of patients with cervical cancer is still unclear. Methods: A total of 128 patients with primary cervical cancer were included in this retrospective study. Quantitative real-time PCR assay was performed to evaluate lncRNA-ATB expression levels. The Kaplan-Meier method was used to estimate survival, log-rank test was used to test differences between the survival curves. Results: As revealed by quantitative RT-PCR analysis, lncRNA-ATB expression was significantly higher in cervical cancer tissues compared with normal adjacent tissues (8.826±3.155 vs. 3.372±1.043, P<0.001). High lncRNA-ATB expression level was observed to be closely correlated with tumor differentiation (P<0.001) and FIGO stage (P<0.001). The overall survival of patients in the high lncRNA-ATB group showed significantly worse survival rates than those who were in the low lncRNA-ATB group (P=0.024). Furthermore, multivariate analysis of the prognosis factors with a Cox proportional hazards model showed that high lncRNA-ATB expression (HR=2.535, CI: 1.372-10.376, P=0.012) was a significant independent predictor of poor survival in cervical cancer. Conclusion: Our results suggested that upregulation of lncRNA-ATB was significantly correlated with tumor progression and might be a potent prognostic marker of cervical cancer.

Keywords: Cervical cancer, lncRNA-ATB, prognosis, biomarker

Introduction

Cervical cancer is the fourth most common cancer and fourth most frequent cause of cancer mortality affecting woman in the world [1]. Despite marked efforts in developing multimodal treatments, the clinical outcome of cervical cancer patients remains unfavorable. Many studies have shown that tumor size, lymph node metastasis and lymph-blood vessel invasion are independent prognostic factors for survival of cervical cancer patients. However, these factors may not accurately estimate prognosis because of heterogeneity in the patient population [2-4]. Thus, it is highly necessary to explore novel biomarkers for the early identification of a more effective, clinical therapeutic strategy against cervical cancer.

Long non-coding RNA (lncRNA) is an RNA molecule with a length of 200 bp-100 kbp that lacks protein-coding potential [5]. Recent studies revealed that lncRNAs play a pivotal role in the regulation of gene expression, such as chromatin modification, transcription and post-transcriptional processing [6-8]. Furthermore, more and more evidences revealed the contribution of lncRNAs as having oncogenic and tumor suppressor roles in tumorigenesis [9-12].

Previously, overexpression of long non-coding RNA activated by TGF-b (lncRNA-ATB) has been observed in several types of cancers, and its overexpression was closely associated with poor survival outcome [13-19]. However, whether the expression level of lncRNA-ATB is associated with the prognosis of patients with cervical cancer is still unclear. The aim of this study was to investigate the clinical significance and prognostic value of lncRNA-ATB in cervical cancer.

Table 1. Association between lncRNA-ATB expression and patients' clinicopathologic features

Clinicopathologic variables	N	lncRNA-ATB level		*P* value
		High (n=67)	Low (n=61)	
Age (years)				
≤55	68	37	31	0.723
>55	60	30	30	
Histology				
Squamous	71	39	32	0.594
Adenocarcinoma	57	28	29	
Tumor size (cm)				
≤4	55	26	29	0.373
>4	73	41	32	
FIGO stage				
I-II	56	19	37	<0.001
II-IV	72	48	24	
Differentiation				
Well-moderate	43	10	33	<0.001
Poor	85	57	28	

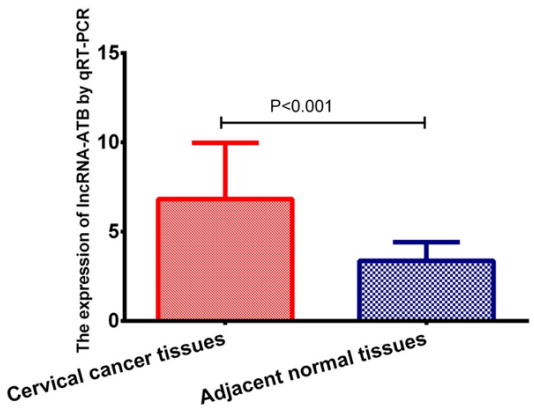

Figure 1. The expression levels of lncRNA-ATB in 128 pairs of primary tumor and normal adjacent samples from 128 cervical cancer patients.

Materials and methods

Patients and tissue samples

A total of 128 patients with primary cervical cancer who underwent surgery at the Department of Gynecology and Obstetrics, The First Affiliated Hospital of Guangxi Medical University, Nanning, were included in this retrospective study. These patients were diagnosed as cervical cancer between 2009 and 2016. None of the patients recruited in this study had chemotherapy or radiotherapy before the surgery. Once the tissues were dissected they were immersed in RNAlater (Qiagen GmbH, Hilden, Germany) for 30 min, and were then stored at -80°C until further use. The collected adjacent normal tissues were 2 cm away from visible cervical cancer lesions. Cervical cancer diagnosis was based on World Health Organization (WHO) criteria. Tumor staging was determined according to the sixth edition of the tumor-node-metastasis (TNM) classification of the International Union against Cancer. The clinicopathological features of 128 patients are summarized in **Table 1**. The study was approved by the Research Ethics Committee of The First Affiliated Hospital of Guangxi Medical University. Informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards.

Evaluation of lncRNA-ATB expression in cervical cancer samples and normal adjacent tissues

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen). For qRT-PCR, RNA was reverse transcribed to cDNA by using a Reverse Transcription Kit (Takara). Real-time PCR analyses were performed with Power SYBR Green (Takara). The PCR primers for lncRNA-ATB or GAPDH were as follows: lncRNA-ATB sense, 5'-CTTCACCAGCACCCAGAGA-3' and reverse, 5'-AAGACAG AAAAACAGTTCCGAGTC-3'; GAPDH sense, 5'-GTCAACGGATTTGGTCTGTATT-3' and reverse, 5'-AGTCTTCTGGGTGGCAGTGAT-3'. Data was collected and analyzed by SDS2.3 Software (Applied Biosystems). The expression level of lncRNA-ATB was internally normalized against that of the GAPDH. The relative quantitative value was expressed by the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicates and repeated three times.

Statistical analysis

All statistical analyses were made using Statistical Package for Social Science (SPSS; version 18.0) for Windows (SPSS Inc, Chicago, IL). Relationships between lncRNA-ATB expression level and other parameters were studied using the chi-square test and Fisher's exact test or independent t test. The Kaplan-Meier method was used to estimate survival; log-rank test was used to test differences between the survival curves. A multivariate survival analysis

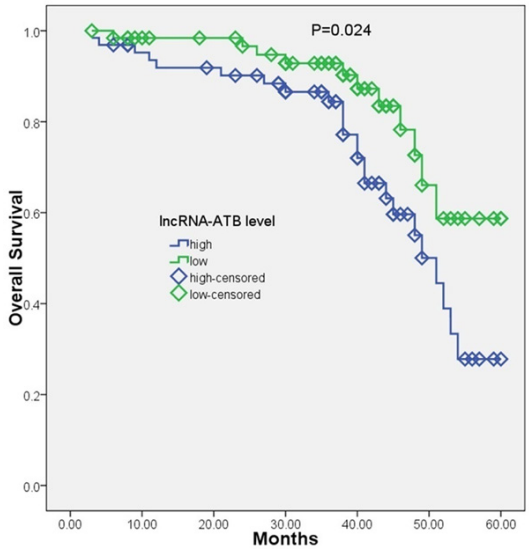

Figure 2. The overall survival of patients in the high lncRNA-ATB group showed significantly worse survival rates than those who were in the low lncRNA-ATB group (P=0.024).

was performed for all parameters that were significant in the univariate analyses using the Cox regression model. Results were considered statistically significant when P≤0.05 was obtained.

Results

The expression level of lncRNA-ATB in cervical cancer samples and normal adjacent tissues

We analyzed the expression levels of lncRNA-ATB in 128 pairs of primary tumor and normal adjacent samples from 128 cervical cancer patients. As revealed by quantitative RT-PCR analysis, lncRNA-ATB expression was significantly higher in cervical cancer tissues compared with normal adjacent tissues (8.826± 3.155 vs. 3.372±1.043, P<0.001, shown in **Figure 1**). The 128 cervical cancer patients were classified into two groups according to the median of lncRNA-ATB expression level as determined by quantitative RT-PCR. 67 cases were placed in the high expression group and 61 in the low expression group.

Correlations of lncRNA-ATB expression with clinicopathologic features of cervical cancer patients

The correlations of lncRNA-ATB expression with clinicopathologic features of patients with cervical cancer were statistically analyzed. As shown in **Table 1**, high lncRNA-ATB expression level was observed to be closely correlated with tumor differentiation (P<0.001) and FIGO stage (P<0.001). However, there were no significant correlations between lncRNA-ATB expression level and other clinicopathologic factors, including age (P=0.723), histology (P=0.594), and tumor size (P=0.373).

Correlation between lncRNA-ATB expression and prognosis of cervical cancer patients

Kaplan-Meier overall survival curve of the cervical cancer patients according to the status of lncRNA-ATB level was examined. The overall survival of patients in the high lncRNA-ATB group showed significantly worse survival rates than those who were in the low lncRNA-ATB group (P=0.024, shown in **Figure 2**), suggesting that high expression of lncRNA-ATB is associated with poor prognosis in cervical cancer patients. A multivariate analysis of the prognosis factors with a Cox proportional hazards model showed that high lncRNA-ATB expression (HR=2.535, CI: 1.372-10.376, P=0.012), high FIGO stage (HR=4.551, CI: 2.017-12.895, P=0.002), and worse tumor differentiation (HR=3.123, CI: 2.128-9.067, P<0.001) were significant independent predictors of poor survival in cervical cancer (shown in **Table 2**).

Discussion

The dysregulation of lncRNA is common in various carcinomas and plays an important role in cancer progression by altering normal gene expression. It has been noted that alterations in single lncRNA expression correlate highly with the progression and prognosis of human tumors. Thus, identification of lncRNA molecular profiles associated with the prognosis of patients with cervical cancer may not only elucidate the underlying biological mechanisms involved in the development or progression of the disease but also provide the opportunity to identify novel targets for cervical cancer therapy.

Overexpression of lncRNA-ATB has been observed in several types of cancer. For example, in the study by Saito et al, they found that lncRNA-ATB was significantly upregulated in gastric cancer tissues, and lncRNA-ATB played an important role in epithelial-mesenchymal transition (EMT) to promote invasion and metastasis through the TGFb/miR-200s/ZEB axis [16]. Shi et al found that lnc-ATB was significantly upregulated in the tissues of trastu-

Table 2. Multivariate analyses of parameters associated with overall survival of 128 cervical cancer patients

Variable	Hazard ratio	95% CI	*P*-value
Age	1.322	0.579-3.542	0.177
Histology	2.263	0.872-7.133	0.091
Tumor size	3.283	0.857-6.765	0.067
FIGO stage	4.551	2.017-12.895	0.002
Differentiation	3.123	2.128-9.067	<0.001
lncRNA-ATB expression level	2.535	1.372-10.376	0.012

zumab resistance breast cancer patients and trastuzumab resistance SKBR-3 cells. Lnc-ATB could promote trastuzumab resistance and invasion-metastasis cascade in breast cancer by competitively biding miR-200c, upregulating ZEB1 and ZNF-217, and then inducing EMT. In addition, the high level of lnc-ATB was correlated with trastuzumab resistance of breast cancer patients. Thus, these findings suggested that lncRNA-ATB, a mediator of TGF-β signaling, could predispose breast cancer patients to EMT and trastuzumab resistance [17]. In agreement with these studies, we confirmed that the expression levels of lncRNA-ATB in cervical cancer tissues were significantly higher than those in normal adjacent tissues. Furthermore, high lncRNA-ATB expression level was observed to be closely correlated with tumor differentiation and FIGO stage, suggesting lncRNA-ATB may be involved in cervical cancer progression. Previously, several studies have found that overexpression of lncRNA-ATB was closely associated with poor survival outcome in some cancers. For example, in the study by Saito et al, they found that the lncRNA-ATB expression level was significantly associated with the overall survival rate after curative surgery for gastric cancer. Patients in the high lncRNA-ATB group had poorer prognoses than patients in the low lncRNA-ATB group (median overall survival, 1.35 vs. 2.08 years; P=0.0074). The results of multivariate analysis for overall survival rate suggested that lncRNA-ATB expression level was independent prognostic factor (hazard ratio 3.50, 95% CI 1.73-7.44, P=0.0004) in patients with gastric cancer [16]. Iguchi et al found that patients in the high-lncRNA-ATB expression group had significantly poorer outcomes than those in the low-expression group in terms of recurrence-free survival (P= 0.022) in colorectal cancer [20]. However, whether the expression level of lncRNA-ATB is associated with the prognosis of patients with cervical cancer is still unclear. In the present study, we found that the overall survival of patients in the high lncRNA-ATB group showed significantly worse survival rates than those who were in the low lncRNA-ATB group, suggesting that high expression of lncRNA-ATB is associated with poor prognosis in cervical cancer patients. Furthermore, multivariate analysis of the prognosis factors with a Cox proportional hazards model showed that high lncRNA-ATB expression was a significant independent predictor of poor survival in cervical cancer.

In conclusion, our results suggested that upregulation of lncRNA-ATB was significantly correlated with tumor progression and may be a potent prognostic marker of cervical cancer. In addition, more high quality studies are needed to confirm our findings.

Acknowledgements

Guangxi health department general scientific research project (Z2015534).

Disclosure of conflict of interest

None.

Address correspondence to: Yan Sun, Department of Gynecology and Obstetrics, The First Affiliated Hospital of Guangxi Medical University, No. 6 Shuangyong Road, Nanning 530021, Guangxi, PR China. Tel: 086-0771-5356697; Fax: 086-0771-5356697; E-mail: sunyangynecology@yeah.net

References

[1] Torre LA, Siegel RL, Ward EM and Jemal A. Global Cancer Incidence and Mortality Rates and Trends--An Update. Cancer Epidemiol Biomarkers Prev 2016; 25: 16-27.

[2] Hillemanns P, Soergel P, Hertel H and Jentschke M. Epidemiology and Early Detection of Cervical Cancer. Oncol Res Treat 2016; 39: 501-506.

[3] Dryden-Peterson S, Bvochora-Nsingo M, Suneja G, Efstathiou JA, Grover S, Chiyapo S, Ramogola-Masire D, Kebabonye-Pusoentsi M, Clayman R, Mapes AC, Tapela N, Asmelash A, Medhin H, Viswanathan AN, Russell AH, Lin LL, Kayembe MK, Mmalane M, Randall TC, Chab-

ner B and Lockman S. HIV Infection and Survival Among Women With Cervical Cancer. J Clin Oncol 2016; [Epub ahead of print].
[4] Chuang LT, Feldman S, Nakisige C, Temin S and Berek JS. Management and Care of Women With Invasive Cervical Cancer: ASCO Resource-Stratified Clinical Practice Guideline. J Clin Oncol 2016; 34: 3354-3355.
[5] Zhang Q, Chen CY, Yedavalli VS and Jeang KT. NEAT1 long noncoding RNA and paraspeckle bodies modulate HIV-1 posttranscriptional expression. MBio 2013; 4: e00596-00512.
[6] Kitagawa M, Kotake Y and Ohhata T. Long noncoding RNAs involved in cancer development and cell fate determination. Curr Drug Targets 2012; 13: 1616-1621.
[7] Yoon JH, Abdelmohsen K and Gorospe M. Posttranscriptional gene regulation by long noncoding RNA. J Mol Biol 2013; 425: 3723-3730.
[8] Yoon JH, Abdelmohsen K, Srikantan S, Yang X, Martindale JL, De S, Huarte M, Zhan M, Becker KG and Gorospe M. LincRNA-p21 suppresses target mRNA translation. Mol Cell 2012; 47: 648-655.
[9] Gibb EA, Brown CJ and Lam WL. The functional role of long non-coding RNA in human carcinomas. Mol Cancer 2011; 10: 38.
[10] Liz J and Esteller M. lncRNAs and microRNAs with a role in cancer development. Biochim Biophys Acta 2016; 1859: 169-176.
[11] Pandey GK and Kanduri C. Long noncoding RNAs and neuroblastoma. Oncotarget 2015; 6: 18265-18275.
[12] Roth A and Diederichs S. Long Noncoding RNAs in Lung Cancer. Curr Top Microbiol Immunol 2016; 394: 57-110.
[13] Li W and Kang Y. A new Lnc in metastasis: long noncoding RNA mediates the prometastatic functions of TGF-beta. Cancer Cell 2014; 25: 557-559.
[14] Qu S, Yang X, Song W, Sun W, Li X, Wang J, Zhong Y, Shang R, Ruan B, Zhang Z, Zhang X and Li H. Downregulation of lncRNA-ATB correlates with clinical progression and unfavorable prognosis in pancreatic cancer. Tumour Biol 2016; 37: 3933-3938.
[15] Richards EJ, Zhang G, Li ZP, Permuth-Wey J, Challa S, Li Y, Kong W, Dan S, Bui MM, Coppola D, Mao WM, Sellers TA and Cheng JQ. Long non-coding RNAs (LncRNA) regulated by transforming growth factor (TGF) beta: LncRNA-hit-mediated TGFbeta-induced epithelial to mesenchymal transition in mammary epithelia. J Biol Chem 2015; 290: 6857-6867.
[16] Saito T, Kurashige J, Nambara S, Komatsu H, Hirata H, Ueda M, Sakimura S, Uchi R, Takano Y, Shinden Y, Iguchi T, Eguchi H, Ehata S, Murakami K, Sugimachi K and Mimori K. A Long Non-coding RNA Activated by Transforming Growth Factor-beta is an Independent Prognostic Marker of Gastric Cancer. Ann Surg Oncol 2015; 22 Suppl 3: S915-922.
[17] Shi SJ, Wang LJ, Yu B, Li YH, Jin Y and Bai XZ. LncRNA-ATB promotes trastuzumab resistance and invasion-metastasis cascade in breast cancer. Oncotarget 2015; 6: 11652-11663.
[18] Xu S, Yi XM, Tang CP, Ge JP, Zhang ZY and Zhou WQ. Long non-coding RNA ATB promotes growth and epithelial-mesenchymal transition and predicts poor prognosis in human prostate carcinoma. Oncol Rep 2016; 36: 10-22.
[19] Yue B, Qiu S, Zhao S, Liu C, Zhang D, Yu F, Peng Z and Yan D. LncRNA-ATB mediated E-cadherin repression promotes the progression of colon cancer and predicts poor prognosis. J Gastroenterol Hepatol 2016; 31: 595-603.
[20] Iguchi T, Uchi R, Nambara S, Saito T, Komatsu H, Hirata H, Ueda M, Sakimura S, Takano Y, Kurashige J, Shinden Y, Eguchi H, Sugimachi K, Maehara Y and Mimori K. A long noncoding RNA, lncRNA-ATB, is involved in the progression and prognosis of colorectal cancer. Anticancer Res 2015; 35: 1385-1388.