European Review for Medical and Pharmacological Sciences 2018; 22: 4524-4531

Long noncoding RNA LINC01296 is associated with poor prognosis in ESCC and promotes ESCC cell proliferation, migration and invasion

B. WANG[1], T. LIANG[2], J. LI[1]

[1]Department of Thoracic Surgery, Chinese PLA General Hospital, Beijing, China
[2]Department of Thoracic Surgery, PLA Rocket Force General Hospital, Beijing, China

Bin Wang and Tao Liang contributed equally to this work

Abstract. – OBJECTIVE: Recent studies have reported that long intergenic non-protein-coding RNA 1296 (LINC01296) regulates the tumorigenesis and the progression of several tumors, but the role of LINC01296 in esophageal squamous cell carcinoma (ESCC) remains unclear. The purpose of this study was to examine the expression, function, and clinical significance of LINC01296 in ESCC.

PATIENTS AND METHODS: Expression of LINC01296 was analyzed in 221 ESCC tissues and three ESCC cell lines by Real-time quantitative RT-PCR. The correlation between LINC01296 levels and other clinical features, disease-free survival (DFS), and overall survival (OS) was analyzed statistically. The function of LINC01296 on cell proliferation, migration, and invasion was confirmed *in vitro* through MTT assay and transwell assay.

RESULTS: We found that LINC01296 was upregulated in ESCC cell lines and cancerous tissues, as compared with normal esophagus cells and adjacent normal tissue samples. High LINC01296 expression was significantly correlated with differentiation grade (p=0.000), lymph nodes metastasis (p=0.002), distant metastasis (p=0.002), and TNM stage (p = 0.015). Moreover, ESCC patients with high LINC01296 expression experienced shorter OS and DFS (p=0.0009 and p=0.0005, respectively). In addition, univariate and multivariate analysis showed that LINC01296 expression was an independent predictor for both OS and DFS in ESCC. Functionally, the results of *in vitro* assay indicated that down-regulation of LINC01296 significantly suppressed ESCC cells proliferation, migration, and invasion, suggesting that LINC01296 contributed to tumorigenesis of ESCC.

CONCLUSIONS: Our findings indicate that LINC01296 exerts a role in promoting the development of human ESCC. Up-regulation of LINC01296 could be considered as a predictor for diagnosis and prognosis of ESCC patients.

Key Words
Long non-coding RNA, LINC01296, Esophageal squamous cell carcinoma, Prognosis, Proliferation, Migration, Invasion.

Introduction

Esophageal carcinoma is one of the most common cancers of the digestive tract with a metastatic potential[1]. In China, esophageal carcinoma is the eighth most common cause of cancer-related mortality[2]. Esophageal squamous cell carcinoma (ESCC) accounted for the most common histological type of esophageal cancer[3]. Despite treatment and perioperative management have evolved in recent years, the five-year survival rates for patients with ESCC remain low at about 10%, due primarily to late diagnosis and metastatic potential of ESCC[4,5]. Therefore, a better understanding of the molecular mechanism of ESCC progression is needed to develop highly sensitive molecular biomarkers with reliable clinical significance.

Long non-coding RNAs (lncRNAs) are a class of RNAs longer than 200 nucleotides without proteins encoding functions[6]. However, some studies have revealed that a number of lncRNAs can regulate gene expression at various levels, including in chromatin modification, transcription, and posttranscriptional processing[7]. Of note, previous functional studies indicated that lncRNAs regulate multiple physiological processes, including differentiation, growth, and cell death[8,9]. In tumor research, evidence has shown that specific lncRNAs serve as oncogenes or tumor suppressor genes[10,11]. Given the important role of lncRNA and its abnormal expression, the potential of lncRNAs as tumor biomarkers for diagnosis and prediction of prognosis in tumors draws researchers' attention[12]. Up to date, many lncRNAs have been reported to be associated with prognosis, tumor cells growth and metastasis in various tumors, including ESCC[13-15]. However, the expression and function of most other lncRNAs remain to be elucidated.

Corresponding Author: Jie Li, MD; e-mail: jlline999@yeah.net

LINC01296, located at chromosome 14q11.2, has been shown to be dysregulated in several tumors[16,17]. However, as a newly identified lncRNA, the function and molecular mechanism of LINC01296 in tumors remain largely unclear. Recently, Dai et al[18] reported that LINC01296 expression was significantly up-regulated in ESCC tissues by microarray analysis. However, to our best knowledge, the specific biological function and clinical significance of LINC01296 have not been investigated. Our present investigation, for the first time, found that LINC01296 may be used as a prognostic biomarker and a novel therapeutic target for ESCC.

Patients and Methods

Patients and Clinical Specimens

221 primary ESCC tissue samples and the adjacent normal esophageal tissues were obtained from patients who were treated at the Chinese PLA General Hospital between 2010 and 2013. The diagnosis was based on clinical examination and histopathological analysis of the samples. None of the patients had received preoperative chemotherapy. To clarify the survival conditions, life-long follow-up was available by direct telephone interview. American Joint Committee on Cancer Staging Manual (7^{th} edition, 2010) was used to assess the tumor stage. This study was approved by the Ethics Committee of Chinese PLA General Hospital. All patients gave written informed consent to participate in the present study. Detailed clinical characteristics of all the patients are shown in Table II.

Cell Culture and Cell Transfection

Three human ESCC cell lines (ECa-109, EC-9706, TE-1) and human esophageal epithelial cells (HEEC) were provided by Chongqing Longhu Technology Co., Ltd. (Nanan, Chongqing, China). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium containing 10% fetal bovine serum (FBS; Gibco, Haidian, Beijing), ampicillin and streptomycin, at 37°C in a humidified cell incubator with an atmosphere of 5% CO_2.

SiRNA-LINC01296 (si-LINC01296) and si-NC were purchased from Invitrogen (Carlsbad, CA, USA). TE-1 cells cultured on six-well plate were transfected with siRNA or negative control using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The sequences of si-LINC01296 were shown in Table I.

Quantitative Real-Time PCR

Total RNA was extracted from ESCC tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Reverse transcript PCR was carried out using PrimeScript RT Master Mix (TaKaRa, Dalian, Niaoling, China) according to the manufacturer's instructions. Quantitation of LINC01296 was performed using SYBR® Green PCR Master Mix (Biosystems, Foster City, CA, USA) on the ABI 7900 Sequence Detection System (Life Technologies, St. Louis, MO, USA). The results were normalized to the expression of glyceraldehydes-3-phosphate dehydrogenase (GAPDH). The primers used were shown in Table I. The relative quantitative value was expressed by the $2^{-\Delta\Delta Ct}$ method. Independent experiments were done in triplicate.

Cell Proliferation Assay

TE-1 cells were plated in a 96-well plate at a density of 4×10^3 cells per well and incubated at 37°C in a 5% CO_2 humidified environment. At different time points (24, 48, 72, or 96 h), the culture medium was removed and replaced with fresh medium containing 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). 150 ul of dimethyl sulfoxide (DMSO, Macklin, Xuhui, Shanghai, China) was then added, and the absorbance was measured at 450 nm.

Table I. The sequences of primers and siRNAs used in this study.

Name		Sequence
LINC01296	Forward	5'-AAGTGGCACCAGCCTCACT-3'
	Reverse	5'-CGGCCAAGT TCTTTACCATC-3'
GAPDH	Forward	5'-ACT GTG CCG ACT TGA CGT TT-3'
	Reverse	5'-ATC GTA GTG AAC GGT CGA TTGT-3'
si-LINC01296		5'-GGCUGGAGAAUAUUUCCUATTTT-3'

Table II. Correlation between LINC01296 expression and different clinicopathological features in ESCC patients.

Parameters	Number	LINC01296 expression		p-value
		Low	High	
Gender				
Male	134	64	70	0.809
Female	87	43	44	
Age (years)				0.271
< 60	99	52	47	
≥ 60	122	55	67	
Tumor location				0.715
Middle	104	49	55	
Lower	117	58	59	
Differentiation grade				0.000
G1	130	76	54	
G2+G3	91	31	60	
Lymph nodes metastasis				0.002
Absence	138	78	60	
Presence	83	29	54	
Distant metastasis				0.002
Yes	91	33	58	
No	130	74	56	
TNM stage				0.015
I + II	152	82	70	
III + IV	69	25	44	

Transwell Assay

For migration assay, a total of 1×10^4 transfected cells in serum-free medium were seeded in the upper chamber. Medium containing 10% fetal bovine serum was added to the lower compartment as a chemo-attractant. For invasion assay, chambers were coated with Matrigel (BD Biosciences, San Jose, CA, USA). After 24 h, the cells moving to the other side were stained with the dye crystal violet. Then, cells that had migrated across the membrane and were counted from five randomly chosen fields in each well using a light microscope.

Statistical Analysis

All statistical analyses were carried out using GraphPad Prism, version 5 (GraphPad Software, Inc., La Jolla, CA, USA). All experiments were independently repeated at least triplicate. The significance of differences between groups was estimated by the Student's *t*-test and the x^2-test. Disease-free survival (DFS) and overall survival (OS) curves were determined by the Kaplan-Meier method, and the survival differences of patients were evaluated by the log-rank test. The influence of each variable on survival was examined by the Cox regression analysis. $p < 0.05$ was considered statistically significant.

Results

LINC01296 was Upregulated in ESCC Tissues and Cell lines

Previous results from microarray data revealed that LINC01296 expression was up-regulated in ESCC tissues. To demonstrate dysregulation of LINC01296 in ESCC patients, we further detected the expression levels of ESCC tissues from the patients in our hospital. As shown in Figure 1A, the results showed that LINC01296 expression was significantly increased in ESCC tissues than in matched normal tissues ($p < 0.01$). In addition, we also determined the expression of LINC01296 in three ESCC cell lines (ECa-109, EC-9706, TE-1) and human esophageal epithelial cells (HEEC). As shown in Figure 1B, it showed that LINC01296 expression was markedly upregulated in all ESCC cell lines compared to that in HEEC.

Correlation Between LINC01296 Expression and Clinicopathological Features

Next, we explored the association of LINC01296 expression level with the clinical pathological factors in ESCC patients. All ESCC patients were subsequently divided into two groups (high ex-

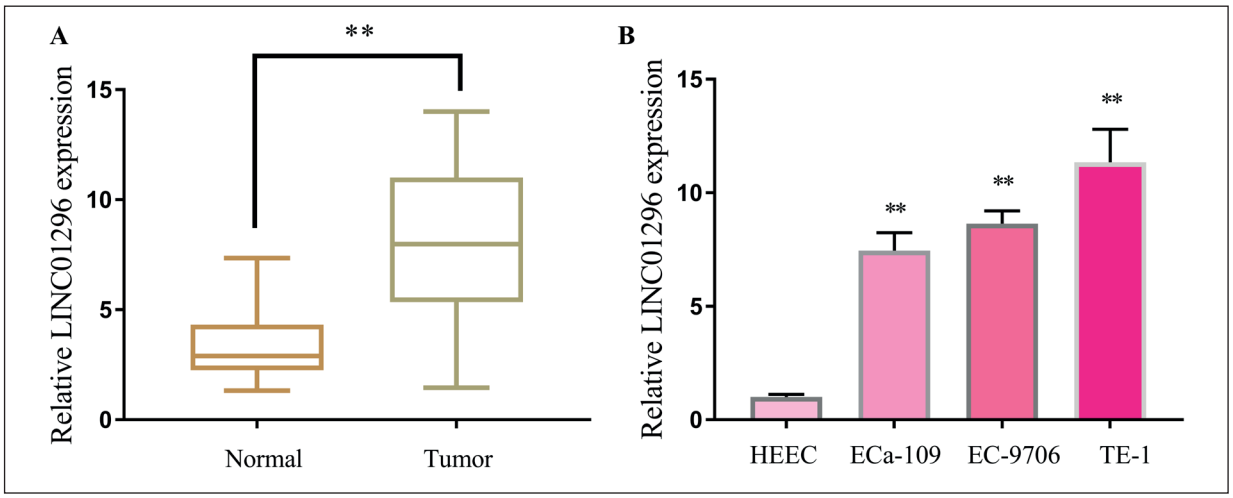

Figure 1. Upregulation of LINC01296 in ESCC tissues and cells. **A**, The expression of LINC01296 was analyzed in ESCC tissues and matched normal tissues by qRT-PCR. **B**, Expression of LINC01296 in ESCC cells (ECa-109, EC-9706, TE-1) and normal esophageal cell line NEEC. **p<0.01.

pression group and low expression group) based on the mean expression of LINC01296. As shown in Table II, the result of chi-square test revealed that high LINC01296 expression was positively correlated with differentiation grade (p = 0.000), lymph nodes metastasis (p = 0.002), distant metastasis (p = 0.002), and TNM stage (p = 0.015) in ESCC patients. However, there were no significant correlations between LINC01296 expression and other clinicopathologic features including gender, age, tumor location (p > 0.05).

Upregulation of LINC01296 Was Correlated With Poor Prognosis in ESCC

Subsequently, Kaplan-Meier and log-rank test analyses were performed to investigate the correlation between LINC01296 expression and patients' OS or DFS. As shown in Figure 2, ESCC patients with high LINC01296 expression had significantly shorter OS (p = 0.009) and DFS (Figure 3, p = 0.005) time than those with low LINC01296 expression. Then, we used Cox regression to investigate the association between

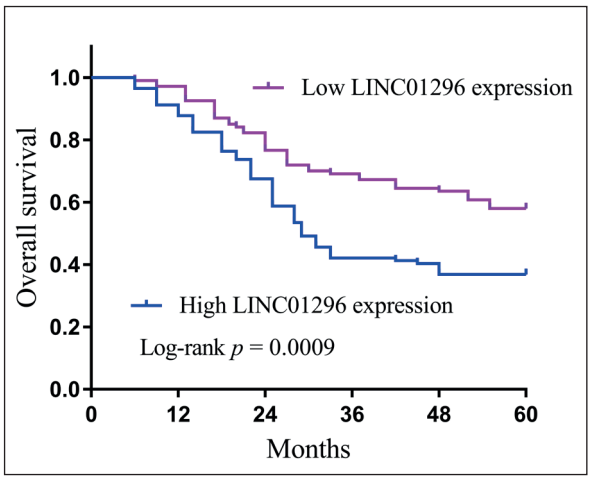

Figure 2. ESCC patients with high LINC01296 expression showed shorter OS compared with patients exhibiting low LINC01296 expression by Kaplan-Meier survival curve (p=0.0009).

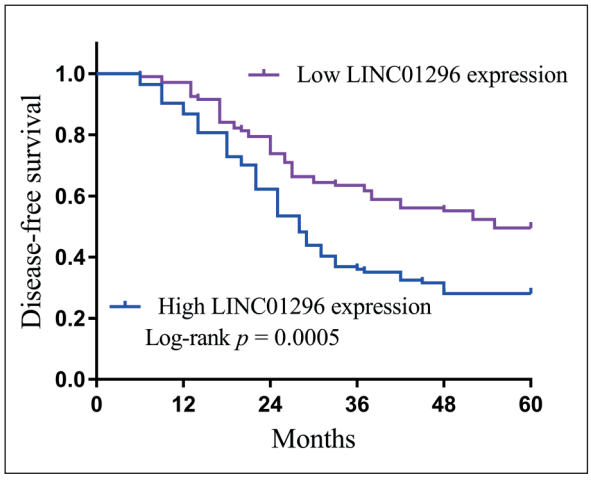

Figure 3. ESCC patients with high LINC01296 expression showed shorter DFS compared with patients exhibiting low LINC01296 expression by Kaplan-Meier survival curve (p=0.0005).

Table III. Univariate and multivariate analysis of prognostic factors in for overall survival.

Variables	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	*p*	Risk ratio	95% CI	*p*
Gender	0.783	0.533-1.648	0.423	-	-	-
Age	0.856	0.637-1.899	0.371	-	-	-
Tumor location	1.231	0.834-2.123	0.189	-	-	-
Differentiation grade	4.323	1.893-9.452	0.001	3.894	1.673-7.673	0.001
Lymph nodes metastasis	3.673	1.563-6.677	0.004	2.783	1.277-4.263	0.008
Distant metastasis	3.251	1.256-5.783	0.006	2.773	1.039-4.563	0.012
TNM stage	2.893	1.348-4.763	0.012	2.563	1.138-3.774	0.025
LINC01296 expression	3.783	1.669-7.693	0.001	2.893	1.253-5.563	0.004

LINC01296 expression and prognosis further. The results of univariate analysis indicated that differentiation grade, lymph nodes metastasis, distant metastasis, TNM stage, and LINC01296 expression were significantly associated with OS (Table III) and DFS (Table IV) of ESCC patients. In addition, multivariate Cox regression analysis enrolling above-mentioned significant parameters confirmed that LINC01296 expression was an independent prognostic marker for OS (Table III, $p = 0.004$) and DFS (Table IV, $p = 0.003$) of ESCC patients.

Effect of LINC01296 Lower Expression on Tumor Growth and Metastasis in vitro

To further assess the biological function of LINC01296 on proliferation, migration, and invasion of ESCC cells, TE-1 cells were transfected with si-LINC01296 or NC. As shown in Figure 4A, the expression of TUG1 was significantly downregulated in si-LINC01296 transfected cells. Then, we performed MTT to explore the effect of LINC01296 on TE-1 cells proliferation. The results showed that after si-LINC01296 transfection, the viability of the TE-1 cells was evidently decreased at 96 h time-point (Figure 4B). Furthermore, we investigated whether LINC01296 affected migration and invasion of TE-1 cells using transwell. We observed that the cell migration and invasion capacity of TE-1 cells transfected with si-LINC01296 was significantly decreased compared with the control group (Figure 4C). These results revealed that LINC01296 promoted ESCC cell growth and metastasis, and served as a tumor oncogene in ESCC tumorigenesis.

Discussion

Although diagnosis and therapy of ESCC have been greatly advanced for decades, the incidence and mortality rates remain very high[19]. An accurate prognostic assay is extremely critical for planning ESCC treatment strategies[20]. Although several clinicopathological features have been used for prognosis prediction for ESCC patients, this classification scheme is not accurate for an in-

Table IV. Univariate and multivariate analysis of prognostic factors in for disease-free survival.

Variables	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	*p*	Risk ratio	95% CI	*p*
Gender	0.934	0.732-2.136	0.163	-	-	-
Age	1.267	0.845-2.325	0.117	-	-	-
Tumor location	1.644	0.548-2.783	0.213	-	-	-
Differentiation grade	4.783	1.932-9.132	0.001	4.219	1.563-8.223	0.001
Lymph nodes metastasis	4.023	1.677-6.745	0.001	3.251	1.352-5.764	0.006
Distant metastasis	3.563	1.793-5.273	0.006	3.211	1.352-4.139	0.009
TNM stage	3.261	1.893-4.732	0.008	2.673	1.564-4.023	0.013
LINC01296 expression	4.213	1.389-8.784	0.001	3.263	1.193-6.763	0.003

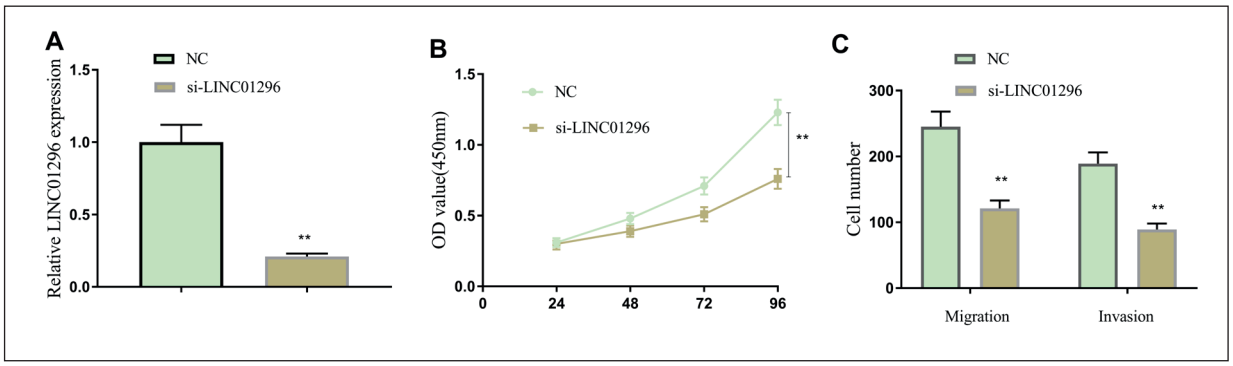

Figure 4. LINC01296 down-regulation suppressed ESCC cells proliferation, migration, and invasion. **A**, Expression of LINC01296 was determined in TE-1 cells transfected with si-LINC01296 or NC. **B**, Cell proliferation was measured by MTT assays in TE-1 cells transfected with si-LINC01296 or NC. **C**, The migration and invasion abilities of TE-1 cells transfected with si-LINC01296 or NC were evaluated by transwell assays. $^{**}p<0.01$.

dividual patient[21]. Thanks to the advances in molecular and cellular biology of tumors, molecular technique has been regarded as an ideal method for prognosis prediction in ESCC[22]. Recently, several research groups reported that functional lncRNAs may be applied for ESCC diagnosis and prognosis, and also may serve as potential therapeutic targets. Various molecular mechanisms were identified. For instance, Xu et al[23] reported that lncRNA HOTAIR was highly expressed in ESCC patients and accelerated the proliferation and metastasis of ESCC cells via acting as a miR-148a sponge. Ren et al[24] reported that the down-regulation of lncRNA MIR31HG was found in ESCC, which often led to a poor prognosis. On the other hand, the diagnostic value of lncRNA POU3F3 was also demonstrated in ESSC patients[25]. Those results encouraged us to identify more lncRNAs.

The possible role and associated molecular mechanism of LINC01296 in several human tumors have been studied. Qiu et al[26] reported that up-regulation of LINC01296 was observed in colorectal cancer, and patients with high expression of LINC01296 have significantly poorer prognosis than those with low expression. Wu et al[27] found that LINC01296 was highly expressed in prostate cancer and its overexpression promoted the proliferation and metastasis of prostate cancer cells by modulating PI3K-Akt-mTOR signaling pathway. Qin et al[28] showed that LINC01296 was up-regulated in GC tissue and correlated with poor prognosis. In addition, functional and mechanistic assay revealed that forced LINC01296 expression promoted gastric cancer cells proliferation, migration, and invasion through targeting miR-122. Those results suggested LINC01296 as a tumor promoter in tumor progression. Dai et al[18] reported that LINC01296 expression was significantly up-regulated in ESCC tissues by microarray analysis. However, to our best knowledge, whether LINC01296 was associated with prognosis of ESCC patients, and its function in ESCC cells growth and metastasis had not been reported.

In the present work, we detected the expression level of LINC01296 in ESCC tissues from the patients of our hospital to further demonstrate whether LINC01296 expression was up-regulated in ESCC patients. Our data were in agreement with the findings of microarray analysis. In addition, up-regulation of LINC01296 was also observed in ESCC cells lines compared to normal esophagus cells. Those results were strongly suggesting the possibility that LINC01296 can be used as a potential biomarker to detect ESCC. Furthermore, the high expression of LINC01296 conferred malignant clinical parameters of ESCC patients including differentiation grade, lymph nodes metastasis, distant metastasis, and advanced TNM stage. More importantly, Kaplan-Meier survival curves revealed that the higher expression level of LINC01296 was closely related to a shorter OS and DFS. Subsequently, both univariate and multivariate analysis confirmed that LINC01296 expression was an independent prognostic marker for DFS and OS in ESCC patients. To investigate the function of LINC01296 in ESCC cells behavior, a loss-of-function experiment was performed *in vitro*. Our data showed that LINC01296 down-regulation suppressed tumor growth by reducing ESCC cell proliferation, migration, and invasion ability. These results supported that LINC01296 was involved in the development and progression of ESCC.

Conclusions

We showed that decreased expression of LINC01296 was associated with poor clinical features of ESCC patients. Functionally, knockdown of LINC01296 expression in ESCC cells leads to inhibition of ESCC cells proliferation and metastasis. These findings suggest that LINC01296 may be a potential candidate for anti-cancer therapy and a prognostic marker for ESCC.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Arnold M, Soerjomataram I, Ferlay J, Forman D. Global incidence of oesophageal cancer by histological subtype in 2012. Gut 2015; 64: 381-387.
2) Lin Y, Totsuka Y, He Y, Kikuchi S, Qiao Y, Ueda J, Wei W, Inoue M, Tanaka H. Epidemiology of esophageal cancer in Japan and China. J Epidemiol 2013; 23: 233-242.
3) Ohashi S, Miyamoto S, Kikuchi O, Goto T, Amanuma Y, Muto M. Recent advances from basic and clinical studies of esophageal squamous cell carcinoma. Gastroenterology 2015; 149: 1700-1715.
4) Napier KJ, Scheerer M, Misra S. Esophageal cancer: a review of epidemiology, pathogenesis, staging workup and treatment modalities. World J Gastrointest Oncol 2014; 6: 112-120.
5) Cobanoğlu U, Dülger C, Kemik O, Celik S, Sayir F. A novel screening test for esophageal squamous cell carcinoma: sirtuin-3. Eur Rev Med Pharmacol Sci 2017; 21: 5399-5401.
6) Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 2014; 15: 7-21.
7) Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 2016; 17: 47-62.
8) Klattenhoff CA, Scheuermann JC, Surface LE, Bradley RK, Fields PA, Steinhauser ML, Ding H, Butty VL, Torrey L, Haas S, Abo R, Tabebordbar M, Lee RT, Burge CB, Boyer LA. Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. Cell 2013; 152: 570-583.
9) Moran VA, Perera RJ, Khalil AM. Emerging functional and mechanistic paradigms of mammalian long non-coding RNAs. Nucleic Acids Res 2012; 40: 6391-6400.
10) Martens-Uzunova ES, Böttcher R, Croce CM, Jenster G, Visakorpi T, Calin GA. Long noncoding RNA in prostate, bladder, and kidney cancer. Eur Urol 2014; 65: 1140-1151.
11) 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, Mimori K. A long noncoding RNA, lncRNA-ATB, is involved in the progression and prognosis of colorectal cancer. Anticancer Res 2015; 35: 1385-1388.
12) Maruyama R, Suzuki H. Long noncoding RNA involvement in cancer. BMB Rep 2012; 45: 604-611.
13) Ono H, Motoi N, Nagano H, Miyauchi E, Ushijima M, Matsuura M, Okumura S, Nishio M, Hirose T, Inase N, Ishikawa Y. Long noncoding RNA HOTAIR is relevant to cellular proliferation, invasiveness, and clinical relapse in small-cell lung cancer. Cancer Med 2014; 3: 632-642.
14) Tan DSW, Chong FT, Leong HS, Toh SY, Lau DP, Kwang XL, Zhang X, Sundaram GM, Tan GS, Chang MM, Chua BT, Lim WT, Tan EH, Ang MK, Lim TKH, Sampath P, Chowbay B, Skanderup AJ, DasGupta R, Iyer NG. Long noncoding RNA EGFR-AS1 mediates epidermal growth factor receptor addiction and modulates treatment response in squamous cell carcinoma. Nat Med 2017; 23: 1167-1175.
15) Wang D, Gao ZM, Han LG, Xu F, Liu K, Shen Y. Long noncoding RNA CASC2 inhibits metastasis and epithelial to mesenchymal transition of lung adenocarcinoma via suppressing SOX4. Eur Rev Med Pharmacol Sci 2017; 21: 4584-4590.
16) Feng L, Houck JR, Lohavanichbutr P, Chen C. Transcriptome analysis reveals differentially expressed lncRNAs between oral squamous cell carcinoma and healthy oral mucosa. Oncotarget 2017; 8: 31521-31531.
17) Seitz AK, Christensen LL, Christensen E, Faarkrog K, Ostenfeld MS, Hedegaard J, Nordentoft I, Nielsen MM, Palmfeldt J, Thomson M, Jensen MT, Nawroth R, Maurer T, Ørntoft TF, Jensen JB, Damgaard CK, Dyrskjøt L. Profiling of long non-coding RNAs identifies LINC00958 and LINC01296 as candidate oncogenes in bladder cancer. Sci Rep 2017; 7: 395.
18) Dai F, Mei L, Meng S, Ma Z, Guo W, Zhou J, Zhang J. The global expression profiling in esophageal squamous cell carcinoma. Genomics 2017; 109: 241-250.
19) Hur C, Miller M, Kong CY, Dowling EC, Nattinger KJ, Dunn M, Feuer EJ. Trends in esophageal adenocarcinoma incidence and mortality. Cancer 2013 15; 119: 1149-1158.
20) Nieman DR, Peters JH. Treatment strategies for esophageal cancer. Gastroenterol Clin North Am 2013; 42: 187-197.
21) Tachibana M, Hirahara N, Kinugasa S, Yoshimura H. Clinicopathologic features of superficial esophageal cancer: results of consecutive 100 patients. Ann Surg Oncol 2008; 15: 104-116.
22) Cao HH, Zheng CP, Wang SH, Wu JY, Shen JH, Xu XE, Fu JH, Wu ZY, Li EM, Xu LY. A molecular prognostic model predicts esophageal squamous cell carcinoma prognosis. PLoS One 2014; 9: e106007.
23) Xu F, Zhang J. Long non-coding RNA HOTAIR functions as miRNA sponge to promote the epithelial to mesenchymal transition in esophageal cancer. Biomed Pharmacother 2017; 90: 888-896.
24) Ren ZP, Chu XY, Xue ZQ, Zhang LB, Wen JX, Deng JQ, Hou XB. Down-regulation of lncRNA MIR31HG correlated with aggressive clinicopathological features and unfavorable prognosis in esophageal squamous cell carcinoma. Eur Rev Med Pharmacol Sci 2017; 21: 3866-3870.

25) Tong YS, Wang XW, Zhou XL, Liu ZH, Yang TX, Shi WH, Xie HW, Lv J, Wu QQ, Cao XF. Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma. Mol Cancer 2015; 14: 3.
26) Qiu JJ, Yan JB. Long non-coding RNA LINC01296 is a potential prognostic biomarker in patients with colorectal cancer. Tumour Biol 2015; 36: 7175-7183.
27) Wu J, Cheng G, Zhang C, Zheng Y, Xu H, Yang H, Hua L. Long noncoding RNA LINC01296 is associated with poor prognosis in prostate cancer and promotes cancer-cell proliferation and metastasis. Onco Targets Ther 2017; 10: 1843-1852.
28) Qin QH, Yin ZQ, Li Y, Wang BG, Zhang MF. Long intergenic noncoding RNA 01296 aggravates gastric cancer cells progress through miR-122/MMP-9. Biomed Pharmacother 2018; 97: 450-457.