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LncRNA-ATB Can Be a Biomarker for Diagnosis and Prognosis Evaluation of Non-Small Cell Lung Cancer

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Abstract: Objective: This study was set out to inquire into the expression and clinical significance of lncRNA activated by transforming growth factor β (LncRNA-ATB) and in cancer tissues of patients with non-small cell lung cancer (NSCLC). Methods: LncRNA-ATB in cancer tissues and adjacent tissues of 89 NSCLC patients was detected by quantitative real-time polymerase chain reaction (qRT-PCR), and its clinical diagnostic value in NSCLC was determined by receiver operating characteristic (ROC) curves. Based on the median expression of LncRNA-ATB in NSCLC tissues, 89 patients were allocated into high- and low-expression groups. The 3-year survival rate was calculated using Kaplan-Meier method and log-rank test, and compared between lncRNA-ATB high and low expression groups by Log-rank test. Cox regression quantitatively analyzed the correlation between lncRNA-ATB and the clinical prognosis of NSCLC patients. Results: LncRNA-ATB presented notably higher expression in NSCLC tissues as compared to adjacent tissues ($P < 0.05$), and was linked to smoking history, TNM stage and greatest tumor diameter (GTD) ($P < 0.05$). The cut-off value, sensitivity and specificity, as well as the area under the curve (AUC) of NSCLC diagnosed by LncRNA-ATB were 2.103, 73.77%, 83.15% and 0.836, respectively. Patients with high lncRNA-ATB expression (40.00%) showed statistically lower than those with low lncRNA-ATB expression (63.36%). TNM stage, differentiation degree and lncRNA-ATB expression were independent variables affecting patient prognosis. Conclusions: LncRNA-ATB, with increased expression profiles in NSCLC tissues, is involved in NSCLC progression and associated with unfavourable prognosis of patients, which can be a plausible tumor marker for NSCLC patients.

Keywords: lncRNA-ATB; NSCLC; diagnosis; prognosis; tumor marker

1 Introduction

Being a member of the most pervasive malignancies in the world with high morbidity and mortality, lung cancer (LC) is a serious threat to human life and health, with approximately 85% of the cases being non-small cell lung cancer (NSCLC) [1]. Driven by the influence of factors such as tobacco and air pollution, the morbidity and mortality of NSCLC are on the rise, and in the meanwhile, its treatment is facing daunting challenges. While due to the occult onset of NSCLC, lack of typical symptoms in the early stage, strong invasion and metastasis, and easy recurrence, most patients have missed the best treatment period when diagnosed, resulting in an unfavorable 5-year survival rate of 17% [2,3]. Therefore, exploring the mechanism of occurrence and progression of NSCLC is paramount for guiding the early diagnosis and

treatment of patients, developing new target therapy and improving the outcomes of patients.

Eukaryotic genomes encode thousands of small and large noncoding RNAs (ncRNA), such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs), all of which have been found to be the main participants in various biological processes [4]. Long non-coding RNAs (lncRNAs) are non-coding RNAs over 200 bases in length that have been proved to be essential in tumorigenesis and development and are related to biological processes such as the occurrence, progression, invasion and metastasis of multiple cancers [5–6]. In recent years, studies have identified that abnormally expressed lncRNAs are closely linked with human diseases and may become new targets for tumor therapy [7,8]. Their critical regulatory roles in occurrence, growth, invasion, metastasis, recurrence and drug resistance of NSCLC have been already well established [9,10]. Of these, lncRNA activated by transforming growth factor β (lncRNA-ATB) is shown to be closely associated with the occurrence and progression of various tumors such as breast cancer, colorectal cancer and pancreatic cancer [11–13]. It was first found to be up-regulated in hepatocellular carcinoma tissue specimens, and its elevated expression promoted the metastasis of hepatocellular carcinoma [14]. However, the clinical significance of lncRNA-ATB in NSCLC remains to be clarified.

In this study, quantitative real-time polymerase chain reaction (qRT-PCR) was applied to test lncRNA-ATB expression in cancerous and corresponding normal lung tissue samples of NSCLC patients, and patient clinicopathological features were analyzed to reveal the possible role of LncRNA-ATB in NSCLC, so as to provide evidence for finding target genes for clinical diagnosis and prognosis of NSCLC patients.

2 Materials and Methods

2.1 Research Participants

Cancer tissues and paracancerous normal tissues (more than 5 cm away from the cancer tissue) of 89 NSCLC patients who underwent radical or palliative resection in the Fourth Hospital of Hebei Medical University from November 2014 to August 2016 were collected, as well as patient personal information and detailed clinical data. All the tissues were pathologically confirmed as NSCLC. The freshly excised specimens were immediately put into liquid nitrogen for cryopreservation. None of the patients received preoperative chemoradiotherapy. Patient clinical data were collected completely. There were 49 males and 40 females, with an average age of (50.14 ± 9.57) years. Classified by TNM staging, 23 cases were in stage I, 27 in stage II, 22 in stage III, and 17 in stage IV; Regarding pathological classification, there were 22 cases of squamous cell carcinoma and others, and 67 cases of adenocarcinoma. The Ethics Committee of the Fourth Hospital of Hebei Medical University approved the experimental protocol, and all patients had provided the informed consent form to participate. Inclusion criteria: All the enrolled patients were diagnosed as NSCLC by imaging examination (including PET/CT) and finally confirmed by histopathology or cytology, including fiberoptic bronchoscopic biopsy, transpleural puncture biopsy, supraclavicular lymph node biopsy and exfoliated cell examination of pleural effusion. All the research participants were recently diagnosed and had not received any intervention measures such as surgical resection, radiotherapy, chemotherapy, molecular targeted therapy or immunotherapy before enrollment. Exclusion criteria: patients with other malignancies or severe dysfunction of heart, liver, kidney or other organs; patients with combined immune system diseases or infections; patients with mental illness.

2.2 qRT-PCR Analysis of LncRNA-ATB Expression

LncRNA-ATB was extracted by qRT-PCR, and the specific protocol was as follows: Total RNA was extracted using the TRIzol kit (ThermoFisher Scientific, Waltham, MA, USA). The total RNA was first added with Trizol reagent for total RNA isolation. After homogenizing and resting for 5 min, chloroform was added and centrifuged at 1200 RPM to extract the supernatant. Then the total RNA was added with isopropyl alcohol, centrifuged again, and the supernatant was discarded to obtain LncRNA precipitation. Thereafter, it was mixed with ethanol, and the absorbance was detected with the Multiskan Sky micro spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) after centrifugation at 7500 revolutions per minute. The

absorbance >1.8 indicated that the purity of RNA extraction was good. A TaqMan miRNA reverse transcription kit (ThermoFisher Scientific, Waltham, MA, USA) was used to reverse transcribe the total RNA into cDNA according to the kit instructions, and PCR quantification was performed by the TaqMan Universal PCR master mix (ThermoFisher Scientific, Waltham, MA, USA). After that, 5 uL extracted RNA sample was added to primers, Buffer solution, dNTP, and RNAse inhibitor. Then the mixture was kept at 94° for 4 min, denatured for 30 s, annealed at 50° for 30 s, and extended at 72° for 40 s for 40 cycles. lncRNA-ATB expression in tissues was calculated by using $2^{-\triangle\triangle Ct}$ with U6 as the internal reference. Primer sequences, which were designed and synthesized by ThermoFisher Scientific, Waltham, MA, USA, are detailed in Tab. 1.

Table 1: Primer sequences

Gene	Forward	Reverse
LncRNA-ATB	5'-TGACAAAGGCAGGAGGTA-3'	5'-ATCTCTGGGTGCTGGTGAAGG-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

2.3 Patient Follow-Up

Patients were followed up through clinic visits and telephone. The follow-up started from the time of diagnosis, and ended with the patient's death or the follow-up cut-off time August 2019, whichever occurred first.

2.4 Statistical Analyses

Data analysis was done by SPSS 22.0 software package (IBM Corp., Armonk, NY, USA), and the counting data were compared by χ^2 test. The clinical diagnostic value of LncRNA-ATB in NSCLC was determined by receiver operating characteristic (ROC) curves. Kaplan-Meier method and Log-rank test were utilized to calculate and compare the survival rate of patients with NSCLC. Univariate and multivariate Cox regression analyses were employed to quantitatively analyze the link between LncRNA-ATB and clinical prognosis of NSCLC patients. $P < 0.05$ was considered significant for all the analyses.

3 Results

3.1 LncRNA-ATB Expression in NSCLC Patients

LncRNA-ATB expression as detected by qRT-PCR showed that the level of LncRNA-ATB in cancer tissues was 2.34 ± 0.52, which was statistically higher than 1.84 ± 0.39 in adjacent tissues ($P < 0.05$) (Fig. 1).

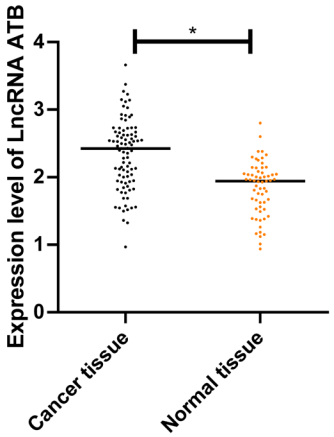

Figure 1: LncRNA-ATB expression in tissues of patients with NSCLC

The expression of LncRNA-ATB in cancer tissues (2.34 ± 0.52) was significantly higher than that in normal tissues (1.84 ± 0.39).

3.2 Association between LncRNA-ATB and Patient Clinicopathological Features

Eighty-nine patients with NSCLC were allocated into high (n = 45) and low (n = 44) expression groups with the the median expression in tissues as the boundary value. The results demonstrated that rather than age, gender, tumor number, differentiation degree, vascular invasion and pathological classification ($P > 0.05$), lncRNA-ATB was observably linked with smoking history, TNM stage and greatest tumor diameter (GTD) ($P < 0.05$).

Table 2: Association between lncRNA-ATB and patient clinicopathological features

Variables	High expression group (n = 45)	Low expression group (n = 44)	t/χ^2 value	P
Gender			3.242	0.072
Male	29 (64.44)	20 (45.45)		
Female	16 (35.56)	24 (54.55)		
Age (years old)			2.574	0.109
≤60	14 (31.11)	21 (47.73)		
>60	31 (68.89)	23 (52.27)		
Smoking history			3.288	0.001
Yes	32 (71.11)	16 (36.36)		
No	13 (28.89)	28 (63.64)		
Number of tumors			0.534	0.593
Single	12 (26.67)	14 (31.82)		
Multiple	33 (73.33)	30 (68.18)		
TNM stage			5.902	0.024
Ⅰ/Ⅱ	20 (44.44)	30 (68.18)		
Ⅲ/Ⅳ	25 (55.56)	14 (31.82)		
Differentiation degree			0.119	0.730
Moderate-high differentiation	26 (57.78)	27 (61.36)		
Low differentiation	19 (42.22)	17 (38.64)		
Vascular invasion			0.331	0.741
No	21 (46.67)	28 (63.64)		
Yes	14 (31.11)	16 (36.36)		
Greatest tumor diameter			15.460	<0.01
≥3 cm	34 (75.56)	15 (34.09)		
<3 cm	11 (24.44)	29 (65.91)		
Pathological classification			0.305	0.581
Adenocarcinoma	35 (77.78)	32 (72.73)		
Squamous cell carcinoma and undifferentiated small cell carcinoma	10 (22.22)	12 (27.27)		

3.3 LncRNA-ATB's Diagnostic Value in NSCLC

LNcRNA-ATB's clinical diagnostic value as evaluated by ROC curves demonstrated that the cut-off value, sensitivity, specificity and AUC were 2.103, 73.77%, 83.15% and 0.836 respectively (Tab. 3, Fig. 2).

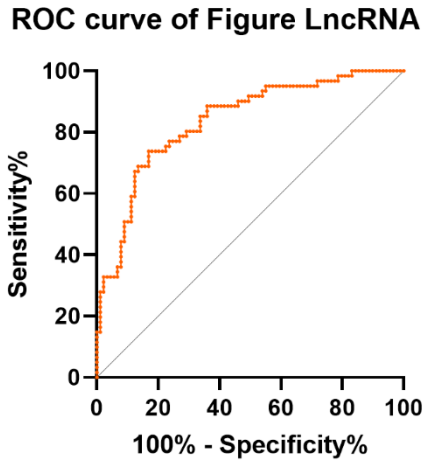

Figure 2: Diagnostic value of lncRNA-ATB in NSCLC

The cut-off value of LncRNA-ATB in diagnosing NSCLC was 2.103, the sensitivity was 73.77%, the specificity was 83.15%, and the area under the curve (AUC) was 0.836.

Table 3: Diagnostic value of lncRNA-ATB in NSCLC

Indicators	AUC	95%CI	Specificity (%)	Sensitivity (%)	Cut-off
LncRNA-ATB	0.836	0.771–0.901	83.15		2.103

3.4 Effects of LncRNA-ATB on Patient Survival

Kaplan-Meier method identified that the 3-year survival rate of patients with high lncRNA-ATB expression was 40.00%, which was statistically lower than that of patients with low expression (63.63%) ($P < 0.05$) (Fig. 3).

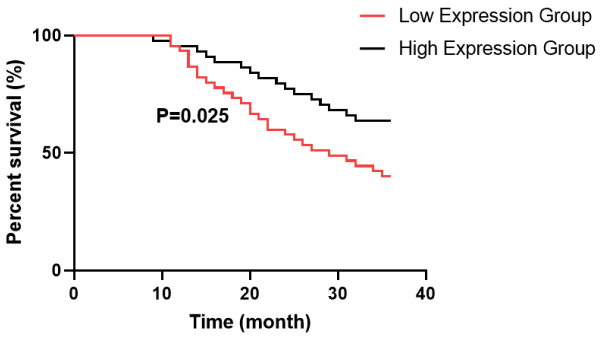

Figure 3: Effects of LncRNA-ATB on patient survival

The 3-year survival rate of patients with high expression of LncRNA-ATB (40.00%) was significantly lower than that of patients with low expression of LncRNA-ATB (63.63%), and the difference was statistically significant ($P < 0.05$).

3.5 Univariate Analysis of Prognosis in Patients with NSCLC

Univariate analysis indicated that patient prognosis was closely linked to GTD, tumor cell differentiation, TNM stage, and lncRNA-ATB expression ($P < 0.05$) (Tab. 4).

Table 4: Univariate analysis of prognosis in patients with NSCLC

Variables	Survival group (n = 46)	Death group (n=43)	t/χ^2 value	P
Gender			0.510	0.475
Male	22 (47.83)	27 (62.79)		
Female	21 (45.65)	19 (44.19)		
Age (years old)			1.597	0.206
≤60	21 (45.65)	14 (32.56)		
>60	25 (54.35)	29 (67.44)		
Smoking history			1.429	0.232
Yes	22 (47.83)	26 (60.47)		
No	24 (52.17)	17 (39.53)		
Number of tumors			1.428	0.232
Single	16 (34.78)	10 (23.26)		
Multiple	30 (65.22)	33 (76.74)		
TNM stage			15.330	<0.01
Ⅰ/Ⅱ	35 (76.09)	15 (34.88)		
Ⅲ/Ⅳ	11 (23.91)	28 (65.12)		
Differentiation degree			3.195	0.001
Moderate-high differentiation	20 (43.48)	33 (76.74)		
Low differentiation	26 (56.52)	10 (23.26)		
Vascular invasion			0.083	0.774
No	26 (56.52)	23 (53.49)		
Yes	20 (43.48)	20 (46.51)		
Greatest tumor diameter			5.158	0.023
≥3 cm	20 (43.48)	29 (67.44)		
<3 cm	26 (56.52)	14 (32.56)		
Pathological classification			0.096	0.757
Adenocarcinoma	34 (73.91)	33 (76.74)		
Squamous cell carcinoma and undifferentiated small cell carcinoma	12 (26.09)	10 (23.26)		
LncRNA-ATB expression			7.050	0.008
Lower expression	29 (63.04)	15 (34.88)		
High expression	17 (36.96)	28 (65.12)		

3.6 Multivariate Analysis of Prognostic Factors in Patients with NSCLC

The indicators with differences in Univariate analysis were included in the assignment (as shown in Tab. 5). Multivariate Cox proportional hazard regression model analysis demonstrated that TNM stage, lncRNA-ATB expression and differentiation degree were independent variables affecting patient prognosis ($P < 0.05$), as shown in Tab. 6.

Table 5: Assignment table

Variables	Assignment
Greatest tumor diameter (cm)	<3 = 1, ≥3 = 0.
Differentiation degree	Low differentiation= 1, moderate and high differentiation = 0.
TNM stage	I/II = 1, III/IV = 0.
LncRNA-ATB expression	High expression= 1, low expression = 0.
Survival	Death = 1, survival = 0

Table 6: Multivariate analysis of prognostic factors in patients with NSCLC

Groups	SE	Wald	Sig value	Exp (B)	95%CI
TNM stage	0.323	6.052	0.019	0.427	0.248-0.862
Differentiation degree	0.276	0.518	0.048	0.808	0.469-1.437
LncRNA-ATB expression	0.341	7.177	0.008	0.365	0.647-0.893

4 Discussion

LC is among the most widespread malignancies globally, and its morbidity and mortality rank first among all kinds of tumors [15]. The main reason for the adverse prognosis of NSCLC patients is the invasion and metastasis of tumor cells, but the pathogenesis, invasion and metastasis of LC are still unclear [16]. Various regulatory genes play crucial roles in the occurrence of NSCLC, so it is of paramount importance to explore the pathogenesis of NSCLC [17,18].

With the penetration into molecular biology, people gradually realize that the occurrence, development and metastasis of LC are complex processes involving multiple genes, stages and factors, integrating various molecular mechanisms [19–21]. As vital regulatory factors, lncRNAs provide a new direction for the study of malignancies and a shortcut for the search for therapeutic targets of NSCLC [21]. LncRNAs are abundant but most of their biological functions are still unknown. Although only a small number of lncRNAs have been studied, they have the potential to be molecular markers for tumor diagnosis and prognosis as their expression levels are related to the clinicopathological characteristics and prognosis of various tumors [22,23]. Located at chromosome 14, lncRNA-ATB is abnormally highly expressed in gastric cancer [24], colon cancer [25], breast cancer [26] and thyroid papillary carcinoma [27], and is closely related to tumor invasion and metastasis.

Our results showed that lncRNA-ATB increased in NSCLC tissues as compared to paracancer tissues, which was consistent with the findings in other tumors. In a study concerning the correlation between lncRNA-ATB and the occurrence and development of NSCLC, Ke et al. identified an abnormally elevated lncRNA-ATB in NSCLC tissues, which was strongly linked to the poor prognosis of NSCLC patients [27]. All these suggest that lncRNA-ATB participates in the malignant transformation of lung adenocarcinoma. Tumor size can reflect tumor load, and when tumor size is larger, its synthesis and release of lncRNA-ATB increase. Further analysis of clinicopathological data showed that rather than gender, age or pathological classification, abnormally expressed lncRNA-ATB was notably associated with smoking history, TNM stage and GTD. Similar to ours, Ke et al. [27] also found that high lncRNA-ATB was closely bound up with larger tumor sizes, lymph node metastases and distant metastases, suggesting that lncRNA-ATB may

promote the invasion and metastasis of NSCLC.

As the early clinical symptoms of NSCLC were not obvious, coupled with the fact that bronchoscopy and other examinations can only diagnose the lesions with imaging changes, and histological examination is not sensitive at the early stage, most patients were already in the advanced stage when they came to see a doctor, missing the best period of radical cure. On the contrary, if the diagnosis rate of LC patients can be improved, the survival rate of patients will be increased. Evidence has identified that serum lncRNA can be used in the diagnosis of malignant tumors [28]. Therefore, ROC curves were utilized for efficacy assessment of serum lncRNA-ATB in the diagnosis of NSCLC. It revealed that, the cut-off value, sensitivity and specificity, as well as AUC of NSCLC diagnosed by LncRNA-ATB were 2.103, 73.77%, 83.15% and 0.836, respectively, indicating that lncRNA-ATB was abnormally expressed in NSCLC and could be a feasible clinical tumor marker.

We also followed up all these 89 NSCLC patients for a period of 3 years, and survival analysis by Kaplan-Meier method indicated that the 3-year survival rate of patients with high LncRNA-ATB expression (40.00%) was statistically lower as compared to those with low expression (63.63%), suggesting that lncRNA-ATB may be a factor affecting patient prognosis. Subsequently, the relationship between LncRNA-ATB and patient clinical prognosis was quantitatively analyzed by univariate and multivariate Cox regression analyses. As demonstrated by univariate analysis, patient prognosis was strongly linked to the GTD, tumor cell differentiation, TNM stage and LncRNA-ATB expression. Cox multivariate survival analysis further showed that TNM stage, LncRNA-ATB expression and differentiation degree were independent risk factors for patients with NSCLC, confirming that LncRNA-ATB is a critical biological index for the prognosis of patients with NSCLC.

Although this study confirmed the diagnostic and prognostic value of lncRNA-ATB in NSCLC, there is still room for improvement. The underlying mechanism remains unclear, and its specific molecular mechanism needs to be further confirmed at the cellular level in the future. Moreover, we only followed up the survival of patients for three years, with only 89 patients included. In future studies, more cases will be included and the time will be extended to further confirm the role of lncRNA-ATB in NSCLC.

Collectively, lncRNA-ATB presents increased expression in NSCLC tissues, and is involved in the development of NSCLC and associated with poor prognosis of patients, which could be a plausible tumor marker for NSCLC patients.

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References

1. Anichini, A., Tassi, E., Grazia, G., Mortarini, R. (2018). The non-small cell lung cancer immune landscape: Emerging complexity, prognostic relevance and prospective significance in the context of immunotherapy. *Cancer Immunology Immunotherapy, 67(6),* 1011–1022. DOI 10.1007/ Cancer Immunol Immunother. 00262-018-2147-7.
2. Qu, J., Zhang, Y., Chen, X., Yang, H., Zhou, C. et al. (2017). Newly developed anti-angiogenic therapy in non-small cell lung cancer. *Oncotarget, 9(11),* 10147–10163. DOI 10.18632/oncotarget.23755.
3. Gong, B. Y., Jiang, N., Yan, G. M., Wang, S. Y., Deng, C. Y. et al. (2016). Predictors for severe acute esophagitis in lung cancer patients treated with chemoradiotherapy: A systematic review. *Current Medical Research and Opinion, 10(32),* 1701–1708.
4. Zheng, L. L., Li, J. H., Wu, J., Sun, W. J., Liu, S. (2016). deepBase v2. 0: Identification, expression, evolution and function of small RNAs, LncRNAs and circular RNAs from deep-sequencing data. *Nucleic Acids Research, 44(D1),* D196–D202.
5. Tu, Z. B., He, D., Deng, X. Z., Xiong, M., Huang, X. X. et al. (2016). An eight-long non-coding RNA signature as a candidate prognostic biomarker for lung cancer. *Oncology Reports, 36(1),* 215–222.

6. Xie, H. B., Ma, B., Gao, Q. J., Zhan, H. J., Liu, Y. C. et al. (2018). Long non-coding RNA CRNDE in cancer prognosis: Review and meta-analysis. *Clinica Chimica Acta, 485, 262–271.* DOI 10.1016/j.cca.2018.07.003.
7. Wang, J. C., Su, Z. L., Lu, S. N., Fu, W., Liu, Z. F. et al. (2018). LncRNA HOXA-AS2 and its molecular mechanisms in human cancer. *Clinica Chimica Acta, 485, 229–233.* DOI 10.1016/j.cca.2018.07.004.
8. Thin, K. Z., Tu, J. C., Raveendran, S. (2019). Long non-coding SNHG1 in cancer. *Clinica Chimica Acta, 494, 38–47.* DOI 10.1016/j.cca.2019.03.002.
9. Yu, W. J., Sun, Z. N., Yang, L., Han, Y. F., Yue, L. et al. (2019). lncRNA PTAR promotes NSCLC cell proliferation, migration and invasion by sponging microRNA 101. *Molecular Medicine Reports, 20(5),* 4168–4174. DOI 10.3892/mmr.2019.10646.
10. Li, Z., Lu, Q., Zhu, D., Han, Y., Zhou, X. et al. (2018). Lnc-SNHG1 may promote the progression of non-small cell lung cancer by acting as a sponge of miR-497. *Biochemical and Biophysical Research Communications, 506(3), 632–640.* DOI 10.1016/j.bbrc.2018.10.086.
11. Guo, F., Li, Y., Liu, Y., Wang, J., Li, Y. et al. (2010). Inhibition of metastasis-associated lung adenocarcinoma transcript 1 in CaSki human cervical cancer cells suppresses cell proliferation and invasion. *Acta Biochimica Et Biophysica Sinica, 42(3),* 224–229.
12. Yamada, K., Kano, J., Tsunoda, H., Okuboc, C., Ishiyama, T. et al. (2006). Phenotypic characterization of endometrial stromal sarcoma of the uterus. *Cancer Science, 97(2),* 106–112.
13. Fellenberg, J., Bernd, L., Delling, G., Witte, D., Zahlten, H. A. et al. (2007). Prognostic significance of drug-regulated genes in high-grade osteosarcoma. *Modern Pathology, 20(10),*1085–1094.
14. Dong, Y. Q., Liang, G. J., Yuan, B., Yang, C. Q., Gao, R. et al. (2015). MALAT1 promotes the proliferation and metastasis of osteosarcoma cells by activating the PI3K/Akt pathway. *Tumor Biology, 36(3),* 1477–1486.
15. Naylor, E. C., Desani, J. K., Chung, P. K. (2016). Targeted therapy and immunotherapy for lung cancer. *Surgical Oncology Clinics of North America, 25(3),* 601–609. DOI 10.1016/j.soc.2016.02.011.
16. Herbst, R. S., Morgensztern, D., Boshoff, C. (2018). The biology and management of non-small cell lung cancer. *Nature, 553(7689),* 446–454. DOI 10.1038/nature25183.
17. Román, M., Baraibar, I., López, I., Nadal, E., Rolfo, C. et al. (2018). KRAS oncogene in non-small cell lung cancer: Clinical perspectives on the treatment of an old target. *Molecular Cancer, 17(1),* 33. DOI 10.1186/s12943-018-0789-x.
18. Prabhu, V. V., Devaraj, S. N. (2017). KAI1/CD82, metastasis suppressor gene as a therapeutic target for non-small-cell lung carcinoma. *Journal of Environmental Pathology, Toxicology and Oncology, 6(3),* 269-275. DOI 10.1615/JEnvironPatholToxicolOncol.2017024619.
19. Zhang, Z., Yan, S., Cui, H., Chen, H., Liu, J. (2019). Correlation between RASSF1A gene promoter hypermethylation in serum or sputum and Non-Small Cell Lung Cancer (NSCLC): A meta-analysis. *Medical Science Monitor, 25,* 5518–5524. DOI 10.12659/MSM.917457.
20. Minchom, A., Thavasu, P., Ahmad, Z., Stewart, A., Georgiou, A. et al. (2017). A study of PD-L1 expression in KRAS mutant non-small cell lung cancer cell lines exposed to relevant targeted treatments. *PLoS One, 12(10),* e0186106. DOI 10.1371/journal.pone.0186106.
21. Wu, W., Zhao, Y., Gao, E., Li, Y., Zhang, H. (2020). LncRNA DLEU2 accelerates the tumorigenesis and invasion of non-small cell lung cancer by sponging miR-30a-5p. *Journal of Cellular and Molecular Medicine, 24(1),* 441–450.
22. Sun, W., Zu, Y., Fu, X., Deng, Y. (2017). Knockdown of lncRNA-XIST enhances the chemosensitivity of NSCLC cells via suppression of autophagy. *Oncology Reports, 38(6),* 3347–3354. DOI 10.3892/or.2017.6056.
23. Chen, Y., Wei, G. Q., Xia, H. W., Tang, Q. L., Bi, F. (2018). Long noncoding RNAATB promotes cell proliferation, migration and invasion in gastriccancer. *Molecular Medicine Reports, 17(1),* 1940–1946.
24. Yue, B., Qiu, S. L., Zhao, S. L., Liu, C. C., Zhang, D. Y. et al. (2016). LncRNA-ATB mediated E-cadherin repression promotes the progression of colon cancer and predicts poor prognosis. *European Journal of Gastroenterology & Hepatology, 31(3),* 595–603.
25. Shi, S. J., Wang, L. J., Yu, B., Li, Y. H., Jin, Y. et al. (2015). LncRNA-ATB promotes trastuzumab resistance and invasion-metastasis cascade in breast cancer. *Oncotarget, 6(13),* 11652–11663.
26. Fu, X. M., Guo, W., Li, N., Liu, H. Z., Liu, J. et al. (2017). The expression and function of long

noncoding RNA lncRNA-ATB in papillary thyroid cancer. *European Review for Medical and Pharmacological Sciences, 21(14),* 3239–3246.

27. Ke, L., Xu, S. B., Wang, J., Jiang, X. L., Xu, M. Q. (2017). High expression of long non-coding RNA ATB indicates a poor prognosis and regulates cell proliferation and metastasis in non-small cell lung cancer. *Clinical and Translational Oncology, 19(5),* 1–7.
28. Tang, Q., Ni, Z., Cheng, Z., Xu, J., Yu, H. et al. (2015). Three circulating long non-coding RNAs act as biomarkers for predicting NSCLC. *Cellular Physiology and Biochemistry, 37(3),* 1002–1009. DOI:10.1159/000430226.