

Original Article

The long noncoding RNA AK139328 promotes the oncogenesis in thyroid cancer

Siqi Li*, Zhijing Zhao*, Yan Li, Yi Zhang, Liying Dong, Ying Liang, Ying Mao, Junfeng Ma

Department of Thyroid and Breast Surgery, The 2nd Affiliated Hospital of Kunming Medical University, Kunming 650101, Yunnan, China. *Co-first authors.

Received February 7, 2017; Accepted June 4, 2017; Epub August 15, 2017; Published August 30, 2017

Abstract: Long noncoding RNAs (lncRNAs) play pivotal roles in various processes especially in oncogenesis. However, the function of lncRNA AK139328 in thyroid cancer development remains poorly understood. In current work, we have unraveled a novel function for AK139328 in thyroid cancer. We found that the expression of AK139328 was frequently increased in cancerous tissues and several cell lines. Meanwhile, upregulating AK139328 expression consistently promotes viability, invasion and cell cycle progression in TPC1 and 8505C cells contrary to the effect in groups with AK139328 knockdown. Higher AK139328 levels also correlate with poor overall and post progression survival. In addition, *in vivo* studies confirmed the oncogenic role of AK139328 as decreasing AK139328 level significantly attenuates Ki-67 staining and xenograft tumor growth. Mechanistic studies implied that upregulating AK139328 expression may promote epithelial-mesenchymal transition. Our study has identified a novel and oncogenic function of AK139328 in thyroid cancer and may provide potential insight into pharmaceutical intervention.

Keywords: lncRNA, AK139328, thyroid cancer, EMT, oncogenesis

Introduction

The thyroid cancer represents a class of tumor which is usually derived from follicular or para-follicular cells in thyroid [1]. About 80% thyroid cancer can be classified into papillary thyroid cancer [1]. Recent reports have demonstrated that the incidence rate of thyroid cancer has been elevated by at least 2 fold [1]. The thyroid cancer ranks among the top ten most frequent cancers in China and therefore poses serious threat to normal survival [2]. Failure to precise diagnosis and specification contribute largely to the ever increasing rate of thyroid cancer incidence [3]. The patients with thyroid cancer also suffer from high recurrence rate despite the five year survival is relatively high [4]. The genetic origin of thyroid cancer is rather complex and can be ascribed to multiple factors [5]. Therefore, elaborate understanding of the mechanisms underlying thyroid cancer development as well as identifying novel biomarkers may play critical roles in efficient diagnosis and treatment of thyroid cancer.

Recent evidence has suggested the mammalian genome can transcribe a significant fraction

of short and long non-coding RNAs (lncRNAs) with limited protein coding activities [6, 7]. The lncRNAs belong to a particular class of RNAs which is longer than 200 nucleotides in length [7]. The lncRNAs may be either distributed in cytoplasm or nucleus and are mainly transcribed by RNA polymerase II. The lncRNAs are actively involved in various processes such as differentiation, proliferation and apoptosis [8-10]. The expression profiles of lncRNAs is significantly reprogrammed in thyroid cancer [11]. Noticeably, the lncRNAs can also play pivotal roles in tumor development. For example, the lncRNA MALAT1 can promote tumor progression via targeting miR-206 [12]. Uzan et al. showed that high expression of lncRNA HULC is also associated with poor diagnosis [13]. The lncRNA PVT1 may also positively correlate with thyroid cancer incidence by recruiting EZH2 [14]. The H19 was reported to bind microRNAs to serve as a competitive endogenous RNA and regulate thyroid cancer [15]. Recently, Sun et al. evaluated a novel lncRNA NR_036575.1 and found that NR_036575.1 can promote proliferation and migration of papillary thyroid cancer (PTC) [16]. Furthermore, they argued that

NR_036575.1 may serve as a diagnostic marker for identifying PTC and noncancerous diseases [16].

Recently, Chen et al. examined the lncRNA profiles after ischemia/reperfusion treatment [17]. They found that among the 71 upregulated lncRNAs, AK139328 displayed the highest expression and was associated with ischemia/reperfusion injury [17]. Silencing AK139328 can annihilate necrosis and caspase-3 activities after ischemia/reperfusion treatment. Therefore, AK139328 can neutralize the injury from ischemia/reperfusion and serve as a potential diagnostic marker. However, whether AK139328 can play a role in thyroid cancer development has not been investigated.

In present work, we showed that AK139328 can promote thyroid cancer progression. The expression of AK139328 is usually upregulated in tumorous tissues compared with normal adjacent tissues. In addition, AK139328 increases cell viability and invasion of thyroid cancer cell lines and si-RNA mediated AK139328 knockdown may significantly induce cell cycle arrest. Patients with higher AK139328 expression exhibited poor overall survival and post progression survival. Xenografts with AK139328 overexpression displayed higher weight whereas si-AK139328 markedly decreased the xenograft tumor growth. Taken together, our results identified an oncogenic role for AK139328 and may provide potential insight into the underlying mechanisms of tumor development in thyroid cancer.

Materials and methods

Cell culture and human samples

The thyroid cancer (TC) cell lines in current study (8505C, FTC133, TPC1, CG3 and BCPAP) and a normal cell line (Nthy-ori 3-1) were obtained from The Shanghai Institute of Cell Biology (Shanghai, China). The 293T cell line was purchased from The Shanghai Institute of Cell Biology (Shanghai, China). The thyroid cancer cells were maintained in RPMI-1640 medium (Sigma, Shanghai, China) supplemented by 3% fetal calf serum (FCS, Sigma, Shanghai, China), plus streptomycin (30 µg/ml, Sigma, Shanghai, China) and penicillin (100 U/ml, Sigma, Shanghai, China) in a culture chamber with 5% CO₂ at 20°C. Matched fresh thyroid cancer specimens and normal adjacent tissues

were collected from surgical archives for 86 patients who have undergone resection at the 2nd Affiliated Hospital of Kunming Medical University between May 2011 and September 2013. Immediately after surgical resection, these tissues were stored at -80°C. None patients have received preoperative chemotherapy or radiotherapy. Consent forms were signed by all patients. The research related to human samples has been formally approved by Ethics Committee of the 2nd Affiliated Hospital of Kunming Medical University (NO. 2011L004).

Quantitative real-time RT-PCR (qRT-PCR)

Total RNAs were isolated from both thyroid cell lines (8505C and TPC1) and human samples with Trizol reagent (Invitrogen, Carlsbad, CA, USA). Totally, 3 ng total RNA in a volume of 10 µl containing 3 mM dNTP Mix (Sigma, Shanghai, China) was used to generate complementary DNA (cDNA). The mixture was maintained in 70°C for 5 min and then a mixture composed of 5×RT buffer, 20 U/µl reverse transcriptase, 200 U/µl RNase inhibitor was added (Sigma, Shanghai, China). *GAPDH* was used as the internal control if not otherwise specified. Reactions were performed by the ABI PRISM® 7000 Sequence Detection System (Applied Biosystem, Foster City, USA) according to the manufacturer's protocols. The expression of AK139328 was quantified by the 2^{-ΔΔCt} method. The experiments were performed triplicates. The primer sequences were: AK139328: sense: 5'-GTAAGCCAGCATT-3'; anti-sense: 5'-TGCGTTAAGCATGGTCT-3'; *GAPDH*: sense: 5'-GATTGCGTACATT-3'; anti-sense: 5'-ATCGAGTCTGAGTT-3'.

AK139328 knockdown and transfection

The cDNA for AK139328 was amplified by PCR and cloned into the pCDNA3.1 vector (Sigma, Shanghai, China). The empty pCDNA3.1 vector was employed as the control. AK139328 small interfering RNAs (si-AK139328) were synthesized by Sigma (Shanghai, China). All transfections were implemented by Lipofectamine 2000 (Invitrogen, CA, USA) according to the manufacturer's protocols.

Cell viability assay

We used the Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) to analyze the cell viability. After treatment for 24 hrs, TPC1 and

8505C cells were suspended and loaded into a 12-well plate (10^5 cells/well) and lasted for 5 days. A total 10 ml MTT solutions were appended into the culture with a final concentration of 15 mg/ml. The crystalline formazan was resolved in 150 μ L sodium dodecyl sulfate (SDS, 10%, Sigma, Shanghai, China) solution for 36 hrs. The optical density was detected at the wavelength of 490 nm.

Transwell invasion assay

Cell invasion assays were performed using the 24-well transwell chambers (8 μ m pore size; BD Biosciences, San Jose, CA, USA). The cell culture surface was firstly coated with Matrigel. About 5×10^5 TPC1 and 8505C cells were suspended in 100 μ l serum-free medium and seeded into top chambers. DMEM (300 μ l) containing 5% FCS was then added into bottom chambers. After 24 h's incubation at 37°C, those cells which did not migrate into the lower chambers were removed by cotton swabs and cells upon lower chambers were stained with crystal violet. We used Leica microscope fluorescent microscope (DM-IRB, Leica, Germany) to visualize and quantify the results.

Cell cycle analysis

After transfection for 48 hrs, TPC1 and 8505C cells were harvested and washed with cold phosphate buffer saline (PBS, Sigma, Shanghai, China). Then, cells were fixed with 75% ethanol at 4°C overnight. Fixed cells were further stained by propidium iodide (PI, Sigma, Shanghai) at 4°C for 30 min in dark. The fraction of cells in G0/G1, S and G2/M phases were measured with fluorescence-activated cell sorting (FACS) (BD Bioscience, Mansfield, MA, USA). The experiments were performed with triplicates.

Western blot

TPC1 and 8505C cells were suspended and harvested with a cell lysis buffer containing 10% glycerol and 3% NP-40 (Sigma, Shanghai, China) for 10 min at 4°C. 20 μ g total proteins were extracted and separated by 10% SDS-PAGE. Then, the proteins were transferred into PVF membrane. The blot was blocked with 5% fat-free milk for 1 h at 20°C. The membrane was incubated with anti-E-cadherin, N-cadherin, vimentin or GAPDH monoclonal antibodies

(dilution 1:1,000, Sigma, Shanghai, China) and horseradish peroxidase-conjugated secondary antibodies (dilution 1:1,000, Sigma, Shanghai, China) overnight at 4°C. After washing with Tris-buffered saline (TBS) containing 0.1% Tween 20, the blots were monitored using a chemiluminescent method kit (Sino-American Biotechnology Company, Shanghai, China). The blots were quantified using ImageJ software.

In vivo implantation and immunohistochemistry

Transfection of TPC1 cells was performed using lenti-virus transfection system. The system was maintained for 12 hrs and then cultured for another 24 hrs. Then, cells were resuspended and totally 1×10^6 cells were injected subcutaneously into the nude mice. Five weeks later, mice were sacrificed by overdose of sodium pentobarbital (3%, 150 mg/kg with intraperitoneal injection; Sigma, Shanghai, China) and solid tumors were weighted. The nude mice were obtained from the Model Animal Research Center (MARC, Nanjing, China). After retrieving antigens in sodium citrate buffer, tissue sections were covered with Ki-67 antibodies (TIANGEN, Shanghai, China). The specimens were washed three times using PBS for 2 min and then blocked with 2% hydrogen peroxide for 15 min at 20°C. The 2- μ m sections were used. The Ki-67 immunostaining was done using primary anti-Ki-67 antibody (Sigma, Shanghai, China). Images were displayed with 100 \times magnification.

Statistical analysis

All experimental results were represented as mean \pm SD. Statistical significance were determined by Student's *t*-test (SPSS, version 16.0, Inc., Chicago, IL, USA) and the significance was identified if $P < 0.05$. Kaplan-Meier survival curve was tested using log-rank test. Fisher exact test was used to evaluate the correlation between AK139328 and clinicopathological features.

Results

Upregulated expression of lncRNA AK139328 in thyroid cancer specimens and cell lines

To identify whether AK139328 may play a role in tumor development of thyroid cancer, we quantified the expression of AK139328 tran-

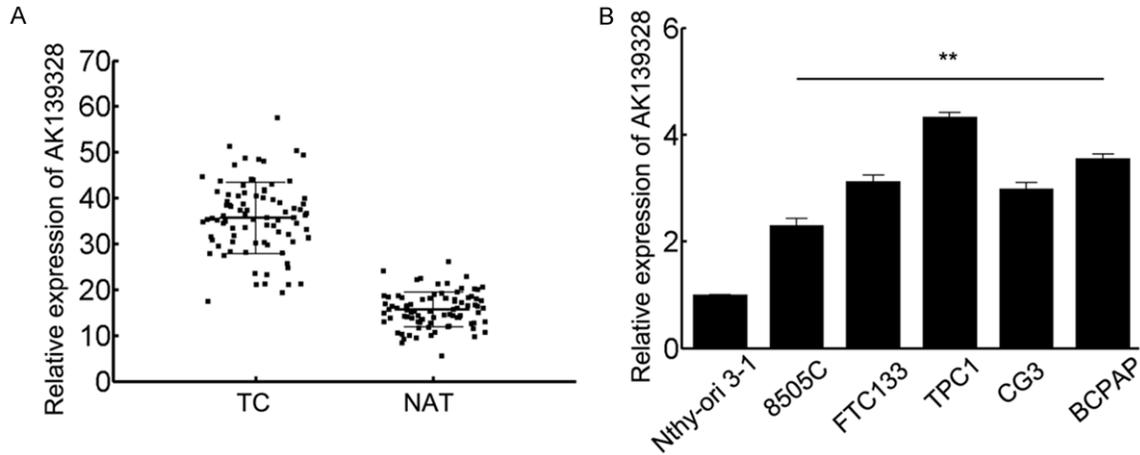


Figure 1. Expression level of AK139328 in thyroid cancer tissues and selected cell lines. A: Relative expression of AK139328 in thyroid cancer and normal adjacent tissues (NATs). The mean \pm SD was shown. B: The expression of AK139328 in thyroid cancer cell lines and normal human thyroid cell line Nthy-ori 3-1. **: $P < 0.01$.

Table 1. AK139328 and different clinicopathological factors

Clinicopathological factor	No.	AK139328 level		P values
		High	Low	
Gender				
Male	45	21 (46.7%)	24 (53.3%)	0.666
Female	41	22 (53.7%)	19 (46.3%)	
Age				
<60	38	16 (42.1%)	22 (57.9%)	0.278
≥ 60	48	27 (56.2%)	21 (43.8%)	
Tumor size				
<3	50	19 (38.0%)	31 (62.0%)	0.008
≥ 3	36	24 (66.7%)	12 (33.3%)	
Pathological type				
Papillary adenocarcinoma	56	26 (46.4%)	30 (53.6%)	0.498
Follicular adenocarcinoma	30	17 (56.7%)	13 (43.3%)	
Clinical stages				
I/II	42	15 (35.7%)	27 (64.3%)	0.009
III/IV	44	28 (63.6%)	16 (36.4%)	
Metastasis				
Absent	30	8 (26.7%)	22 (63.3%)	0.001
Present	56	35 (62.5%)	21 (37.5%)	

The median value was used as the cutoff (35.6601).

scripts in thyroid cancer specimens and normal adjacent tissues. We observed that the expression of AK139328 was substantially increased compared with paired adjacent tissues (Figure 1A). Furthermore, we also evaluated the expression of AK139328 in thyroid cancer as well as normal thyroid cell lines. AK139328 consistently showed elevated expression in thyroid cancer cell lines compared with normal thyroid cells (Figure 1B). We also found that high

expression of AK139328 was significantly associated with tumorigenic factors such as tumor size, TNM grade and metastasis (Table 1). Other factors such as age, gender and pathological type were not significantly correlated with AK139328 expression (Table 1). Therefore, AK139328 may serve as a potential oncogenic factor in thyroid cancer. We chose TPC1 and 8505C cells for further analysis since they showed relatively highest and lowest expression of AK139328.

The AK139328 promotes cell viability, invasion and cell cycle progression

We further evaluated whether AK139328 has *in vitro* effect in thyroid cancer cells. Therefore, we overexpressed and knocked down AK139328 levels in TPC1 and 8505C cells. The overexpression

and knockdown efficiency were verified (Figure 2A and 2B). The results confirmed that pcDNA3.1 vector mediated overexpression and siRNA induced knockdown can significantly alter intrinsic AK139328 levels in both TPC1 and 8505C cells (Figure 2A and 2B). AK139328 overexpression markedly increased the viability of TPC1 cells (Figure 2C). Consistently, AK139328 knockdown decreased TPC1 cell viability compared with controls (Figure 2C).

lncRNA AK139328 promotes TC

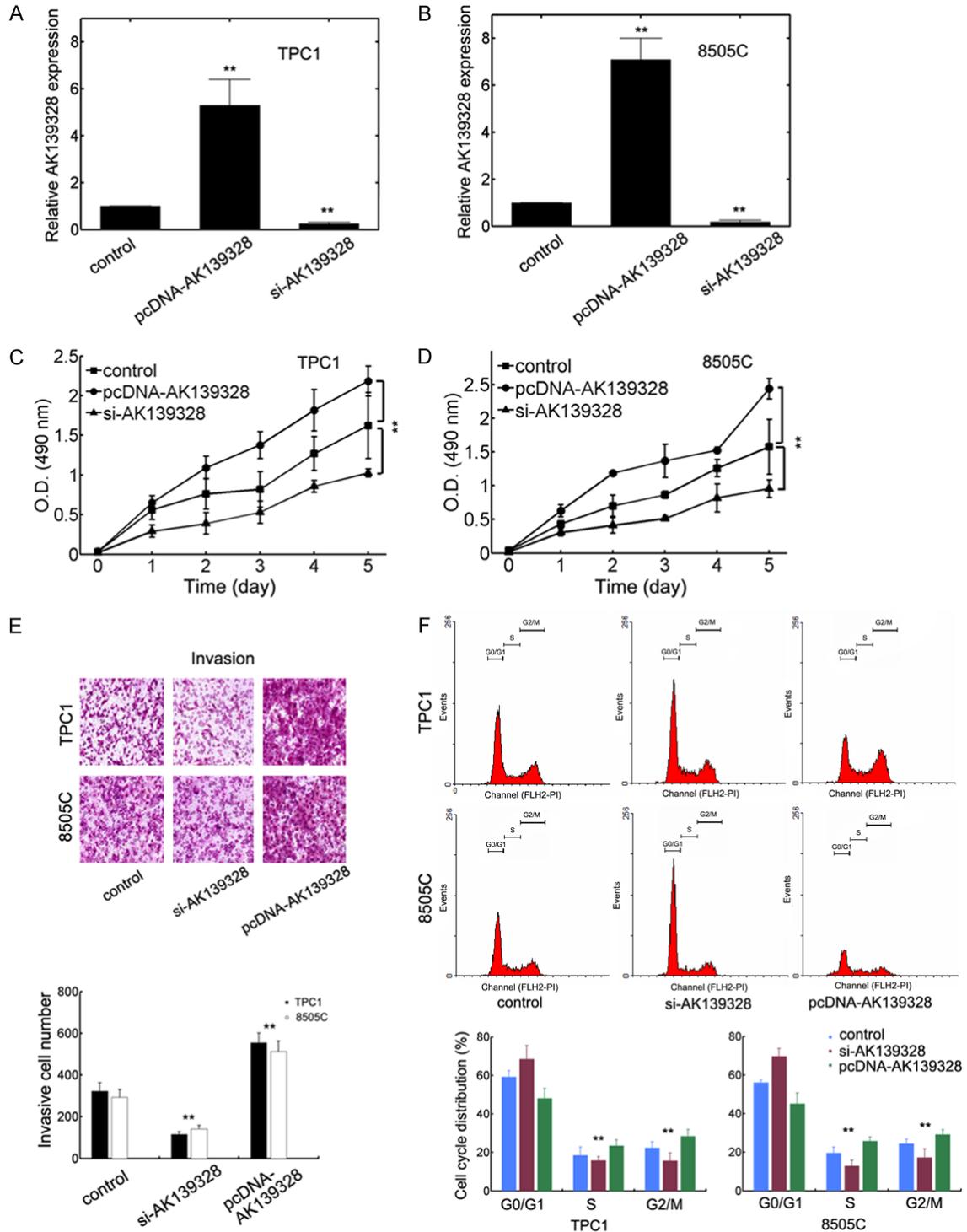


Figure 2. AK139328 increases the oncogenesis of thyroid cancer cells *in vitro*. Verification on the transfection efficiency of si-AK139328 or pcDNA-AK139328 in (A) TPC1 and (B) 8505C cells. **: $P < 0.01$. The viability assay for (C) TPC1 and (D) 8505C cells left untreated or transfected with either si-AK139328 or pcDNA-AK139328 and the five-day cell viability was evaluated. **: $P < 0.01$. (E) Transwell invasion assays for TPC1 and 8505C cells either left untreated or transfected with si-AK139328 or pcDNA-AK139328 plasmids. The quantification results were shown at bottom. (F) Cell cycle distribution for TPC1 and 8505C cells either left untreated or treated with si-AK139328 or pcDNA-AK139328. The sub-G1 fraction was quantified and the statistical significance was determined. Quantification was displayed at bottom. **: $P < 0.01$.

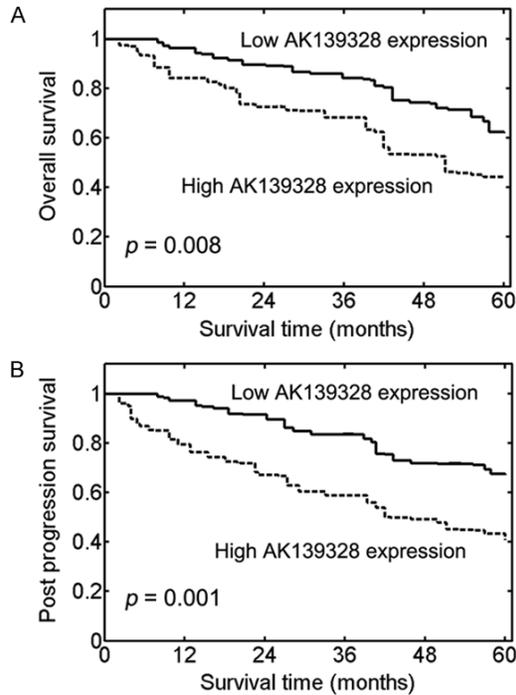


Figure 3. Kaplan-Meier survival curves for patients with thyroid cancer. Log-rank test was used. A: Patients with higher AK139328 expression had a significantly poor overall survival ($P = 0.008$). B: Post progression survival for patients with thyroid cancer ($P = 0.001$). The median level was used as the cut-off (35.6601).

The *in vitro* effect of changing AK139328 levels exhibited similar effects upon 8505C cells (Figure 2D). The invasive capacity of thyroid cancer cells was also measured using transwell invasion assay. As a result, increasing AK139328 expression promoted the invasion of TPC1 (top) as well as 8505C (bottom) cells (Figure 2E). The results were even more significant in TPC1 cells possibly due to higher intrinsic expression of AK139328 (Figures 1B and 2E). Lowering AK139328 levels by si-RNA may also induce cell cycle arrest in TPC1 and 8505C cells (Figure 2F). On the contrary, increasing AK139328 expression decreased the fraction of cells in G0/G1 phase in TPC1 and 8505C cells (Figure 2F). Collectively, data above suggested that AK139328 might exert its oncogenic role by promoting cell viability, invasion and reprogram cell cycle distribution.

AK139328 expression correlates with decreased survival

To confirm whether AK139328 levels correlated with patient survival, we evaluated the prognostic value of AK139328 by plotting the

Kaplan-Meier curves. We found that lower AK139328 expression indicated better survival while elevated AK139328 may result in poor overall survival (Figure 3A, $P = 0.008$). Consistently, higher AK139328 levels correlated with poor post progression survival (Figure 3B, $P = 0.001$). The difference in survival rate was generally enlarged post evaluation (Figure 3). These data suggested that higher AK139328 expression positively correlated with poor survival in thyroid cancer patients.

AK139328 functions as an oncogenic factor in vivo

We next checked *in vivo* effect of AK139328 by implantation study. TPC1 cells were transfected with si-AK139328 or pcDNA-AK139328 plasmid for 48 hrs. Then, genetically modified TPC1 cells were injected into nude mice. We found that si-AK139328 transfection significantly decreased the tumor volume compared with control while AK139328 overexpression increased the xenograft tumor growth (Figure 4A). The tumor weight after 5 weeks was quantified and the results showed that AK139328 knockdown markedly decreased the tumor weight (Figure 4B, $P < 0.01$). The tumor weight was substantially increased in the group with AK139328 overexpression (Figure 4B, $P < 0.01$). The fraction of Ki-67 positive cells was also increased with AK139328 overexpression indicative of enhanced cell viability (Figure 4C and 4D). We also evaluated whether AK139328 expression correlated with epithelial-mesenchymal transition (EMT) progression, we measured the level of E-cadherin, N-cadherin and Vimentin. The results showed that N-cadherin and Vimentin expression were increased with AK139328 overexpression while knocking down AK139328 decreased their expression accordingly in TPC1 and 8505C cells (Figure 4E and 4F). The E-cadherin displayed the opposite effect compared with N-cadherin and Vimentin (Figure 4E and 4F). The effect in TPC1 cells was slightly less significant compared with that in 8505C cells possibly owing to higher intrinsic AK139328 level in TPC1 cells (Figure 1B). These results further suggested that AK139328 has oncogenic role *in vivo* possibly by promoting EMT process.

Discussion

In current work, the expression of AK139328 was relatively higher in thyroid cancer tissues compared with normal adjacent ones.

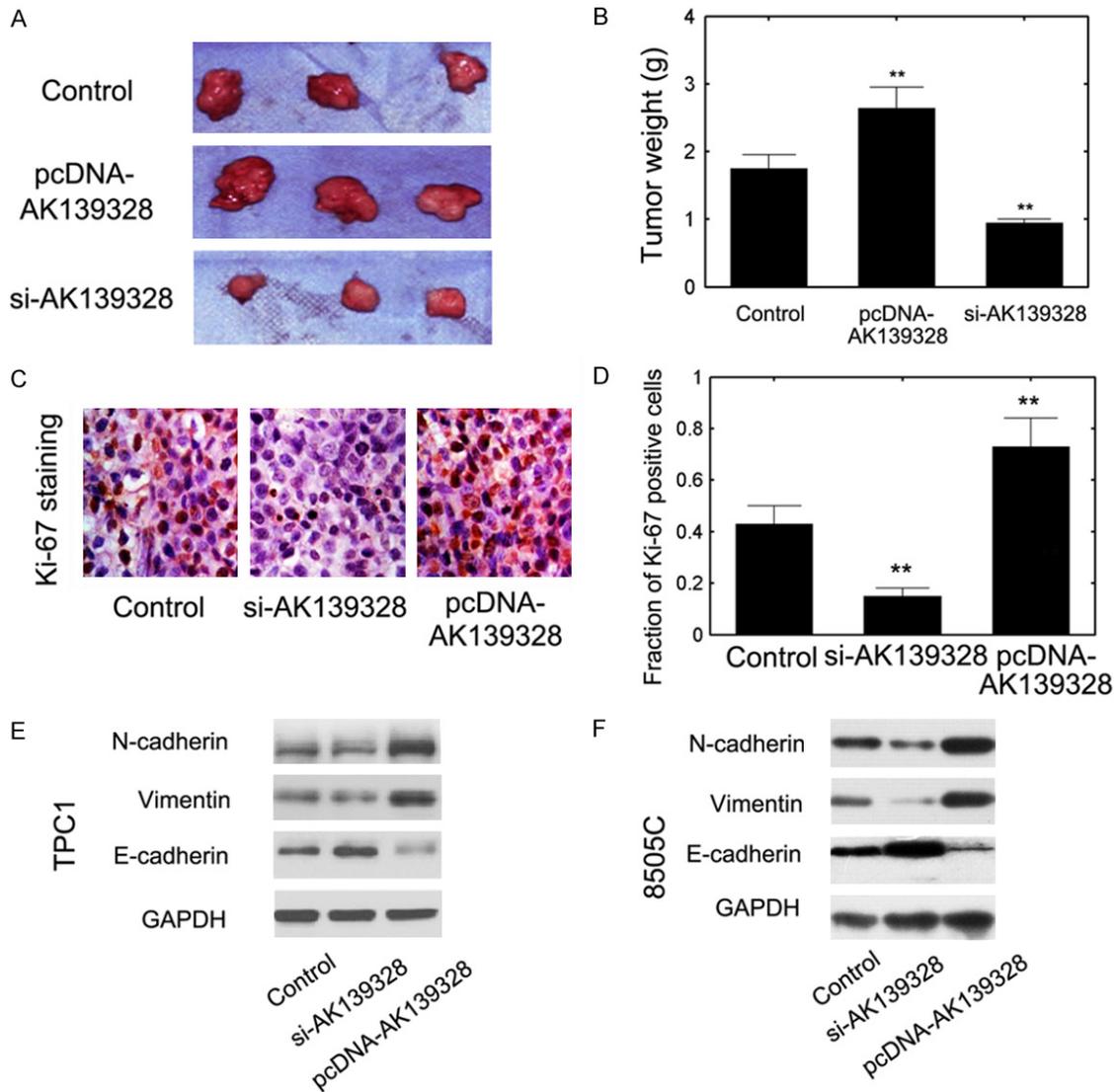


Figure 4. AK139328 promotes thyroid cancer oncogenesis *in vivo*. (A) The tumor growth of TPC1 xenografts either untreated or transfected with si-AK139328 or pcDNA-AK139328. (B) Tumor weight for different conditions. After 5 weeks, the xenografts were resected and weighted. (C) The Ki-67 staining for xenografts under control or transfection with si-AK139328 or pcDNA-AK139328 conditions. (D) Quantifying the results in (C) Western blots of N-cadherin, Vimentin and E-cadherin for TPC1 (E) and 8505C (F) cells either untreated or transfected with si-AK139328 or pcDNA-AK139328. Data were representative of three independent replicates.

Significant association between AK139328 expression and clinicopathological factors has been identified for metastasis, TNM stage arguing that AK139328 might serve as a potential oncogenic factor in thyroid cancer. Previous report has demonstrated that deregulated lncRNAs can contribute to oncogenesis and genomic profiling studies have provided guidelines for in-depth investigation of tumor related lncRNAs especially in thyroid cancer [2, 18]. Furthermore, accessing the role of lncRNA in thyroid cancer progression has uncovered sev-

eral contributors. For example, the lncRNA ANRIL has been upregulated in thyroid cancer tissues as well as several tumorous cell lines [2]. The lncRNA HOTAIR and its functional single nucleotide polymorphisms (SNPs) have also been investigated in thyroid cancer and a bipartite association in gender was intriguingly identified [19]. Instead, the lncRNA BANCR can inhibit thyroid cancer development and serve as a tumor suppressor by inactivating ERK1/2 and p38 pathways [20]. Recently, Xu et al. detected the lncRNA ENST00000426615 as a

potential tumor suppressive factor in papillary thyroid cancer as it markedly inhibits cell motility, proliferation and induces cell cycle arrest [21]. However, the involvement of AK139328 in thyroid cancer pathogenesis has caught little attention. Our study showed that AK139328 indeed promoted the oncogenesis of thyroid cancer possibly by promoting cell viability and invasion. Meanwhile, *in vivo* studies also confirmed that AK139328 may play tumorigenic role in thyroid cancer. The growth of tumor xenografts with lower AK139328 transfection was significantly attenuated. Therefore, we have identified a novel function for AK139328 in thyroid cancer.

Using microarray technology strategy, Chen et al. recently showed that AK139328 displayed the highest expression after ischemia/reperfusion injury [17]. Reducing AK139328 attenuates the lesions from ischemia/reperfusion treatment by increasing the survival signaling such as elevated phosphorylation of Akt (Akt), glycogen synthase kinase 3 (GSK3) together with endothelial nitric oxide synthase (eNOS) [17]. However, whether AK139328 fulfill its role in thyroid cancer remains largely elusive. Meanwhile, to date, few reports have focused on the function of AK139328 in thyroid cancer. As a result, we have implicated AK139328 in the pathogenesis of thyroid cancer and indicated that AK139328 might be of diagnostic value at least in thyroid cancer.

We have also shown that the EMT process was significantly advanced by upregulating AK139328 level due to the fact that N-cadherin and Vimentin protein expression were substantially increased. Accumulating evidence has identified EMT as an important contributor to metastasis, invasion and prognosis [22]. A myriad of studies have described a functional association between EMT and lncRNA. Matouk et al. indicated that H19 lncRNA emerges as a key player by regulating critical events during EMT and mesenchymal to epithelial transitions (MET) [23]. Furthermore, Xiao et al. demonstrated that lncRNA UCA1 is capable of inducing EMT in breast cancer cells by shaping Wnt/ β -catenin signaling [24]. Using a lncRNA-mining approach with reliable profiling, Li et al. clarified that the expression of SLC25A25-AS1 is significantly decreased in colorectal cancer (CRC) specimens and cell lines [25]. Decreasing SLC25A25-AS1 expression obviously enhanced the EMT *in vitro* suggesting that SLC25A25-AS1

may exert tumor suppressive function in CRC [25]. Therefore, the tumorigenic function of lncRNA can possibly be ascribed to its modulation through the EMT process. Our results have unraveled a novel facet in this correlation and may provide further insight into combinatorial intervention by targeting multiple lncRNAs to regulate tumor development.

We did not determine the exact signaling pathway in which AK139328 serves its oncogenic function. Whether AK139328 can antagonize or synergize with other lncRNAs remains to be investigated. However, we have provided the first evidence that AK139328 promotes oncogenesis in thyroid cancer by increasing cell viability, invasion as well as *in vivo* xenograft tumor growth. Patients with higher AK139328 expression correlate with poor overall and post progression survival. Collectively, our current study implicated AK139328 as a putative diagnostic marker in thyroid cancer. Identifying the intricate signaling pathway in which AK139328 acts as an oncogenic lncRNA as well as the therapeutic efficiency by targeting AK139328 demands further evaluation.

Disclosure of conflict of interest

None.

Authors' contribution

SQL, ZJZ and JFM conceived the study. SQL, ZJZ, YL, YZ, LYD, YL and YM performed the experiments. SQL, ZJZ, YL and YZ analyzed the data. SQL, LYD, YL and YM prepared the figures. SQL, ZJZ and JFM wrote the paper. All authors have read and approved the final version.

Address correspondence to: Dr. Junfeng Ma, Department of Thyroid and Breast Surgery, The 2nd Affiliated Hospital of Kunming Medical University, No.374, Dianmian Avenue, Wuhua District, Kunming 650101, Yunnan, China. Tel: 86-871-63402570; Fax: 86-871-63402570; E-mail: majfkunming@yeah.net

References

- [1] Ukrainski MB, Pribitkin EA and Miller JL. Increasing incidence of thyroid nodules and thyroid cancer: does increased detection of a sub-clinical reservoir justify the associated anxiety and treatment? *Clin Ther* 2016; 38: 976-985.
- [2] Zhao JJ, Hao S, Wang LL, Hu CY, Zhang S, Guo LJ, Zhang G, Gao B, Jiang Y, Tian WG and Luo

lncRNA AK139328 promotes TC

- DL. Long non-coding RNA ANRIL promotes the invasion and metastasis of thyroid cancer cells through TGF-beta/Smad signaling pathway. *Oncotarget* 2016; 7: 57903-57918.
- [3] Chou A, Fraser S, Toon CW, Clarkson A, Sioson L, Farzin M, Cussigh C, Aniss A, O'Neill C, Watson N, Clifton-Bligh RJ, Learoyd DL, Robinson BG, Selinger CI, Delbridge LW, Sidhu SB, O'Toole SA, Sywak M and Gill AJ. A detailed clinicopathologic study of ALK-translocated papillary thyroid carcinoma. *Am J Surg Pathol* 2015; 39: 652-659.
- [4] Vigneri R, Malandrino P and Vigneri P. The changing epidemiology of thyroid cancer: why is incidence increasing? *Curr Opin Oncol* 2015; 27: 1-7.
- [5] Santini F, Marzullo P, Rotondi M, Ceccarini G, Pagano L, Ippolito S, Chiovato L and Biondi B. Mechanisms in endocrinology: the crosstalk between thyroid gland and adipose tissue: signal integration in health and disease. *Eur J Endocrinol* 2014; 171: R137-152.
- [6] Wang KC and Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell* 2011; 43: 904-914.
- [7] Ernst C and Morton CC. Identification and function of long non-coding RNA. *Front Cell Neurosci* 2013; 7: 168.
- [8] Jain S, Thakkar N, Chhatai J, Bhadra MP and Bhadra U. Long non-coding RNA: functional agent for disease traits. *RNA Biol* 2017; 14: 522-535.
- [9] DeOcesano-Pereira C, Amaral MS, Parreira KS, Ayupe AC, Jacysyn JF, Amarante-Mendes GP, Reis EM and Verjovski-Almeida S. Long non-coding RNA INXS is a critical mediator of BCL-XS induced apoptosis. *Nucleic Acids Res* 2016; 44: 9518.
- [10] Zhu XX, Yan YW, Chen D, Ai CZ, Lu X, Xu SS, Jiang S, Zhong GS, Chen DB and Jiang YZ. Long non-coding RNA HoxA-AS3 interacts with EZH2 to regulate lineage commitment of mesenchymal stem cells. *Oncotarget* 2016; 7: 63561-63570.
- [11] Yang M, Tian J, Guo X, Yang Y, Guan R, Qiu M, Li Y, Sun X, Zhen Y, Zhang Y, Chen C and Fang H. Long noncoding RNA are aberrantly expressed in human papillary thyroid carcinoma. *Oncol Lett* 2016; 12: 544-552.
- [12] Wang SH, Zhang WJ, Wu XC, Zhang MD, Weng MZ, Zhou D, Wang JD and Quan ZW. Long non-coding RNA Malat1 promotes gallbladder cancer development by acting as a molecular sponge to regulate miR-206. *Oncotarget* 2016; 7: 37857-37867.
- [13] Uzan VR, Lengert A, Boldrini E, Penna V, Scapulatempo-Neto C, Scrideli CA, Filho AP, Cavalcante CE, de Oliveira CZ, Lopes LF and Vidal DO. High expression of HULC is associated with poor prognosis in osteosarcoma patients. *PLoS One* 2016; 11: e0156774.
- [14] Zhou Q, Chen J, Feng J and Wang J. Long non-coding RNA PVT1 modulates thyroid cancer cell proliferation by recruiting EZH2 and regulating thyroid-stimulating hormone receptor (TSHR). *Tumour Biol* 2016; 37: 3105-3113.
- [15] Liu L, Yang J, Zhu X, Li D, Lv Z and Zhang X. Long noncoding RNA H19 competitively binds miR-17-5p to regulate YES1 expression in thyroid cancer. *FEBS J* 2016; 283: 2326-2339.
- [16] Sun W, Lan X, Wang Z, Dong W, He L, Zhang T and Zhang H. Overexpression of long non-coding RNA NR_036575.1 contributes to the proliferation and migration of papillary thyroid cancer. *Med Oncol* 2016; 33: 102.
- [17] Chen Z, Jia S, Li D, Cai J, Tu J, Geng B, Guan Y, Cui Q and Yang J. Silencing of long noncoding RNA AK139328 attenuates ischemia/reperfusion injury in mouse livers. *PLoS One* 2013; 8: e80817.
- [18] Lan X, Zhang H, Wang Z, Dong W, Sun W, Shao L, Zhang T and Zhang D. Genome-wide analysis of long noncoding RNA expression profile in papillary thyroid carcinoma. *Gene* 2015; 569: 109-117.
- [19] Zhu H, Lv Z, An C, Shi M, Pan W, Zhou L, Yang W and Yang M. Onco-lncRNA HOTAIR and its functional genetic variants in papillary thyroid carcinoma. *Sci Rep* 2016; 6: 31969.
- [20] Liao T, Qu N, Shi RL, Guo K, Ma B, Cao YM, Xiang J, Lu ZW, Zhu YX, Li DS and Ji QH. BRAF-activated LncRNA functions as a tumor suppressor in papillary thyroid cancer. *Oncotarget* 2017; 8: 238-247.
- [21] Xu B, Shao Q, Xie K, Zhang Y, Dong T, Xia Y and Tang W. The Long non-coding RNA ENST00000537266 and ENST00000426615 influence papillary thyroid cancer cell proliferation and motility. *Cell Physiol Biochem* 2016; 38: 368-378.
- [22] De Craene B and Berx G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer* 2013; 13: 97-110.
- [23] Matouk IJ, Halle D, Raveh E, Gilon M, Sorin V and Hochberg A. The role of the oncofetal H19 lncRNA in tumor metastasis: orchestrating the EMT-MET decision. *Oncotarget* 2016; 7: 3748-3765.
- [24] Xiao C, Wu CH and Hu HZ. LncRNA UCA1 promotes epithelial-mesenchymal transition (EMT) of breast cancer cells via enhancing Wnt/beta-catenin signaling pathway. *Eur Rev Med Pharmacol Sci* 2016; 20: 2819-2824.
- [25] Li Y, Huang S, Zhang W, He K, Zhao M, Lin H, Li D, Zhang H, Zheng Z and Huang C. Decreased expression of LncRNA SLC25A25-AS1 promotes proliferation, chemoresistance, and EMT in colorectal cancer cells. *Tumour Biol* 2016; 37: 14205-14215.