European Review for Medical and Pharmacological Sciences 2020; 24: 11148-11153

Expression of lncRNA-ATB in laryngeal carcinoma and its relationship with prognosis

D.-N. HUANG, H.-W. LIU, Z.-D. LI

Department of Head and Neck Area, Cancer Hospital of China Medical University, Liaoning Cancer Hospital, Shenyang, China

Abstract. – OBJECTIVE: The aim of this study was to investigate the expression of long non-coding RNA (lncRNA)-ATB in laryngeal carcinoma (LNCa) and its relationship with the prognosis.

PATIENTS AND METHODS: The expression of lncRNA-ATB was examined in laryngeal carcinoma tissue specimens, as well as in normal ones by quantitative real-time polymerase chain reaction (qRT-PCR), and the interplay between lncRNA-ATB levels and clinical indicators was analyzed. In addition, the diagnostic value of lncRNA-ATB for LNCa was assessed by receiver operating characteristic (ROC) curve analysis. The patients were followed up for 5 years and the survival analysis was conducted by Kaplan-Meier test. Finally, the Cox regression model was used to analyze the factors affecting the prognosis of patients.

RESULTS: LncRNA-ATB expression was markedly enhanced in laryngeal carcinoma tissue samples compared to the corresponding normal ones, which was relevant to T grade and clinical stage. For the diagnosis of laryngeal carcinoma using lncRNA-ATB, the area under the ROC curve (AUC) was 0.8672, the diagnostic threshold was 3.895, and the sensitivity and specificity were 83.02% and 76.42%, respectively. In addition, the overall survival rate of patients with high expression of lncRNA-ATB was markedly lower than those in low expression group. Meanwhile, T grade, clinical stage and lncRNA-ATB are found as three independent factors influencing the prognosis of LNCa.

CONCLUSIONS: LncRNA-ATB was highly expressed in laryngeal carcinoma tissues, which was not conducive to the prognosis of patients. Therefore, this molecular marker has potential to become a new biomarker for the diagnosis and prognosis prediction of patients with LNCa.

Key Words:
Laryngeal carcinoma, LncRNA-ATB, Prognosis, Biomarkers.

Introduction

Laryngeal cancer (LNCa) is a common malignant tumor of head and neck, accounting for about 13.9% of head and neck tumors and 2.1% of systemic malignant tumors. It can be divided into glottic type, supraglottic type and subglottic type, among which, glottic type is the most common, accounting for about 60%.The majority of patients were male, and the male-female ratio was about 9:1[1]. Squamous cell carcinoma (SCC) accounts for 96%-98% of laryngeal malignancies, while others, such as adenocarcinoma, basal cell carcinoma, poorly differentiated carcinoma, lymphosarcoma and malignant lymphoma, are less common. The specific pathogenesis of LNCa still remains elusive, which may be related to smoking, drinking, and other bad habits, as well as human papillomavirus (HPV) infection, environmental factors, radiation, and other factors[2]. Early LNCa can be treated clinically by surgery combined with radiotherapy, while middle and advanced LNCa is mainly treated by surgery combined with radiotherapy and chemotherapy, but the therapeutic efficacy is not satisfactory. Patients' breathing, pronunciation, eating, and other functions were severely damaged after surgery, which reduced the quality of life of patients to a large extent. Therefore, the research on LNCa has profound clinical significance.

Long non-coding RNA (lncRNA) generally refers to RNA with a length greater than 200 nt, which is located in the nucleus or cytoplasm[3]. The occurrence of LNCa is mostly due to the influence of external environment and lifestyle, which is a complex multi-gene change process. LncRNA expression is up-regulated or down-regulated in LNCa cells and surrounding tissues and plays a potential role in promoting or inhibiting cancer by participating in various biological processes, and is related to the metastasis and differentiation degree of tumor[4-6]. Li et al[6] detected the expression level of HOTAIR in cancer tissues and paracancerous tissues of 72 patients with laryngeal squamous cell carcinoma by quantitative real-time polymerase chain reaction (qRT-PCR),

Corresponding Author: Zhendong Li, BM; e-mail: 1349946150@qq.com

and found that the expression level of HOTAIR in tumor tissues was significantly higher than that in adjacent tissues (16 times); in addition, its expression level was statistically correlated with clinical stage, degree of differentiation, cervical lymph node metastasis, and prognosis. Mirisola et al[7] analyzed tissue samples from 20 patients with LNCa using gene expression microarray and found that H19 presented a low expression in the samples of patients with LNCa who were more prone to recurrence, suggesting that H19 may have an anti-cancer effect.

LncRNA-ATB, known as a LncRNA activated by TGF-β, is the first discovered long-stranded non-coding RNA activated by transforming growth factors, with a length of about 2.4 KB, located on human chromosomes 13, 14 and 22[8]. LncRNA-ATB plays a significant regulatory role in phenotypic transformation of human peritoneal mesothelial cells[9-11], HCV-related cirrhosis, preeclampsia, and other diseases. In addition, lncRNA-ATB is abnormally expressed in various tumors and diseases, such as hepatic cell carcinoma, thyroid cholangiocarcinoma, breast cancer, osteosarcoma, and other tumors[12-15]. Also, lncRNA-ATB is a potential carcinogenic molecule. In cervical cancer cells and tissues, researches confirmed that the expression of lncRNA-ATB was also abnormally up-regulated, which was closely related to tumor volume, lymph node metastasis, and FIGO stage. Of note, lncRNA-ATB is closely associated with postoperative adverse prognosis of patients with cervical cancer and can be used as an independent prognostic indicator for patients with cervical cancer[16]. However, there have been no relevant studies on the interaction of lncRNA-ATB and LNCa. Therefore, we aim to explore the association between lncRNA-ATB and LNCa, so as to provide new research ideas and guiding strategies for the clinical diagnosis and treatment of LNCa.

Patients and Methods

Sample Collection

A total of 106 specimens of LNCa tissues and matched para-tumor normal ones were collected from the Liaoning Cancer Hospital, and then quickly frozen in liquid nitrogen immediately after excision and transferred to -80°C for preservation. Clinical and pathological data of all patients were collected at the same time. The investigation was approved by Ethics Committee of Liaoning Cancer Hospital.

QRT-PCR Detection

Total RNA was extracted from the tissue using TRIzol (Invitrogen, Carlsbad, CA, USA), and the expression level of the relevant gene was detected using a PCR detection kit (TaKaRa, Otsu, Shiga, Japan). The relative expression level was calculated by the relative quantitative PCR method $2^{-\Delta\Delta Ct}$ (RQ value), ΔΔCt=mean of the test group (Ct lncRNA-AT-Ct GAPDH)-control group (Ct lncRNA-AT-Ct GAPDH). lncRNA-ATB Fr: 5'-TCTGGCTGAGGCTGGTTGAC-3', lncRNA-ATB R: 5'-ACACAGAATAAAATAACAC-3'; GAPDH (internal reference) F: 5'-GGTGAAGGTCGGAGTCAACGG-3', GAPDH R: 5'-GAGGTCAATGAAGGGGTCATTG -3'.

Postoperative Follow-Up

Patients were followed up by outpatient review, telephone or e-mail. They were usually performed once in the first month after surgery, every 3 months in the first year, every 6 months in the second year, and once a year thereafter. The follow-up rate reached 100%. The follow-up period lasted for 5 years and the deadline was December 2018.

Statistical Analysis

Data analysis was performed using Statistical Product and Service Solutions (SPSS) 20.0 statistical software (IBM, Armonk, NY, USA). Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$), *t*-test was used for comparison between the two groups; χ^2-test was used for counting data. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of lncRNA-ATB for tumors. Kaplan-Meier survival curve method and Log-Rank test were used to analyze the difference between lncRNA-ATB expression and overall 5-year survival of patients. Cox regression analysis was used to analyze prognostic factors. The difference was statistically significant when $p<0.05$, $^*p<0.05$.

Results

High Expression of LncRNA-ATB in Laryngeal Carcinoma

QRT-PCR detected that the expression of lncRNA-ATB in laryngeal carcinoma tissue samples was markedly higher than that in normal ones (Figure 1), suggesting that lncRNA-ATB might act as a tumor-promoting gene and thus be involved in the progression of laryngeal carcinoma.

Evaluation of the Sensitivity and Specificity of LncRNA-ATB to Predict LNCa

We explored whether lncRNA-ATB can be used as a biomarker for the diagnosis of LNCa. The results showed that the area under the ROC curve of lncRNA-ATB for the diagnosis of LNCa was 0.8672 (p<0.001) with 95% CI: 0.8199-0.9145 (Figure 2). When the cut-off value is 3.895, the sensitivity is 83.02% and the specificity is 76.42%, which suggested that lncRNA-ATB ≥3.895 will increase the chance of occurrence of LNCa.

Correlation Between Expression of LncRNA-ATB and Clinicopathological Features

To investigate the clinical significance of lncRNA-ATB in patients with LNCa, we divided the above tissue samples into lncRNA-ATB high expression group (n=84) and low expression group (n=22), with the cut-off value 3.995 as a boundary. Subsequently, it was found that the expression level of lncRNA-ATB had no significant relationship with the gender, age, N grade, and pathological differentiation of the patients (p>0.05), but with T stage and clinical stage. The expression of lncRNA-ATB in patients in T3+4, III + IV stage was higher than those in T1+2, I +II stage (p<0.05; Table I), suggesting that lncRNA-ATB level was correlated with T grade and clinical stage.

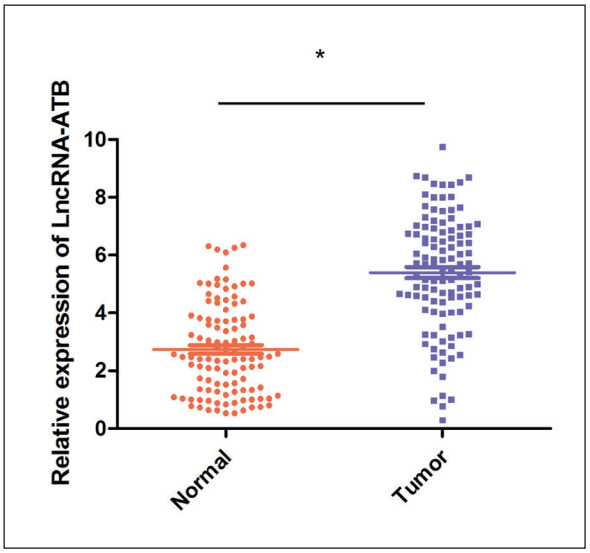

Figure 1. LncRNA-ATB is under-expressed in laryngeal carcinoma tissue. Compared with normal tissues, qRT-PCR showed that the expression of lncRNA-ATB was significantly increased in laryngeal carcinoma tissues.

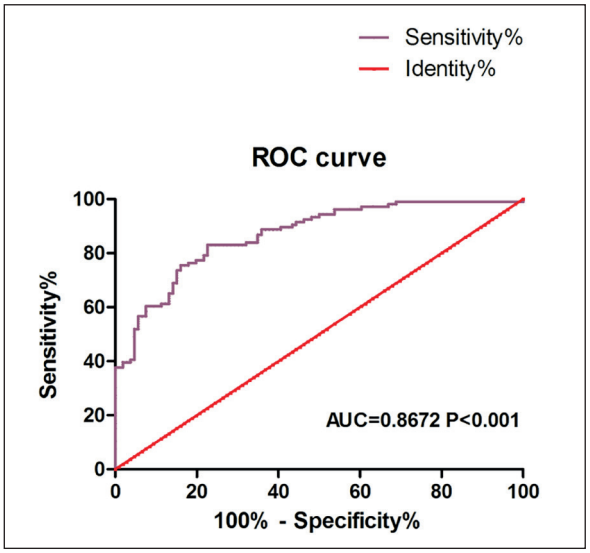

Figure 2. The sensitivity and specificity of lncRNA-ATB was assessed to predict laryngeal cancer. The area under the ROC curve was AUC=0.8672, p<0.001. When the cut-off value was 3.895, the sensitivity was 83.02% and the specificity was 76.42%.

Effect of LncRNA-ATB on Overall Survival Rate In Patients With LNCa

To further figure out the impact of lncRNA-ATB on overall survival of patients with LNCa, we performed a 5-year follow-up, and the Kaplan-Meier survival analysis showed that the survival rate of laryngeal cancer patients with high lncRNA-ATB was conspicuously lower than those with lowly-expressed lncRNA-ATB, HR=4.473, p=0.0344 (Figure 3). Therefore, it was indicated that lncRNA-ATB was not conducive to the prognosis of patients with LNCa.

Cox Regression Analysis of Factors Affecting Survival of Patients With LNCa

To determine the factors that influence the survival of patients with LNCa, we used the COX risk regression model for analysis. The results showed that the risk of death in patients in T3+4, clinical III + IV stage or those with high lncRNA-ATB expression was 1.834 times, 2.671 times, and 2.071 times higher than those in T1+2, I + II stage, and those with low lncRNA-ATB expression, respectively (Table II). The above results demonstrated that high T grade, high clin-

Table I. Correlation between expression of lncRNA-ATB and clinicopathological features.

Variable	No.	lncRNA-ATB		*p*
		High (n = 84)	Low (n = 22)	
Sex				
Male	68	50	18	0.079
Female	38	34	4	
Age				
< 55	45	37	8	0.630
≥ 55	61	47	14	
T classification				
T1+2	35	23	12	0.022*
T3+4	71	61	10	
N classification				
N0+1	58	45	13	0.810
N2+3	48	39	9	
Pathological grade				
Low	38	28	10	0.324
Medium+High	68	56	12	
Clinical stage				
I +II	43	29	14	0.016*
III +IV	63	55	8	

*p<0.05.

ical stage, and high lncRNA-ATB expression are three independent risk factors for the prognosis of LNCa.

Discussion

As one of the common head and neck malignancies, LNCa is also one of the malignancies with relatively high invasive degree, accounting for about 2.4% of new tumors every year[17]. Despite the continuous optimization and development of surgical techniques and comprehensive treatment regimens, early LNCa can also be treated by surgery combined with radiotherapy to achieve the expected treatment. However, due to its hidden clinical manifestations, the disease has always been found to in advanced stage. Patients with advanced LNCa are mainly treated by surgery combined with radiotherapy,

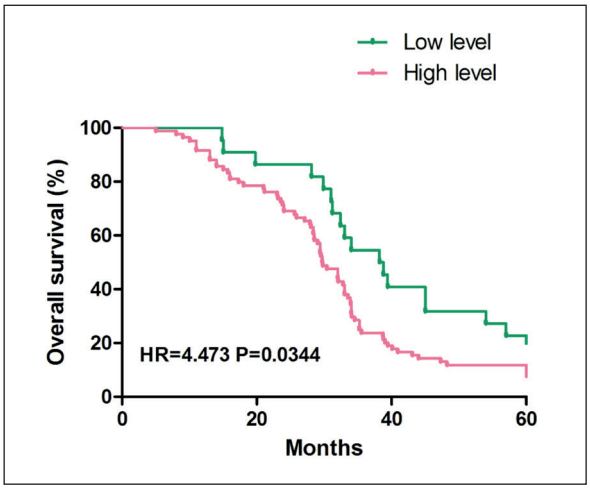

Figure 3. Effect of lncRNA-ATB expression on overall survival in patients with laryngeal cancer. The overall survival rate of laryngeal cancer patients with lncRNA-ATB high expression was significantly lower than those with lncRNA-ATB low expression. (HR=4.473, p=0.0344).

Table II. Cox regression analysis of survival factors in patients with laryngeal cancer.

Variable	HR (95% CI)	*p*-value
T classification (T1+2, T3+4)	1.834 (1.031-2.899)	0.037
Clinical stage (I +II , III +IV)	2.671 (1.326-4.931)	0.021
lncRNA-ATB (Low, High)	2.071 (1.723-3.873)	0.031

HR=hazard ratios, CI=confidence interval

but their appearance, breathing, swallowing, and pronunciation function are greatly affected. In the course of postoperative combined radiotherapy, some patients may need reoperation due to laryngeal edema and poor healing of the surgical area, and various postoperative complications may occur, which also has a serious impact on the quality of life of patients[18]. LncRNAs can be engaged in various biological processes including epigenetic, transcriptional and post-transcriptional regulation as a signal molecule, bait molecule, guide molecule, and skeleton molecule, and plays a significant role in modulating tumor biological activity[19,20].

LncRNA-ATB is a new long non-coding RNA reported and named for the first time by Chinese scholars; it can enhance the invasiveness and metastasis ability of cancer cells *via* regulating phenotypic transformation of cells[21]. Jang et al[22] used qRT-PCR to quantitatively detect tissue samples from 100 patients with liver cancer and found that the expression level of lncRNA-ATB was significantly up-regulated in liver cancer tumor tissues compared with adjacent normal tissues. In addition, abnormal expression of lncRNA-ATB was closely related to portal vein thrombosis, intrahepatic metastasis, mUICC staging, and staging of clinical liver cancer in Barcelona, and the overall survival and progression-free survival of patients with high expression of lncRNA-ATB were relatively poor[22]. The correlation analysis between the abnormal expression level of LncRNA-ATB and the clinicopathological data of liver cancer patients showed that larger tumors (>5 cm in diameter) and high expression level of lncRNA-ATB could be independent factors for the overall survival of liver cancer patients[23]. Additionally, Qi et al[24] have demonstrated that the overall survival time of patients with renal cell carcinoma in the group with high expression of lncRNA-ATB is shorter, suggesting that lncRNA-ATB can be an independent determinant of the prognosis of patients with this cancer.

We found that lncRNA-ATB expression was markedly up-regulated in LNCa tissues, which may accelerate the progression of LNCa. Meanwhile, further statistical analysis showed that lncRNA-ATB levels were markedly upregulated in patients who had been in T3+4 grade or clinical III +IV stage, suggesting that T grade and clinical stage were closely relevant to the progression of LNCa. In addition, the prognostic evaluation of lncRNA-ATB in patients with LNCa showed that the survival condition of patients with high lncRNA-ATB expression was markedly worse than that of the group with low lncRNA-ATB expression. Finally, the COX model revealed that lncRNA-ATB was an independent factor affecting the prognosis of patients.

Conclusions

In summary, we showed that lncRNA-ATB may serve as an oncogene to promote the occurrence and progression of LNCa, which is expected to be a molecular marker for predicting the prognosis of LNCa patients, so as to provide new ideas for the target diagnosis and treatment of LNCa.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Dziegielewski PT, O'Connell DA, Klein M, Fung C, Singh P, Alex MM, Fung D, Harris JR, Seikaly H. Primary total laryngectomy versus organ preservation for T3/T4a laryngeal cancer: a population-based analysis of survival. J Otolaryngol Head Neck Surg 2012; 41 Suppl 1: S56-S64.
2) Edefonti V, Nicolussi F, Polesel J, Bravi F, Bosetti C, Garavello W, La Vecchia C, Bidoli E, Decarli A, Serraino D, Calza S, Ferraroni M. Nutrient-based dietary patterns and nasopharyngeal cancer: evidence from an exploratory factor analysis. Br J Cancer 2015; 112: 446-454.
3) Liu T, Zuo JJ, Li F, Xu YC, Zheng AY, Tao ZZ. LncRNA SNHG1 promotes cell proliferation in laryngeal cancer via Notch1 signaling pathway. Eur Rev Med Pharmacol Sci 2019; 23: 6562-6569.
4) Fang XY, Pan HF, Leng RX, Ye DQ. Long noncoding RNAs: novel insights into gastric cancer. Cancer Lett 2015; 356: 357-366.
5) Bolton EM, Tuzova AV, Walsh AL, Lynch T, Perry AS. Noncoding RNAs in prostate cancer: the long and the short of it. Clin Cancer Res 2014; 20: 35-43.
6) Li D, Feng J, Wu T, Wang Y, Sun Y, Ren J, Liu M. Long intergenic noncoding RNA HOTAIR is overexpressed and regulates PTEN methylation in laryngeal squamous cell carcinoma. Am J Pathol 2013; 182: 64-70.
7) Mirisola V, Mora R, Esposito AI, Guastini L, Tabacchiera F, Paleari L, Amaro A, Angelini G, Dellepiane M, Pfeffer U, Salami A. A prognostic multigene classifier for squamous cell carcinomas of the larynx. Cancer Lett 2011; 307: 37-46.

8) Fu N, Zhao SX, Kong LB, Du JH, Ren WG, Han F, Zhang QS, Li WC, Cui P, Wang RQ, Zhang YG, Nan YM. LncRNA-ATB/microRNA-200a/beta-catenin regulatory axis involved in the progression of HCV-related hepatic fibrosis. Gene 2017; 618: 1-7.

9) Liu X, Chen H, Kong W, Zhang Y, Cao L, Gao L, Zhou R. Down-regulated long non-coding RNA-ATB in preeclampsia and its effect on suppressing migration, proliferation, and tube formation of trophoblast cells. Placenta 2017; 49: 80-87.

10) Liu Y, Li Y, Xu Q, Yao W, Wu Q, Yuan J, Yan W, Xu T, Ji X, Ni C. Long non-coding RNA-ATB promotes EMT during silica-induced pulmonary fibrosis by competitively binding miR-200c. Biochim Biophys Acta Mol Basis Dis 2018; 1864: 420-431.

11) Qiu J, Chen Y, Huang G, Zhang Z, Chen L, Na N. Transforming growth factor-beta activated long non-coding RNA ATB plays an important role in acute rejection of renal allografts and may impacts the postoperative pharmaceutical immunosuppression therapy. Nephrology (Carlton) 2017; 22: 796-803.

12) Jang SY, Kim G, Park SY, Lee YR, Kwon SH, Kim HS, Yoon JS, Lee JS, Kweon YO, Ha HT, Chun JM, Han YS, Lee WK, Chang JY, Park JG, Lee B, Tak WY, Hur K. Clinical significance of lncRNA-ATB expression in human hepatocellular carcinoma. Oncotarget 2017; 8: 78588-78597.

13) Lin H, Yang L, Tian F, Nie S, Zhou H, Liu J, Chen W. Up-regulated LncRNA-ATB regulates the growth and metastasis of cholangiocarcinoma via miR-200c signals. Onco Targets Ther 2019; 12: 7561-7571.

14) Zhang Y, Li J, Jia S, Wang Y, Kang Y, Zhang W. Down-regulation of lncRNA-ATB inhibits epithelial-mesenchymal transition of breast cancer cells by increasing miR-141-3p expression. Biochem Cell Biol 2019; 97: 193-200.

15) Han F, Wang C, Wang Y, Zhang L. Long noncoding RNA ATB promotes osteosarcoma cell proliferation, migration and invasion by suppressing miR-200s. Am J Cancer Res 2017; 7: 770-783.

16) Cao W, Peng T, Zhou Y. Long noncoding RNA activated by transforming growth factor-beta promotes cancer development and is a prognostic marker in cervical cancer. J Cancer Res Ther 2017; 13: 801-806.

17) Christensen A, Kristensen E, Therkildsen MH, Specht L, Reibel J, Homoe P. Ten-year retrospective study of head and neck carcinoma in situ: incidence, treatment, and clinical outcome. Oral Surg Oral Med Oral Pathol Oral Radiol 2013; 116: 174-178.

18) Edefonti V, Bravi F, Garavello W, La Vecchia C, Parpinel M, Franceschi S, Dal Maso L, Bosetti C, Boffetta P, Ferraroni M, Decarli A. Nutrient-based dietary patterns and laryngeal cancer: evidence from an exploratory factor analysis. Cancer Epidemiol Biomarkers Prev 2010; 19: 18-27.

19) Yao C, Yu B. Role of Long noncoding RNAs and circular RNAs in nerve regeneration. Front Mol Neurosci 2019; 12: 165.

20) de Lima DS, Cardozo LE, Maracaja-Coutinho V, Suhrbier A, Mane K, Jeffries D, Silveira E, Amaral PP, Rappuoli R, de Silva TI, Nakaya HI. Long noncoding RNAs are involved in multiple immunological pathways in response to vaccination. Proc Natl Acad Sci U S A 2019; 116: 17121-17126.

21) He L, Lou W, Ji L, Liang W, Zhou M, Xu G, Zhao L, Huang C, Li R, Wang H, Chen X, Sun S. Serum response factor accelerates the high glucose-induced epithelial-to-mesenchymal transition (EMT) via snail signaling in human peritoneal mesothelial cells. PLoS One 2014; 9: e108593.

22) Jang SY, Kim G, Park SY, Lee YR, Kwon SH, Kim HS, Yoon JS, Lee JS, Kweon YO, Ha HT, Chun JM, Han YS, Lee WK, Chang JY, Park JG, Lee B, Tak WY, Hur K. Clinical significance of lncRNA-ATB expression in human hepatocellular carcinoma. Oncotarget 2017; 8: 78588-78597.

23) Li Y, Ye Y, Chen H. Astragaloside IV inhibits cell migration and viability of hepatocellular carcinoma cells via suppressing long noncoding RNA ATB. Biomed Pharmacother 2018; 99: 134-141.

24) Qi JJ, Liu YX, Lin L. High expression of long non-coding RNA ATB is associated with poor prognosis in patients with renal cell carcinoma. Eur Rev Med Pharmacol Sci 2017; 21: 2835-2839.