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SP1-induced upregulation of long noncoding RNA LINC00313 contributes to papillary thyroid cancer progression via the miR-422a

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Abstract. – OBJECTIVE: Long noncoding RNA LINC00313 (LINC00313) has been reported to be dysregulated in several tumors, including papillary thyroid carcinoma (PTC). Our present study aimed to further explore the potential mechanism of LINC00313 in the progression of papillary thyroid carcinoma (PTC).

PATIENTS AND METHODS: RT-PCR was performed to detect the expression of LINC00313 in both PTC tissues and cell lines. Luciferase reporter and chromatin immunoprecipitation (ChIP) assays were performed to explore whether SP1 could bind to the promoter region of LINC00313 and activate its transcription. The biological functional correlation of LINC00313 was determined by down-regulating the expression of LINC00313 on PTC cell proliferation, apoptosis, migration and invasion. The regulating relationship between LINC00313 and miR-422a was investigated in PTC cells using luciferase reporter assays.

RESULTS: We observed that LINC00313 expression was significantly up-regulated in both PTC tissues and cell lines. Next, the results of bioinformatics analysis and luciferase reporter assays indicated that the transcription factor SP1 can bind to the promoter region of LINC00313 resulting in the overexpression of LINC00313 in PTC. Moreover, functional study revealed that knockdown of LINC00313 significantly suppressed cells proliferation, migration, invasion and EMT. Finally, our results indicated that LINC00313 functioned as an oncogene in PTC in part through serving as a competing endogenous RNA to modulate mi-422a expression.

CONCLUSIONS: Overall, our data demonstrated that SP1-induced LINC00313 contributed to PTC progression by via competitively binding to miR-422a, which may provide a novel therapeutic strategy for PTC.

Key Words:
LINC00313, Papillary thyroid carcinoma, SP1, miR-422a, Tumorigenesis.

Introduction

Thyroid cancer is a cancer that develops from the tissues of the thyroid gland and the most common endocrine malignancy with 300,000 new cases worldwide per year, and nearly 40,000 deaths[1,2]. The most common type of thyroid cancer is papillary thyroid cancer (PTC), comprising 80% of all cases[3]. The incidence of PTC continues to rise in China, mostly as a result of the development of diagnostic methods[4]. Although the prognosis of most PTC patients is favorable by surgical resection combined with radioiodine and levothyroxine treatment, around 15% of cases present recurrence in local/regional and distant sites in the next decade leading to death[5,6]. With the deepening of research, PTC results from the dysregulation of many tumor-related genes[7]. Thus, more efforts are desired to investigate the molecular mechanisms underlying the progression and metastasis of PTC to develop novel anticancer treatment options. Long non-coding RNAs (lncRNAs) are more than 200 nucleotides in length with limited or no protein-coding capacity[8]. It has been showed by various studies that lncRNAs are involved in the regulation of a series of biological processes such as transcription, cell cycle regulation, cellular differentiation, apoptosis and chromatin modification[9,10]. Novel evidence in clinical progress and basis experiments indicates that lncRNAs participate in

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tumorigenesis and functions as either oncogene or tumor suppressor gene according to the types of tumors, providing new insight into the biology of tumors[11-13]. More and more lncRNAs were well-studied, such as lncRNA HOTAIR, lncRNA PVT-1 and lncRNA MALAT-1. They have been reported to be dysregulated and serve as important regulators in almost all types of tumors[14-16]. In addition, in clinical research, lncRNAs have emerged as novel candidates of biomarkers or therapeutic targets for various types of human cancers. However, the biological function and potential mechanism of most lncRNAs in PTC progression remains largely unclear. LINC00313, located in 21q22.3, was a newly identified lncRNA whose dysregulation had been reported in several tumors[17-19]. However, its researches in biological function are limited. Wu et al[20] reported that LINC00313 was highly expressed in PTC and acted as a positive regulator by performing a series of cells experiments. However, the evidences are limited and the potential mechanism by which LINC00313 displayed its tumor-promotive role in PTC remains largely unclear.

Patients and Methods

Tissue Samples

46 paired tumor specimens and non-tumor adjacent tissues were obtained from National Cancer Center/ National Clinical Research Center for Cancer/ Cancer Hospital between 2015 and 2017. No local or systemic treatment had been conducted prior to the operation. All samples were immediately frozen in liquid nitrogen and stored at -80°C until use. Informed consent was obtained from each patient, and the experimental protocol was reviewed and approved by National Cancer Center/ National Clinical Research Center for Cancer/ Cancer Hospital.

Cell lines and cell transfection

In this study, we detected LINC00313 expression in three human PTC cell lines (8505C, SW1736 and TPC-1), and one human thyroid follicular epithelial cell line, Nthy-ori3-1. These cells were obtained from Shanghai Fuheng Biotechnology Co., Ltd. (Xuhui, Shanghai, China). These cells were all maintained at 37°C with 5% CO_2 using RPMI-1640 medium containing 10% fetal bovine serum (FBS). A NanoFect transfection reagent (Comiike, Nantong, Jiangsu, China) was applied to transfect siRNAs, miRNA mimic or plasmids into TPC-1 and SW1736 cells. The small interfering RNAs (siRNAs) targeting SP1 (si-SP1#1 and si-SP1#2), LINC00313 (siRNA#1 and siRNA#2), negative control siRNA for SP1 (si-NC) and negative control siRNA for LINC00313 (NC siRNA) were all obtained from Transheep Co., Ltd. (Suzhou, Jiangsu, China). In addition, the miR-422a mimic and negative control (NC) mimic were all synthesized by Biomics Biotechnology Co., Ltd. (Nantong, Jiangsu, China). Moreover, the sequence of LINC00313 was constructed into pcDNA3.1 empty vector by Genecreate Co., Ltd. (Wuhan, Hubei, China) to overexpress LINC00313.

Quantitative Real-Time PCR (qRT-PCR)

A Direct-zol RNA purification kit (Zymo Research, Chaoyang, Beijing, China) was used to extract total RNA from the PTC cell lines. A HiScript II One Step qRT-PCR SYBR Green kit (Vazyme Biotech, Nanjing, Jiangsu, China) was then applied to examine the SP1 or LINC00313 expression on a Q4 Thmorgan qRT-PCR instrument (Thmorgan, Changping, Beijing, China). The data were normalized to the expression of GAPDH. For miR-422a examination, an All-in-One miRNA qRT-PCR Detection kit (Bioeasy Biotechnology, Xuhui, Shanghai, China) was used. The expression levels of miR-422a were normalized to U6. The $2^{-\Delta\Delta Ct}$ method was applied to analyze the relative expression changes. The primers were all listed in Table I.

Cell Counting Kit-8 (CCK-8) Assays

After the TPC-1 and SW1736 cells were transfected with SP1 siRNAs, LINC00313 siRNAs, miRNA mimics or plasmids, they were collected, resuspended and placed in 96-well plates (2000 cells/well). After culturing for 24 h, each well was added with 10 μl CCK-8 solution (Biotechwell, Xuhui, Shanghai, China) and the cells were further incubated at 37°C for 1-2 h. Then, a PerkinElmer EnVision microreader (PerkinElmer, Pudong, Shanghai, China) was used to detect the absorbance at a wavelength of 450 nm.

EdU Assays

An EdU (5-Ethynyl-2'-deoxyuridine) kit (Bersinbio, Guangzhou, Guangdong, China) was also utilized to evaluate the proliferation of TPC-1 and SW1736 cells. Briefly, the TPC-1 and SW1736 cells were seeded into 24-well plates and continue to be cultured for 24 h. Then, the cells were transfected with LINC00313 siRNAs. After

Table I. The primer sequences included in this study.

Genes	Primer sequences (5′–3′)
LINC00313:forward	GCGGGAAACCTCGATGAACA
LINC00313: reverse	ACATTCTTTCCCATCGGGCT
miR-422a:forward	ACUGGACUUAGGGUCAGAAGGC
miR-422a: reverse	GCCUUCUGACCCUAAGUCCAGU
GAPDH: forward	GACTCATGACCACAGTCCATGC
GAPDH: reverse	AGAGGCAGGGATGATGTTCTG

24 h, completed medium containing EdU (final concentration: 10 μM) was added into the cells and the cells were maintained at 37°C for an additional 2 h. Next, the cells were fixed with 4% paraformaldehyde and washed with PBS for three times. Finally, an inverted fluorescence microscope (MF53, Mshot, Guangzhou, Guangdong, China) was employed to take photographs.

Colony Formation Assays

In brief, the treated TPC-1 and SW1736 cells were resuspended in complete medium and placed into 6-well plates. After culturing for about two weeks, 0.1% crystal violet solution (Baomanbio, Xuhui, Shanghai, China) was added into the plates, and the plates were then washed with PBS for three times. Finally, an inverted fluorescence microscope (MF53, Mshot, Guangzhou, Guangdong, China) was employed to take photographs.

Western Blot Assays

The treated TPC-1 and SW1736 cells were lysed using a total protein extraction kit (G-CLONE, Yizhuang, Beijing, China). The proteins were then separated on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and sequentially transferred onto polyvinylidene difluoride (PVDF) (ZikerBio, Shenzhen, Guangdong, China) membranes. The membranes were then probed overnight at 4°C with primary antibodies and corresponding secondary antibodies. Finally, the signals of the corresponding molecules were visualized by an ABSIN enhanced ECL kit (Pudong, Shanghai, China). The primary antibodies targeting N-Cadherin, vimentin and GAPDH were all purchased from Abcam Co., Ltd. (Abcam, Pudong, Shanghai, China).

Chromatin Immunoprecipitation (ChIP) Assays

A One-Step ChIP kit (Amyjet Scientific, Guangzhou, Guangdong, China) was utilized to perform the ChIP assays. In short, 1% formaldehyde was firstly used to fix the TPC-1 and SW1736 cells for 15 min, and 125 nM glycine was then added into the cells incubating for additional 5 min. Next, chromatin was sonicated into DNA fragments, which ranged from 200 to 300 bp. Subsequently, chromatin was immunoprecipitated by anti-SP1 antibody (Cell Signaling Technology, Beverly, MA, USA) or anti-IgG antibody (ProteinTech, Wuhan, Hubei, China). The precipitated DNA was analyzed by qPCR.

RNA Immunoprecipitation (RIP) Assays

A BersinBio RIP assay kit (BersinBio, Guangzhou, Guangdong, China) was utilized to conduct the RIP experiments. In brief, the RIP lysis buffer was firstly used to lyse the cells transfected with pcDNA3.1-LINC00313 or miR-422a mimics. After that, the magnetic beads with anti-Argonaute-2 (Ago2) antibody (Cell Signaling Technology, Beverly, MA, USA) was added into the cell lysates. Finally, the qRT-PCR assays were employed to analyze the co-precipitated RNAs.

Apoptosis Assays

A flow cytometry apoptosis analysis kit (ZeYe Biotechnology, Songjiang, Shanghai, China) was applied to perform the cell apoptosis detection. Briefly, TPC-1 and SW1736 cells transfected with LINC00313 siRNAs were firstly digested by trypsin and the resuspended in binding buffer. Afterwards, staining with propidium iodide (PI) as well as Annexin V was conducted for 15 min and the samples needed to be protected from light. Finally, the apoptotic TPC-1 and SW1736 cells were assessed by a CytoFLEX LX flow cytometer (Beckman-Coulter Inc., Brea, CA, USA).

Dual-Luciferase Reporter Assay

Firstly, the full promoter sequence of LINC00313 (pGL3-LINC00313 full promoter), the site#1 as well as site#3 sequences of LINC00313 (pGL3-LINC00313 partial promot-

er), the site#2 sequence of LINC00313 (pGL3-LINC00313 site#2 promoter) were all constructed into pGL3 reporter plasmids by MiaolingBio Co., Ltd. (Wuhan, Hubei, China). Besides, the LINC00313 wild type (LINC00313-wt) vector and LINC00313 mutant (LINC00313-mut) vector were constructed by Saierbio Co., Ltd. (Jinnan, Tianjin, China). Next, corresponding plasmids were transfected into TPC-1 and SW1736 cells. Finally, a GeneCopoeia LucPair Duo-Luciferase Assay Kit 2.0 (FulenGen, Guangzhou, Guangdong, China) was applied to evaluate the luciferase activities in TPC-1 and SW1736 cells.

Transwell Invasion Assays

Briefly, TPC-1 and SW1736 cells were transfected with LINC00313 siRNAs or NC siRNAs. Next, a Millipore transwell insert was pre-treated with Matrigel (80 μl), and the treated TPC-1 as well as SW1736 cells were resuspended in the culture medium without fetal bovine serum (FBS) and sequentially added into the upper chamber of the transwell insert. Besides, the 15% FBS containing medium (350 μl) was added into the lower chambers. Twenty-four hours later, 0.2% crystal violet dye solution (Baomanbio, Xuhui, Shanghai, China) was used to stain the invaded TPC-1 or SW1736 cells. Finally, an inverted fluorescence microscope (MF53, Mshot, Guangzhou, Guangdong, China) was used to take photographs of these invasive cells.

Wound Healing Assays

The LINC00313 siRNAs were transfected into TPC-1 and SW1736 cells. Then, 70 μl of the treated TPC-1 or SW1736 cells at a cell density of 5×10^5 cells per ml was added into the two reservoirs of an inserts in an Ibidi 3.5 cm μ-dish (Koster Scientific, Guangzhou, Guangdong, China). Twenty-four hours later, the culture inserts were removed and an inverted fluorescence microscope (MF53, Mshot, Guangzhou, Guangdong, China) was used to observe and image the wounded areas at 0 h and 24 h.

Statistical Analysis

All statistical analysis in our experiments was conducted by the SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). Two-tailed Student's t-test was applied to compare the differences between two groups and one-way ANOVA was conducted when analyzing more than two groups. *p*-values less than 0.05 were considered significant.

Results

Comprehensive Bioinformatics Analysis Indicated that LINC00313 was Highly Expressed in PTC Tissues and Cell Lines

To investigate aberrant lncRNAs in TPC, we firstly analyzed the microarray data from TCGA datasets and Hierarchical clustering and Volcano plots showed systematic variations in the expression of lncRNAs between PTC and normal thyroid samples (Figure 1A and B). In addition, the expression levels of LINC00313 were significantly up-regulated in PTC tissues according to TCGA datasets (Figure 1C). Next, in order to demonstrate the results from TCGA datasets, we further performed RT-PCR to explore whether LINC00313 was dysregulated in PTC samples from our hospital, finding that LINC00313 expression was significantly up-regulated in PTC tissues compared to matched normal tissues (Figure 1D). Moreover, up-regulation of LINC00313 expression was also observed in PTC cell lines (8505C, SW1736 and TPC-1) compared to normal thyroid cells (Nthy-ori 3-1) (Figure 1E). Taken together, our results, together with online data, showed that LINC00313 expression was increased in PTC patients and may act as a tumor promoter.

LINC00313 was Induced by SP1 in PTC Cells

Accumulating evidence indicated that transcription factors (TFs) exerted essential roles in lncRNA dysregulation. Therefore, we conducted bioinformatics analysis using two online TFs prediction websites, JASPAR (http://jaspar.genereg.net/) and PROMO (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3), to predict the potential TFs which could bind the promoter of LINC00313. The data suggested that there were four TFs (SP1, USF2, YY1 and SRY) in the predicting results of both JASPAR and PROMO (Figure 2A). In addition, we focused on transcription factor, SP1, which had several predicted binding sites in the promoter of LINC00313 (Figure 2B). The qRT-PCR assays revealed that the relative mRNA levels of SP1 in TPC-1 and SW1736 cells were remarkably inhibited by transfection of SP1 siRNAs (si-SP1#1 and si-SP1#2), while transfection of SP1 overexpressing plasmid (pcDNA3.1-SP1) trended opposite roles (Figure 2C and D). Besides, silence of SP1 resulted in a significantly reduction of LINC00313 expressing levels in TPC-1 and SW1736 cells, whereas enhancing

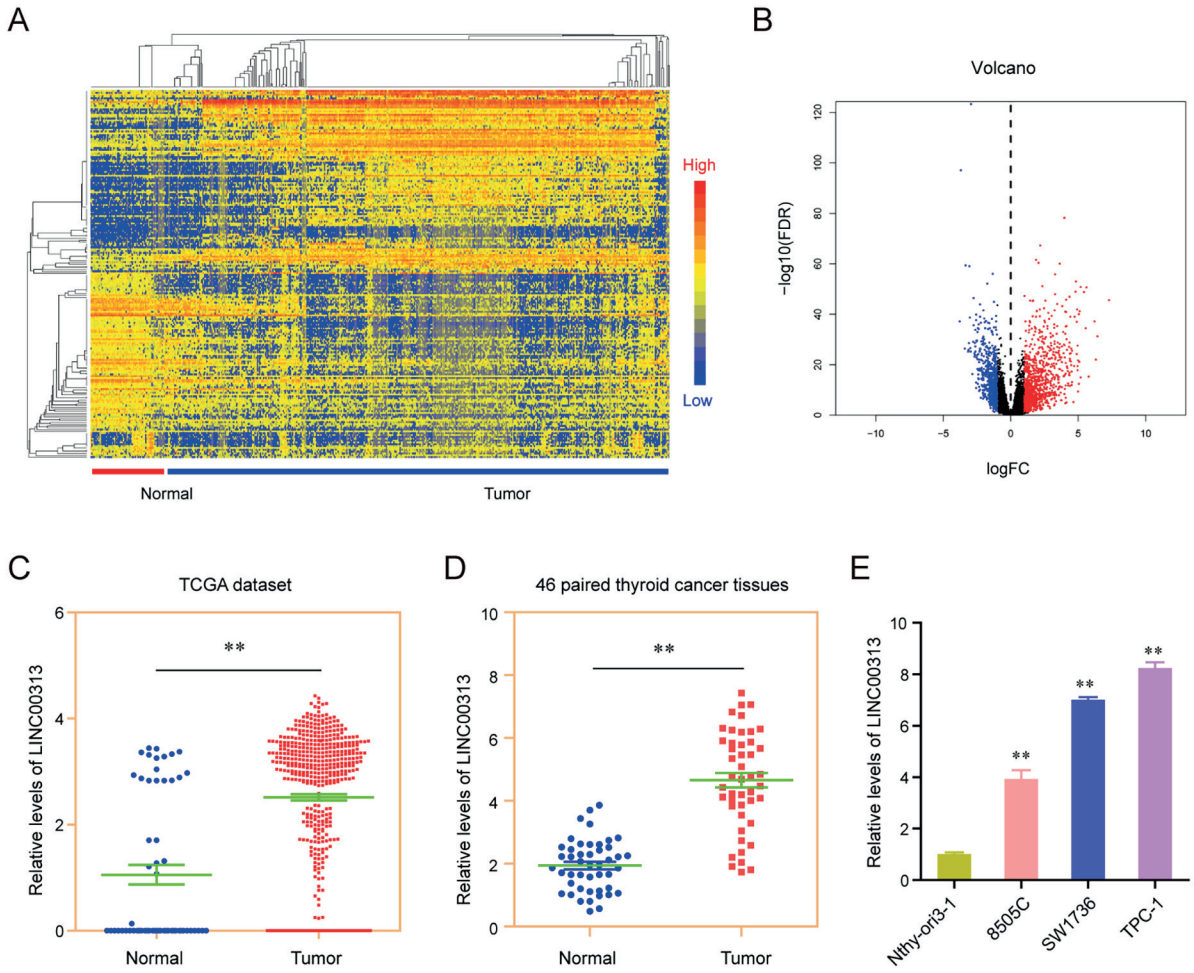

Figure 1. Bioinformatics analysis of differentially expressed lncRNAs in PTC tissues. ***(A)*** Heat map analysis of the lncRNAs expression of groups was created using a method of hierarchical clustering by GeneSpring GX, version 7.3. Microarray data were obtained from TCGA. ***(B)*** Volcano plots showing expression profiles of lncRNAs in PTC. ***(C)*** The expression levels of LINC00313 in PTC tissues and normal thyroid tissues in TCGA datasets. ***(D)*** RT-PCR analysis of LINC00313 expression in 46 paired PTC/non-tumor tissue specimens. ***(E)*** LINC00313 expression was upregulated in the PTC cell lines 8505C, SW1736 and PTC-1 compared with Nthy-ori3-1. *$p < 0.05$, **$p < 0.01$.

the expression of SP1 trended opposite roles (Figure 2E). Furthermore, the results of ChIP assays demonstrated that SP1 could effectively bind to site#2 region (Figure 2F). To further validate this, we firstly constructed the full promoter sequence of LINC00313 (pGL3-LINC00313 full promoter), the site#1 as well as site#3 sequences of LINC00313 (pGL3-LINC00313 partial promoter) and the site#2 sequence of LINC00313 (pGL3-LINC00313 site#2 promoter) into pGL3 reporter plasmids (Figure 2G). Moreover, the results of luciferase activities showed that co-transfection of SP1 overexpressing plasmids with pGL3-LINC00313 site#2 promoter or pGL3-LINC00313 full promoter significantly increased the luciferase activities of TPC-1 and SW1736 cells, which indicated that site#2 region of LINC00313 promoter was the exact binding site of SP1 (Figure 2H). Taken together, our data validated that the up-regulation of LINC00313 in PTC was mediated by SP1.

Knockdown of LINC00313 Suppressed the Proliferation of PTC Cells and Accelerated Cell Apoptosis

We next explored whether LINC00313 affected the biological behaviors of PTC cells. The qRT-PCR assays revealed that transfection of LINC00313 siRNAs (siRNA#1 and siRNA#2) dramatically reduced the expression levels of

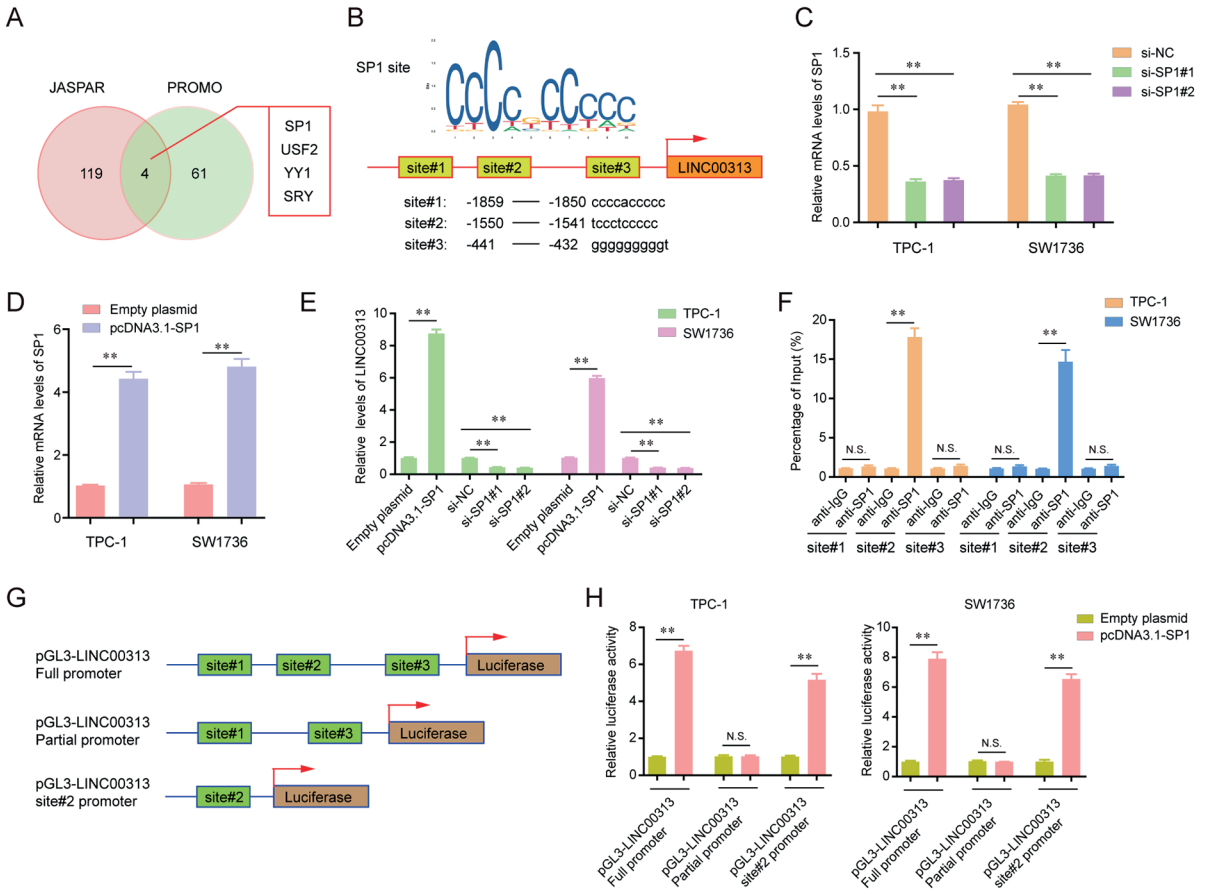

Figure 2. The transcription factor SP1 was involved in LINC00313 upregulation. ***(A)*** The transcription factors (SP1, USF2, YY1 and SRY) were in the predicting results of both JASPAR and PROMO. ***(B)*** The predicted positions of putative SP1 binding motif in -2000 bp human LINC00313 promoter ***(C*** and ***D)***. The relative mRNA levels of SP1 in TPC-1 and SW1736 cells after transfection of SP1 siRNAs or SP1 overexpressing plasmids, pcDNA3.1-SP1. ***(E)*** The relative expression levels of LINC00313 in TPC-1 and SW1736 cells after transfection of SP1 siRNAs or pcDNA3.1-SP1. ***(F)*** ChIP analysis of SP1 occupancy in the LINC00313 promoter in TPC-1 and SW1736 cells. ***(G)*** The construction of the luciferase reporter plasmids. (H) Dual luciferase reporter assays were applied to detect the SP1 binding site on the LINC00313 promoter region. $*p < 0.05$, $**p < 0.01$.

LINC00313 in TPC-1 and SW1736 cells (Figure 3A). Moreover, the proliferative rates evaluated by CCK-8 assays confirmed that silence of LINC00313 remarkably inhibited the cell growth of TPC-1 and SW1736 cells (Figure 3B and C). Similarly, the EdU assays further confirmed that the knockdown of LINC00313 suppressed the proliferation of both TPC-1 and SW1736 cells (Figure 3D and E). Besides, the cell colony formation assays suggested that transfection of LINC00313 siRNAs reduced the clonogenic abilities of TPC-1 and SW1736 cells (Figure 3F). Additionally, the results of flow cytometry indicated that the apoptotic rates of TPC-1 and SW1736 cells were remarkably elevated after the cells were transfected with LINC00313 siRNAs (Figure 3G). Collectively, these data demonstrated that LINC00313 exerted crucial roles in modulating the development of PTC.

Silence of LINC00313 Impaired the Migratory and Invasive Abilities of PTC Cells

We next aimed to investigate the effects of LINC00313 on the invasion and migration of PTC cells. According to the results of transwell invasion assays, transfection of LINC00313 siRNAs led to a significantly decline of invasive TPC-1 and SW1736 cells (Figure 4A and B). In addition, the wounded areas of TPC-1 and SW1736 cells transfected with LINC00313 siRNAs were remarkably wider than that of the cells transfected with the negative control (NC) siRNAs (Figure 4C). Furthermore, we performed Western blot as-

says to examine the roles of LINC00313 on EMT pathway, finding that the protein levels of N-cadherin and vimentin were notably decreased after repression of LINC00313 (Figure 4D). In summary, these data proved that LINC00313 affected the progression of PTC via epithelial-mesenchymal transition.

LINC00313 Served as a ceRNA Sponge of miR-422a in PTC Cells

A plethora of studies had confirmed that lncRNA could function as competing endogenous RNA (ceRNA) of specific miRNAs. Thus, we next searched the webserver "starbase" (http://starbase.sysu.edu.cn/) to predict the potential miRNAs, which could be directly interacted with LINC00313. The results of prediction revealed that miR-422a was a potential target of LINC00313 (Figure 5A). Hence, we next conducted the dual-luciferase reporter assays to demonstrate whether miR-422a was the exact target of LINC00313. The data suggested that co-transfection with LINC00313-wt vectors and miR-422a mimics dramatically reduced the luciferase activities of TPC-1 and SW1736 cells (Figure 5B). In addition, the results of RIP assays proved that LINC00313 and miR-422a were significantly enriched in Ago2-containing beads compared with

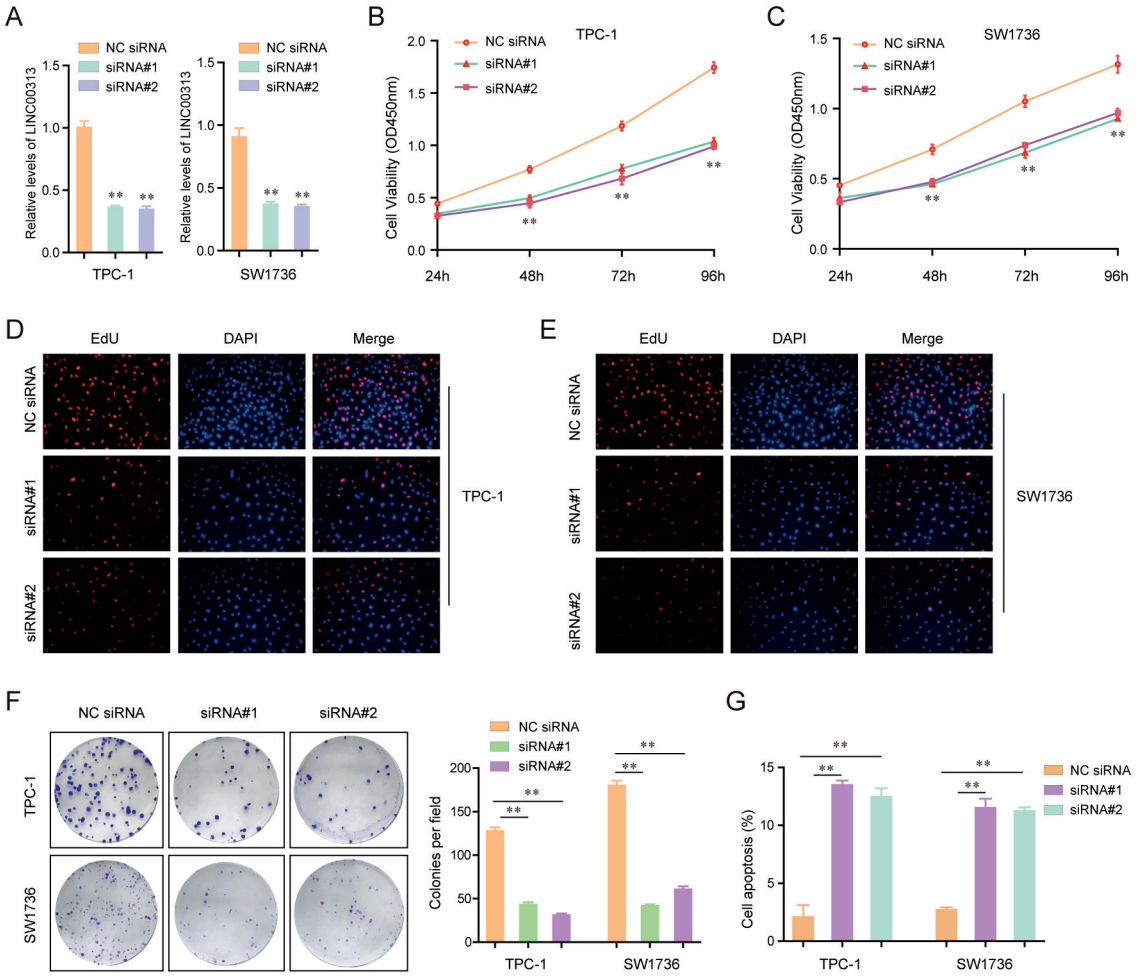

Figure 3. LINC00313 knockdown suppressed the proliferation and accelerated the apoptosis of TPC-1 and SW1736 cells. ***(A)*** The relative expression levels of LINC00313 in TPC-1 and SW1736 cells transfected with LINC00313 siRNAs (siRNA#1 and siRNA#2) or negative control siRNAs (NC siRNA). ***(B*** and ***C)*** Transfection of LINC00313 siRNAs reduced the proliferation of TPC-1 and SW1736 cells. ***(D*** and ***E)*** EdU assays detected the proliferation of TPC-1 and SW1736 cells after transfection of LINC00313 siRNAs or NC siRNA. The positive cells (proliferative cells) were labeled with red fluorescence; nuclear fractions were labeled with DAPI (blue). ***(F)*** Silence of LINC00313 reduced colony formation abilities of TPC-1 and SW1736 cells. ***(G)*** The cells apoptosis was detected by flow cytometry. $*p < 0.05$, $**p < 0.01$.

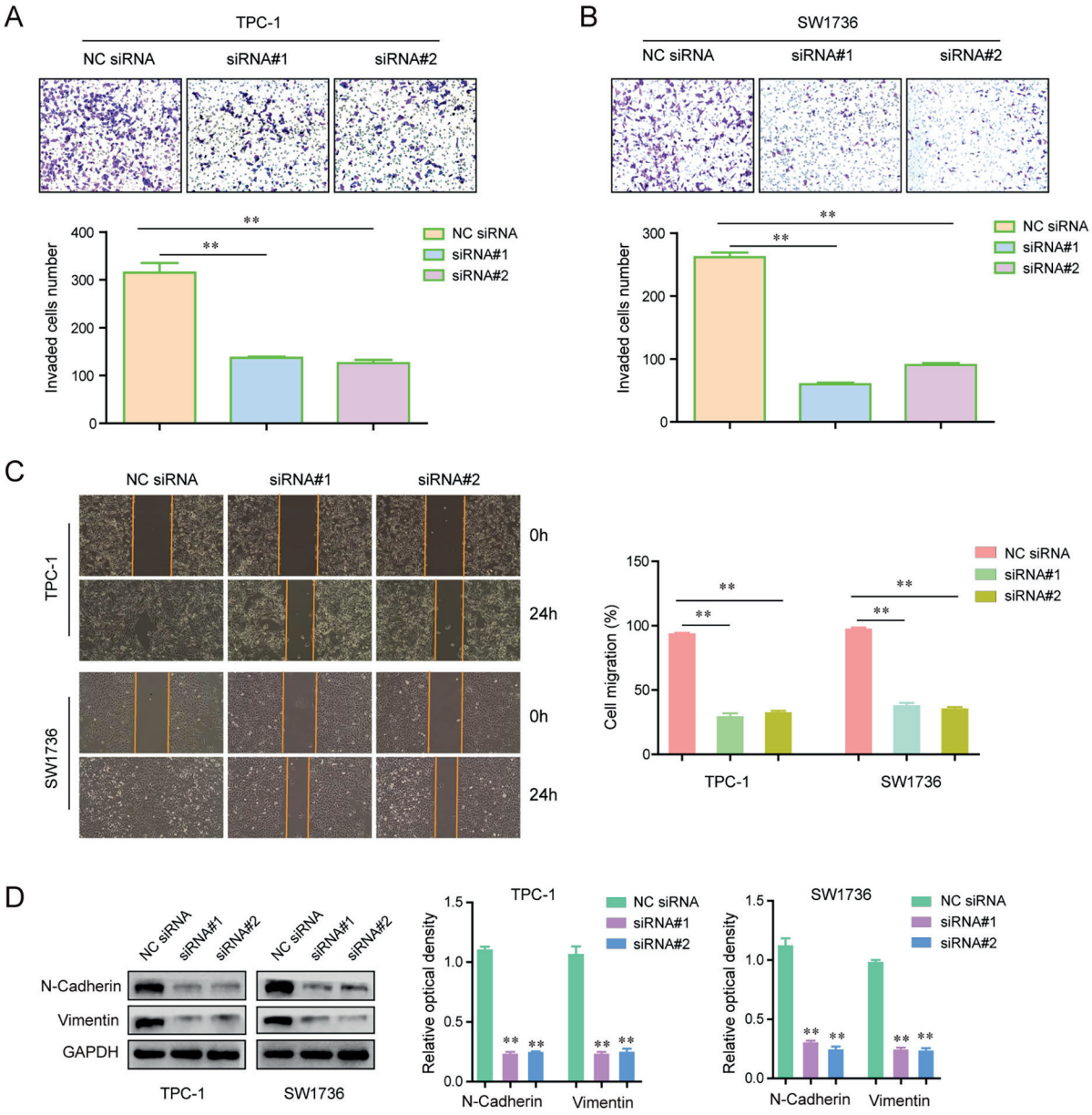

Figure 4. Silence of LINC00313 repressed the invasion and migration of TPC-1 and SW1736 cells. ***(A*** and ***B)*** Transfection of LINC00313 siRNAs remarkably reduced the invasive abilities of TPC-1 and SW1736 cells. ***(C*** and ***D)*** Knockdown of LINC00313 significantly reduced the migration of TPC-1 and SW1736 cells. ***(E*** and ***F)*** Transfection of LINC00313 siRNAs dramatically decreased the protein levels of N-cadherin and vimentin. *$p < 0.05$, **$p < 0.01$.

the input group, which further confirmed that LINC00313 was directly associated with miR-422a (Figure 5C). Besides, we further applied qRT-PCR assays to examine the alteration of miR-422a in TPC-1 and SW1736 cells. The data revealed that enhancing expression of LINC00313 in TPC-1 and SW1736 cells remarkably reduced the expression levels of miR-422a, while silence of LINC00313 notably accelerated miR-422a expression (Figure 5D). Overall, our data provided evidence that miR-422a was directly interacted with LINC00313, and LINC00313 modulated the development as well as progression of PTC via sponging miR-422a.

Discussion

PTC is one of the fastest growing cancer diagnoses in China. To date, the conundrum for PTC is that a small number of patients with aggressive PTC develop invasive tumors and/or distant metastases[21]. More understanding about the biological mechanism involved in PTC is critical to find better therapeutic strategies for PTC patients with metastasis. Recently, the studies of lncRNAs became a hotspot because of its wild regulation in various biological progression[22,23]. In this study, we firstly analyzed RNA sequencing data of PTC and para-cancerous tissues downloaded from TCGA, finding that LINC00313 was one of the most up-regulated lncRNA in PTC. In addition, our results from RT-PCR also showed that the expression levels of LINC00313 were significantly high in both PTC tissues and cell lines. Thus, our findings, indicated that up-regulation of LINC00313 may be involved in the progression of PTC. Specificity protein 1 (Sp1) is a transcription factor that is ubiquitously expressed in various tissues and involved in several biological processes, such as cell differentiation, cell cycle, immune responses and response to DNA damage[24,25]. Increasing evidence demonstrated that SP1 could activate the transcription of downstream targets including lncRNAs. For instance, lncRNA AGAP2-AS1, upregulated by SP1, promoted cell proliferation and invasion in gastric cancer[26]. SP1 could up-regulate lncRNA TINCR expression to promote cell growth and suppress apoptosis by epigenetic regulation of KLF2 mRNA[27]. In this study, using two online TFs prediction websites, we observed that SP1 could interact with the promoter of LINC00313. Then, we performed luciferase reporter assays and ChIP, and observed that SP1 could bind to LINC00313 promoter region and induce its transcription. Overall, our results revealed that overexpression of LINC00313 may be modulated by SP1. Recently, the dysregulation of LINC00313 has been reported in several tumors, such as lung cancer[19], clear cell renal carcinoma[17] and gliomas[28]. Importantly, recent study by Wu et al[20] firstly reported that LINC00313 was highly expressed in PTC and its downregulation suppressed PTC cells proliferation and migration via sponging miR-4429. In this study, we also performed lost-of-function assay to explore the

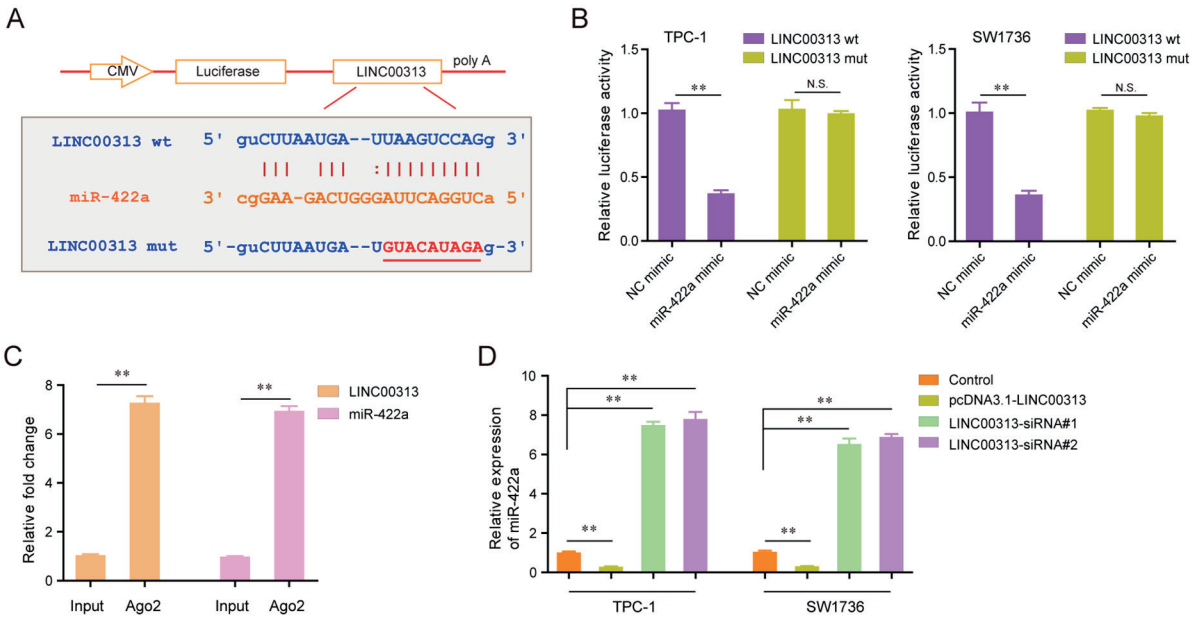

Figure 5. miR-422a directly targeted LINC00313. ***(A)*** The putative miR-422a binding site was predicted by "starbase". ***(B)*** The luciferase activities of TPC-1 and SW1736 cells after co-transfecting with LINC00313 wt or LINC00313 mut plasmids as well as negative control (NC) mimic or miR-422a mimic. ***(C)*** The enrichment of LINC00313 and miR-422a Ago2-containing beads detected by RIP assays. ***(D)*** The relative expression of miR-422a in TPC-1 and SW1736 cells after transfection of LINC00313 siRNAs or overexpressing plasmid, pcDNA3.1-LINC00313. *$p < 0.05$, **$p < 0.01$.

roles of LINC00313 in PTC behaviors, finding that knockdown of LINC00313 significantly suppressed cells proliferation, migration and invasion in PTC cells, which was consistent with previous findings. In addition, the results of Western blot showed that LINC00313 may display its tumor-promotive role by modulating EMT signaling which play an important role in carcinogenesis[21]. It has been confirmed that lncRNAs act as miRNA sponges, which interact with miRNAs and modulate the expression of miRNA target genes. Then, we found that LINC00313 may be a target of miR-422a by analyzing the online software program starbase v2.0. Previously, miR-422a had been detected to be down-regulated in PTC and its overexpression suppressed PTC cells proliferation and metastasis[29]. Following luciferase reporter assay and qPCR verified that LINC00313 is a genuine target of miR-422a. Taken together, our findings revealed that LINC00313 promoted proliferation and metastasis through sponging miR-422a.

Conclusions

We demonstrated that LINC00313 was up-regulated and activated by SP1 regulator in PTC. Knockdown of LINC00313 suppressed PTC cell proliferation, migration and invasion by at least in part through modulating miR-422a. In the future, LINC00313 could be considered as a potential target for the PTC therapies.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.
2) Kitahara CM, Sosa JA. The changing incidence of thyroid cancer. Nat Rev Endocrinol 2016; 12: 646-653.
3) Cabanillas ME, McFadden DG, Durante C. Thyroid cancer. Lancet 2016; 388: 2783-2795.
4) Li Q, Lin X, Shao Y, Xiang F, Samir AE. Imaging and screening of thyroid cancer. Radiol Clin North Am 2017; 55: 1261-1271.
5) Grewal RK, Ho A, Schoder H. Novel approaches to thyroid cancer treatment and response assessment. Semin Nucl Med 2016; 46: 109-118.
6) Hadoux J, Pacini F, Tuttle RM, Schlumberger M. Management of advanced medullary thyroid cancer. Lancet Diabetes Endocrinol 2016; 4: 64-71.
7) Azar FK, Lee SL, Rosen JE. Medullary thyroid cancer: an update for surgeons. Am Surg 2015; 81: 1-8.
8) Jandura A, Krause HM. The new RNA world: growing evidence for long noncoding RNA functionality. Trends Genet 2017; 33: 665-676.
9) Meller VH, Joshi SS, Deshpande N. Modulation of chromatin by noncoding RNA. Annu Rev Genet 2015; 49: 673-695.
10) Kung JT, Colognori D, Lee JT. Long noncoding RNAs: past, present, and future. Genetics 2013; 193: 651-669.
11) Luo ML. Methods to study long Noncoding RNA biology in cancer. Adv Exp Med Biol 2016; 927: 69-107.
12) Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. Cancer Cell 2016; 29: 452-463.
13) Fu XM, Guo W, Li N, Liu HZ, Liu J, Qiu SQ, Zhang Q, Wang LC, Li F, Li CL. The expression and function of long noncoding RNA lncRNA-ATB in papillary thyroid cancer. Eur Rev Med Pharmacol Sci 2017; 21: 3239-3246.
14) Bhan A, Mandal SS. LncRNA HOTAIR: a master regulator of chromatin dynamics and cancer. Biochim Biophys Acta 2015; 1856: 151-164.
15) Gao KT, Lian D. Long non-coding RNA MALAT1 is an independent prognostic factor of osteosarcoma. Eur Rev Med Pharmacol Sci 2016; 20: 3561-3565.
16) Cui M, You L, Ren X, Zhao W, Liao Q, Zhao Y. Long non-coding RNA PVT1 and cancer. Biochem Biophys Res Commun 2016; 471: 10-14.
17) Yin H, Wang X, Zhang X, Wang Y, Zeng Y, Xiong Y, Li T, Lin R, Zhou Q, Ling H, Zhou F, Zhou Y. Integrated analysis of long noncoding RNA associated-competing endogenous RNA as prognostic biomarkers in clear cell renal carcinoma. Cancer Sci 2018; 109: 3336-3349.
18) Wei MM, Zhou GB. Long Non-coding RNAs and their roles in non-small-cell lung cancer. Genomics Proteomics Bioinformatics 2016; 14: 280-288.
19) Li M, Qiu M, Xu Y, Mao Q, Wang J, Dong G, Xia W, Yin R, Xu L. Differentially expressed protein-coding genes and long noncoding RNA in early-stage lung cancer. Tumour Biol 2015; 36: 9969-9978.
20) Wu WJ, Yin H, Hu JJ, Wei XZ. Long noncoding RNA LINC00313 modulates papillary thyroid cancer tumorigenesis via sponging miR-4429. Neoplasma 2018; 65: 933-942.
21) Rebasmall UM, Rebasmall UA. Molecular genetics of thyroid cancer. Genet Res (Camb) 2016; 98: e7.
22) Renganathan A, Felley-Bosco E. Long noncoding RNAs in cancer and therapeutic potential. Adv Exp Med Biol 2017; 1008: 199-222.
23) Yang L, Froberg JE, Lee JT. Long noncoding RNAs: fresh perspectives into the RNA world. Trends Biochem Sci 2014; 39: 35-43.

24) Vizcaino C, Mansilla S, Portugal J. Sp1 transcription factor: a long-standing target in cancer chemotherapy. Pharmacol Ther 2015; 152: 111-124.

25) Beishline K, Azizkhan-Clifford J. Sp1 and the 'hallmarks of cancer'. FEBS J 2015; 282: 224-258.

26) Qi F, Liu X, Wu H, Yu X, Wei C, Huang X, Ji G, Nie F, Wang K. Long noncoding AGAP2-AS1 is activated by SP1 and promotes cell proliferation and invasion in gastric cancer. J Hematol Oncol 2017; 10: 48.

27) Xu TP, Liu XX, Xia R, Yin L, Kong R, Chen WM, Huang MD, Shu YQ. SP1-induced upregulation of the long noncoding RNA TINCR regulates cell proliferation and apoptosis by affecting KLF2 mRNA stability in gastric cancer. Oncogene 2015; 34: 5648-5661.

28) Hassan A, Mosley J, Singh S, Zinn PO. A comprehensive review of genomics and noncoding RNA in gliomas. Top Magn Reson Imaging 2017; 26: 3-14.

29) Wang J, Yang H, Si Y, Hu D, Yu Y, Zhang Y, Gao M, Zhang H. Iodine promotes tumorigenesis of thyroid cancer by suppressing Mir-422a and up-regulating MAPK1. Cell Physiol Biochem 2017; 43: 1325-1336.