European Review for Medical and Pharmacological Sciences 2019; 23: 9803-9813

Long non-coding RNA LINC00152 is up-regulated in ovarian cancer tissues and regulates proliferation and cell cycle of SKOV3 cells

H. NI[1], L.-L. NIU[1], S.-C. TIAN[2], L.-K. JING[1], L.-T. ZHANG[2], Q.-Q. LIN[1], Y.-H. CAI[1], H.-M. LIANG[1], Q. DU[2], H. LI[1]

[1]Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China
[2]State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, China

Huamao Liang, Quan Du, and *Hua Li* contributed equally to this paper

Abstract. – OBJECTIVE: To characterize functions of long non-coding RNA (lncRNA) in the progression of epithelial ovarian cancer.

PATIENTS AND METHODS: Epithelial ovarian cancer tissues and matching normal tissues were collected from two individual patients for RNA microarray analysis. Besides, twenty-two ovarian cancer samples and ten healthy ovarian epithelial tissues were collected for Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR). Microarray assay suggested that a list of cancer relating mRNAs and lncRNAs were upregulated. The identified lncRNAs were validated *via* RT-qPCR, which led to the identification of long intergenic non-protein coding RNA 152 (LINC00152). To determine the function of LINC00152 in ovarian cancer, we knocked down the expression of LINC00152 in epithelial ovarian cancer cell line SKOV3 with small interference RNAs (siRNAs). The effects of LIN00152 on the proliferation and cell cycle were determined by comparing the cell viability of SKOV3 cells with LIN00152 knockdown and the control cells with negative siRNA. The cell viability was assessed using Cell Counting Kit-8 (CCK-8) and flow cytometry assay. RNA microarray assay was used again in control and LINC00152 knockdown SKOV3 cells to identify downstream signaling pathways.

RESULTS: Fourteen ovarian cancer relating lncRNAs were identified by RNA microarray assay. Up-regulation of LINC00152 was validated *via* RT-qPCR. A higher expression of LINC00152 in late cancer stage (III-IV) compared to the early stage tumors was also demonstrated. Inhibition of LINC00152 in SKOV3 cells inhibited cell proliferation and induced cell cycle arrest that involved prolonged G1 phase and shortened S phase. The microarray assay data of SKOV3 cells suggested that Cyclin-Dependent Kinase Inhibitor 1C (CDKN1C) was a potential downstream target of LINC00152.

CONCLUSIONS: LINC00152 is upregulated in epithelial ovarian cancer tissues comparing to normal tissues. Knockdown of LINC00152 expression inhibits cell proliferation and induces cell cycle arrest. LINC00152 possibly interacts with Tumor Necrosis Factor (TNF) signaling pathway. CDKN1C is a potential downstream target of LINC00152.

Key Words:
Ovarian cancer, LncRNA, LINC00152, Microarray.

Introduction

Ovarian cancer is a fatal gynecological disease. Despite the progress in research and treatment of ovarian cancer, challenges remain for the development of early diagnosis and practical treatment approaches.

With the recent development of high throughput sequencing, the critical roles of long non-coding Ribonucleic acid (lncRNA) in physiology and pathology have been revealed[1,2]. In addition to its engagement in multiple biological processes such as proliferation, cell cycle, and metastasis[3], studies also indicated that lncRNAs play essential roles in tumor occurrence and development[4]. However, in the field of gynecological cancers, very few functional lncRNAs have been investigated. Among them, the aberrant expression of HOX

Corresponding Author: Hua Li, MD; e-mail: hual_gyn@163.com

transcript antisense Ribonucleic acid (HOTAIR)[5], H19[6], X-inactive specific transcript (XIST)[7], Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1)[8], Urothelial Cancer Associated 1 (UCA1)[9], and Maternally Expressed Gene 3 (MEG3)[10] were reported in ovarian cancer tissues. They may play different roles in the progression of ovarian cancer.

To investigate the roles of lncRNA in cancer progression, we performed gene expression assays with paired cancer and normal tissues. Cancer relating lncRNAs were then investigated by RT-qPCR, leading to the identification of LINC00152.

Patients and Methods

Ethics Statement

This investigation was conducted according to the protocols approved by the Ethics Review Board of Peking University Third Hospital (Beijing, China). Written informed consent was obtained for sample collection and analysis.

Sample Acquisition and Processing

Tissue samples were obtained from thirty-four surgical patients in Peking University Third Hospital. Twenty-six samples were collected from epithelial ovarian cancer patients, including twenty-four tumor tissues and two matched normal counterparts derived from adjacent normal ovarian tissues or opposite ovarian. Ten tissue samples were collected from the normal ovarian epithelial tissues of ten additional patients. The samples were collected during surgery and immediately snap-frozen in liquid nitrogen and stored. More than two pathologists examined the histological type. Total RNAs were extracted from the samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA quantity and quality were determined using NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and electrophoresis, then stored at –80°C freezer until use.

Microarray of Tissues and Computational Analysis

Total RNA of two ovarian cancer tissues and normal counterparts was quantified by the NanoDrop-2000 (Thermo Fisher Scientific, Waltham, MA, USA). The RNA integrity was assessed using Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Sample labeling, microarray hybridization, and washing were performed according to the manufacturer's standard protocols. Briefly, total RNA was transcribed to double strand cDNA, then synthesized into cRNA and labeled with Cyanine-3-CTP. The labeled cRNAs were hybridized onto the microarray. After washing, the arrays were scanned by the Agilent Scanner G2505C (Agilent Technologies, Santa Clara, CA, USA). Feature Extraction software (version 10.7.1.1, Agilent Technologies, Santa Clara, CA, USA) was used to analyze array images to get raw data. GeneSpring (version 13.1, Agilent Technologies, Santa Clara, CA, USA) was employed for primary analysis of raw data. Briefly, raw data were normalized with the quantile algorithm. The probes that at least 1 out of 2 terms have flags in "positive (P)" were chosen for further data analysis. Differentially expressed mRNAs or lncRNAs were then identified through fold change. The threshold set for up- and down-regulated genes was≥ 2.0-fold change. Afterward, GO analysis and KEGG analysis were applied to determine the roles of these differentially expressed mRNAs. Genes were identified based on a fold change of ≥ 4.0.

Quantitative Reverse Transcription PCR

Total RNAs and random RT primer (TIANGEN, Beijing, China) was used for cDNA synthesis. Synthesized cDNAs were analyzed using quantitative real-time PCR assay with kit from Promega (Madison, WI, USA). U6 was used as internal control.

The sequences of the primers used in the assay are as follows: U6 sense, 5'-GCTTCGGCAGCACATATACTAAAAT-3'; antisense: 5'-CGCTTCACGAATTTGCGTGTCAT-3'; LINC00152, sense: 5'-ATCCACATTCCAACCTCCGT-3'; antisense: 5'-CTGAGTCGTGATTTTCGGTGT-3'; UCA1 sense: 5'-ACGCTAACTGGCACCTTGTT-3'; antisense: 5'-CCGGACTGCTTCAAGTGTGA-3'. CDKN1C sense: 5'-GGCTCTAAACTGCGAGGAGA-3'; antisense: 5'-CGGTGAGCCAAGTGAGTACA-3'. CDK1 sense: 5'-GAACACCACTTGTCCCTCTAAGAT-3'; antisense: 5'-CTGGCAAGGCCAAAATCAGC-3'. CDK2 sense: 5'-CGTCAACGTGGGTCTTGGTA-3'; antisense: 5'-ATCAGTGTGCCAACAGGGAG-3'. CDK3 sense: 5'-TCAGGTGACTCGAAAAGCCC-3'; antisense: 5'-GTCAGGAAAGAGACCCTGGC-3'. CDK6 sense: 5'-CATTTTGTGGCTGCTTGGCA-3'; antisense: 5'-AAAAGTGTCCCCTCACGACC-3'.

Cell Culture and Transfection

SKOV3 ovarian cancer cells (Peking University Third Hospital, Beijing, China) were cultured in DMEM/F12, were supplemented with 100 units/mL penicillin (Life Technologies, Carlsbad, CA, USA) and 100 μg/mL streptomycin (P/S), and 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA). The cells were incubated in 5% CO_2 at 37°C.

When the cell density reached about 50%, siRNA targeting LINC00152 and negative control siRNA designed by RiboBio (Guangzhou, China) were transfected into cells using Lipofectamine® imax Reagent (Invitrogen, San Diego, CA, USA). After incubation for 4-6 hours at 37°C, the culture medium was replaced with refresh culture medium containing 10% FBS and P/S after transfection. Silencing efficiency was observed *via* RT-qPCR. The following tests were performed 48 hours after transfection.

Cell Proliferation Assays

Cell proliferation assays were performed using Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Kyushu, Japan) according to the manufacturer's protocol. After transfection of siRNAs, 1500 cells per well were seeded into 96-well plates and incubated for 48 hours. Viable cells were evaluated by the 450 nm absorbance at the indicated time points.

Flow Cytometry Analysis for Cell Cycle

Cells were collected and fixed in chilled 70% ethanol at 4°C for 12 h, followed by washing with phosphate-buffered saline (PBS; Thermo Fisher Scientific, Waltham, MA, USA). The fixed cells were stained with 50 mg/ml propidium iodide (PI; Thermo Fisher Scientific, Waltham, MA, USA) at 4°C in the darkness for 20 min before analysis. The flow cytometry analysis was performed using Beckman CytoFLEX FCM (Beckman, Brea, CA, USA).

Statistical Analysis

Statistical analysis was performed using Statistical Program in SPSS 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Armonk, NY, USA) or GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). For data that complied with normal distribution, two-tailed Student's *t*-test was used. For data that did not belong to normal distribution, nonparametric rank sum test was performed. Regarding enumeration data, Chi-square test was performed when case number was more than 40; alternatively, Fisher exact test was performed when case number was less than 40. Three independent experiments were carried out for each assay. $p < 0.05$ was considered statistically significant.

Results

Identification of Differentially Expressed LncRNAs in Ovarian Cancer Tissues

Microarray assay was performed in two ovarian cancer tissues and their matching adjacent normal counterpart to characterize lncRNAs relating to ovarian cancer. In this assay, 46506 lncRNA probes and 30656 mRNA probes were applied for each pair of tissues. Ovarian cancer relating RNAs were determined by comparing cancer and normal tissues (Table I), while the threshold fold change value was 2-fold (up or down). Accordingly, 28.81% of the probed mRNAs and 25.51% of the probed lncRNAs showed different expression levels in cancer tissues comparing to normal tissues (Table II). To narrow down the target IncRNAs for further inve-

Table I. The differentially expressing lncRNAs and mRNAs.

Cancer	Normal counterpart	mRNA (up)	mRNA (down)	mRNA (total)	lncRNA (up)	lncRNA (down)	lncRNA (total)
1	1a	4149 (46.84%)	4709 (53.16%)	8858 (100%)	4339 (36.80%)	7452 (63.20%)	11791 (100%)
5	5a	4999 (56.75%)	3810 (43.25%)	8809 (100%)	6764 (56.64%)	5178 (43.36%)	11942 (100%)

Up means up-regulated; down means down-regulated. For each sample, 46506 lncRNA probes and 30656 mRNA probes were applied. In relative to their normal counterpart, differentially expressing RNAs in cancer tissues were determined according to a 2-fold change in gene expression.

Table II. The differently expressed lncRNAs and mRNAs.

RNA	Sum: Difference	Sum: No difference	Total	Differential rate	χ^2-value	*p*-value
lncRNA	23733	69279	93012	25.51%		
mRNA	17667	43645	61312	28.81%	204.86	< 0.001*

By means of Pearson's chi-squared test, differently expressed lncRNAs and mRNAs were calculated.

stigation, the threshold was increased to 4-fold (up or down). This led to the identification of fourteen lncRNAs, among which the expression of nine lncRNAs was higher and five lncRNAs was lower in the tumor tissues than in the normal counterparts. Five of these lncRNAs have been studied in human diseases (Table III).

Validation of LINC00152's Up-Regulation in Ovarian Cancer Tissues

To validate microarray data, we assessed the expression of LINC00152 in twenty-two epithelial ovarian cancer tissues and ten normal ovarian epithelial tissues *via* RT-qPCR. In the meantime, UCA1 was measured as a positive control since it is a well-established ovarian cancer-related lncRNA[9]. According to RT-qPCR result, LINC00152 was up-regulated in ovarian cancer tissues ($p < 0.001$, Figure 1A). UCA1 was also up-regulated in ovarian cancer tissues ($p < 0.001$, Figure 1B). Moreover, to determine whether the increase of LINC00152 expression was related to ovarian cancer progression, the LINC00152 expression level was further compared within cancer patients. Clinical features including age, tumor stage, and tumor grade were documented, while each one was assigned a cut-point to group patients. LINC00152 exhibited higher expression levels in patients with more advanced tumor stage ($p < 0.05$, Table IV), whereas age and tumor grade were not relevant to LINC00152's expression.

The effects of LINC00152 Knockdown on SKOV3 Cells

To investigate the potential functions of LINC00152 in ovarian cancer progression, we applied gene silencing targeting LINC00152 in SKOV3 cells (an ovarian cancer cell line). Spe-

Table III. Characterization of differentially expressed lncRNAs with a threshold of 4-fold change.

No.	Type	lncRNA	Chr.	FC (1/1a)	FC (5/5a)	Relative disease
1	Up	LINC00152	chr2	71.73	11.84	Gastric cancer[17], hepatocellular carcinoma[13], colon cancer[18], gall-bladder cancer[14], renal cell carcinoma[15]
2	Up	MIR4435-1HG	chr2	42.47	8.98	##
3	Up	LOC100507351	chr17	29.84	37.81	##
4	Up	KRT18P55	chr17	14.92	12.01	Intestinal-type gastric cancer[23]
5	Up	SNAR-D	chr19	9.16	37.86	##
6	Up	SNAR-A1	chr19	7.46	45.69	##
7	Up	SNAR-A3	chr19	7.29	52.63	##
8	Up	SNAR-B2	chr19	6.81	40.97	##
9	Up	DUXAP10	chr14	5.16	8.98	Non-small cell lung cancer[24], colorectal cancer[25], bladder cancer[26], oral squamous cell carcinoma[27]
10	Down	UBE2Q2P1	chr15	-4.49	-8.75	##
11	Down	NR2F2-AS1	chr15	-8.92	-9.46	##
12	Down	PRRT2	chr16	-9.44	-18.25	Paroxysmal diseases[28]
13	Down	EPB41L4A-AS1	chr5	-11.15	-7.00	##
14	Down	VLDLR-AS1	chr9	-14.37	-24.82	Fat loss of cancer cachexia[29]

FC represents expression fold change. A positive value represents expression higher than control normal tissue, while a negative value represents lower expression than control normal tissue. For tissues, 1 and 5 represent cancer tissues, while 1a and 5a represent normal counterparts. ##Means no evidence yet.

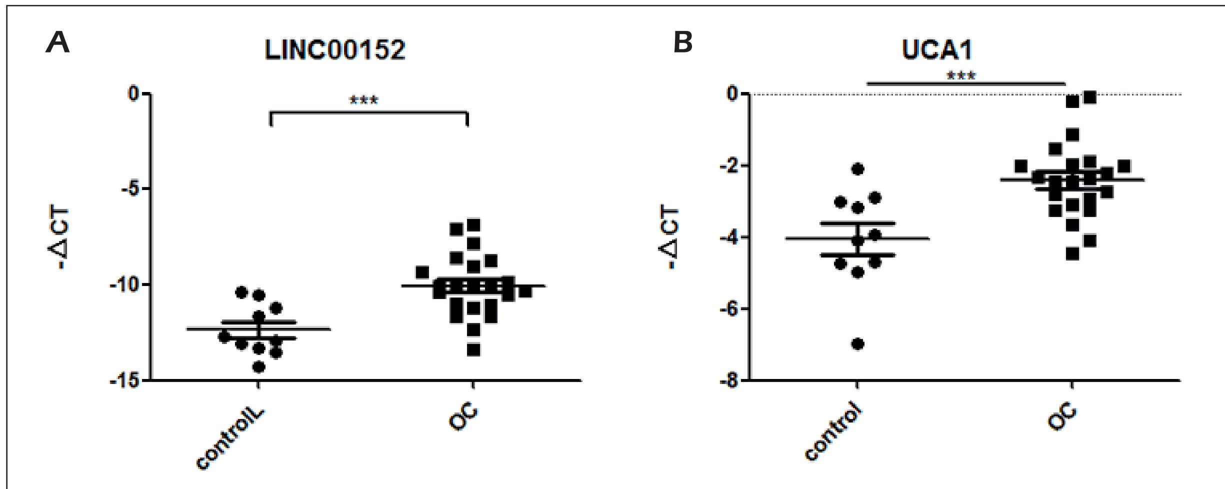

Figure 1. Validation of ovarian relating lncRNAs *via* RT-qPCR. **A**, LINC00152 is up-regulated in ovarian cancer tissue; **B**, UCA1 is up-regulated in ovarian cancer tissues. Data are presented as Mean±SD, *$p < 0.05$, ** $p < 0.01$, ***$p < 0.005$.

cifically, LINC00152-specific siRNAs were transfected into SKOV3 to knock down the expression of LINC00152. According to the results of CCK-8 assays, knockdown of LINC00152 expression inhibited cell proliferation (Figure 2A). Furthermore, flow cytometry data suggested that LINC00152 knockdown resulted in shortened S phase and prolonged of G1 phase (Figure 2B) in the cell cycle.

Whole-Genome Transcriptome Analysis of LINC00152

To reveal the mechanism of LINC00152 function in cell proliferation and cell cycle, we compared the global gene expression profile between control and LINC00152-silencing SKOV3 cells using microarray analysis. In three independent experiments, total RNA samples of cells with or without LINC00152 inhibition were collected and analyzed. Principal component analysis (PCA) (Figure 3A) and unsupervised hierarchical clustering analysis (Figure 3B) were performed with the raw data to confirm the similarity within group and distinction across groups (S6, S7, and S8 belong to LINC00152 silencing group, S10, S11, S12, and S13 belong to control group). Overall analysis showed that in LINC00152 knockdown cells, the expression of 149 mRNAs was up-regulated and the expression of 292 mRNAs was down-regulated. Gene Ontology (GO) analysis was performed to categorize these mRNAs into diverse function groups (Figure 4A), cellular components (Figure 4B), and biological processes (Figure 4C). Accordingly, the most affected molecular function was "structural constituent of muscle"'. The most affected cellular component was "nucleus". The most significant biological process was "cellular response to mechanical stimulus". Besides, the Kyoto Encyclopedia of Genes and Genomes

Table IV. LINC00152 expression in groups with different clinical features.

Clinical feature		-ΔCT	2-ΔΔCT	*p*-value
Age	≥ 60 (n=11)	-5.727 ± 0.3578	0.695	$p = 0.2651$
	< 60 (n=11)	-5.202 ± 0.2857		
Stage	Early (I-II) (n=8)	-10.94 ± 0.4549	0.381	$p = 0.0475$*
	Late (III-IV) (n = 14)	-9.547 ± 0.4244		
Grade	G1/G2 (n = 13)	-9.930 ± 0.4747	1.231	$p = 0.6736$
	G3 (n=9)	-10.23 ± 0.5085		

The histological type of all samples is serous epithelial ovarian cancer. Values were shown as mean±SEM. Independent *t*-test was performed to calculate the *p*-value. For comparison, the second group was used as control.

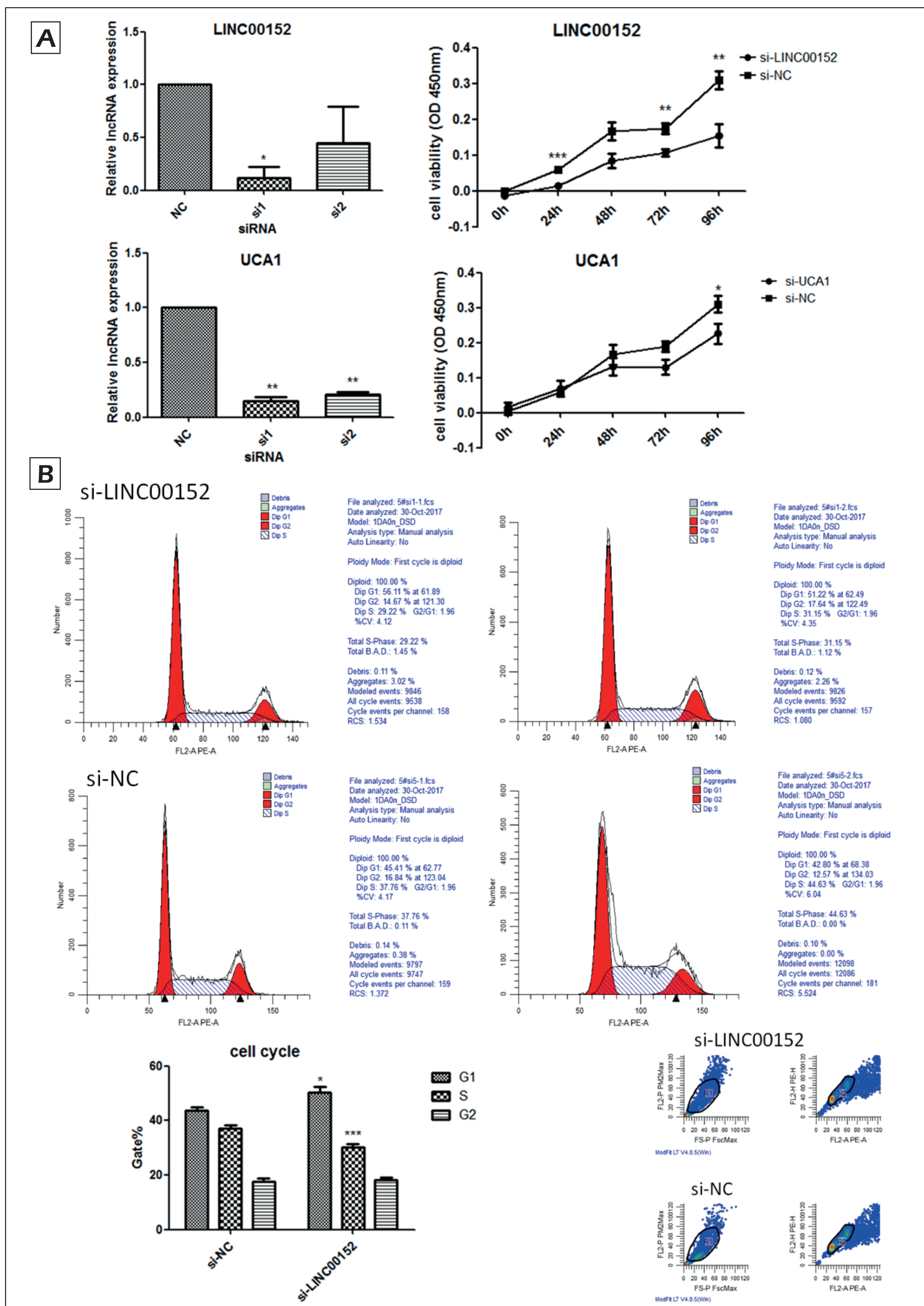

Figure 2. The effects of LINC00152 knockdown on cells proliferation and cell cycle. **A**, CCK-8 assays determined the cell viability in the condition of LINC00152 knockdown, while UCA1 knockdown was indicated as a positive control. **B**, Flow cytometry assays analyzed the change of cell cycle in the condition of LINC00152 knockdown. Data are presented as Mean±SD, *$p < 0.05$, ** $p < 0.01$, ***$p < 0.005$.

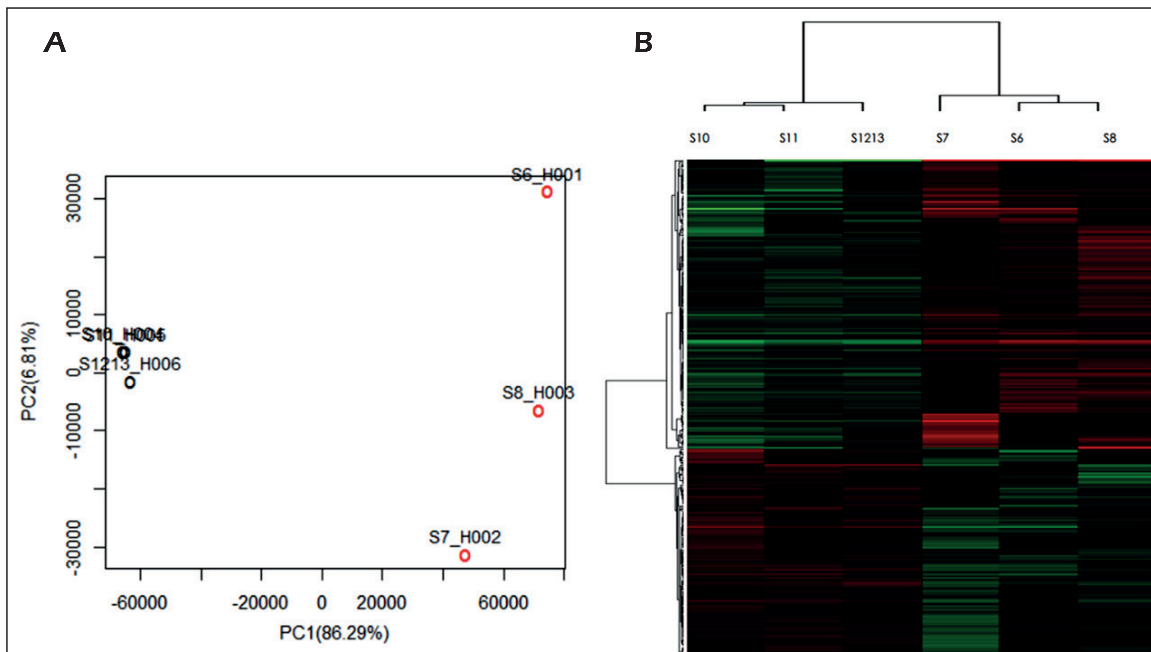

Figure 3. Results of principal component analysis (PCA) and unsupervised hierarchical clustering analysis of mRNAs in ovarian cancer cells. S6, S7, and S8 belong to the LINC00152 silencing group, and S10, S11, S12, and S13 belong to the control group. **A**, PCA was performed with the mRNA expression raw data to indicate the general similarity within group and distinction across groups. **B**, Unsupervised hierarchical clustering showed the cluster of genes in each group.

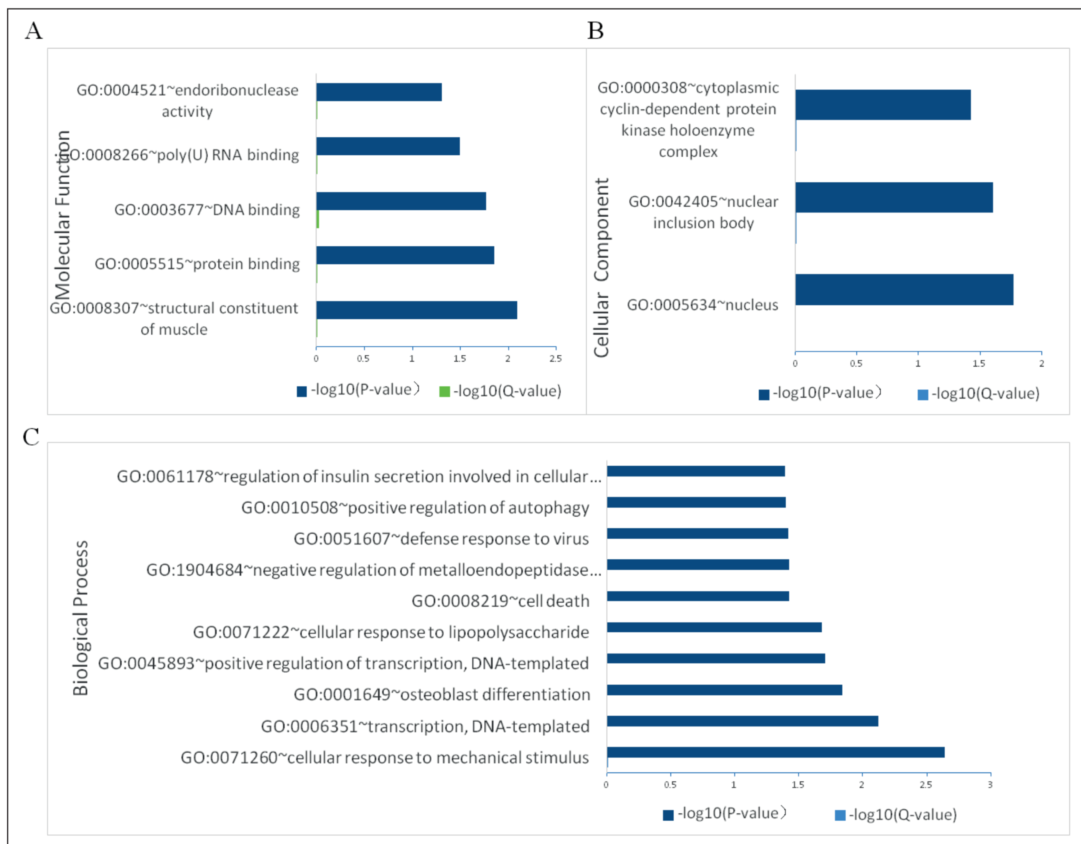

Figure 4. Gene Ontology (GO) analysis of mRNAs that were regulated by LINC00152 in ovarian cancer cells. **A**, The most relevant molecular functions. **B**, The most relevant cellular components. **C**, The most relevant biological process.

(KEGG) analysis was utilized to check the relevant signaling pathways. "Tumor Necrosis Factor (TNF) signaling pathway" was identified as the most relevant pathway (Table V).

Negative Correlation Between CDKN1C and LINC00152

It is noteworthy that Cyclin-Dependent Kinase Inhibitor 1C (CDKN1C) and Ribonuclease (RNase) are the only genes of which expressions correlate to LINC00152 in both *in vivo* and *in vitro* analyses. CDKN1C expression was reversely correlated to the expression of LINC00152 (Table III and Table V). Meanwhile, LINC00152 knockdown resulted in an increase of CDKN1C expression in SKOV3 cells (Table VI), which was confirmed by both microarray data and RT-qPCR. Interestingly, we found that CDK2 expression was also up-regulated by LINC00152 silencing in SKOV3 cells (Figure 5). It has been reported that CDKN1C interacts with Cyclin-Dependent Kinase 1 (CDK1), Cyclin-Dependent Kinase 2 (CDK2), Cyclin-Dependent Kinase 3 (CDK3), and Cyclin-Dependent Kinase 6 (CDK6). We hypothesize that LINC00152 regulates the expression and functions of the cyclin-dependent kinase family.

Discussion

Long non-coding RNA (lncRNA) is recently a thriving gene class involving a variety of biological functions[1]. The abnormal expression of lncRNAs has been revealed in many human diseases, including cancer[3]. Nonetheless, very few lncRNAs have been studied in ovarian cancer. This gap of knowledge needs to be filled to discover new biomarkers for the detection and prognosis of ovarian cancer. LINC00152, an 828 bp lncRNA mapped to chromosome 2p11.2, was initially detected as differentially hypomethylated lncRNA in hepatocarcinogenesis[11]. Later on, it was proposed to be an oncogene in variant cancers, namely gastric cancer[4,12], hepatocellular carcinoma[13], gall-bladder cancer[14], renal cell carcinoma[15] and colon cancer[16]. High expression of LINC00152 contributes to malignant tumor stage, reduced survival, deeper invasion, and lymph node metastasis[4,12,13,15,16]. Moreover, *in vitro* experiments demonstrated that LINC00152 promotes cancer by suppressing cell apoptosis and accelerating cell proliferation, migration, invasion, and epithelial to mesenchymal transition (EMT)[4,12,17]. Notably, our research is the first report to suggest that LINC00152 drives the progression of ovarian cancer and to analyze transcriptome profile relating to LINC00152 in cancer cells. Based on microarray analysis of ovarian cancer tissues and matched adjacent normal epithelial tissues, we identified fourteen lncRNAs with over four folds change, including nine up-regulated lncRNAs, LINC00152, MIR4435-2 Host Gene (MIR4435-1HG), Uncharacterized LOC100507351 (LOC100507351), Keratin 18 Pseudogene 55 (KRT18P55), Small NF90 Associated Ribonucleic acid D (SNAR-D), Small NF90 Associated Ribonucleic acid A1 (SNAR-A1), Small NF90 Associated Ribonucleic acid A3 (SNAR-A3), Small NF90 Associated Ribonucleic acid B2 (SNAR-B2), and Double Homeobox A Pseudogene 10 (DUXAP10). Five lncRNAs were down-regulated, including Ubiquitin-Conjugating Enzyme E2 Q2 Pseudogene 1 (UBE2Q2P1), Nuclear Receptor Subfamily 2 Group F Member 2-Antisense 1 (NR2F2-AS1), Proline-Rich Transmembrane Protein 2 (PRRT2), Erythrocyte Membrane Protein Band 4.1 Like 4A-Antisense1 (EPB41L4A-AS1), and Very Low-Density Lipoprotein Receptor-Antisense1 (VLDLR-AS1). We, then, further detected the increased expression of LINC00152 in ovarian cancer tissues. It is promising that the expression level of LINC00152 is significantly higher in tumor samples of late stages (III-IV) than that of early stages (I-II). In addition, silencing LINC00152 suppressed the proliferation of SKOV3 cells, caused cell cycle

Table V. Pathway analysis on differently regulated mRNAs.

Gene set name	Genes in overlap (k)	*p*-value
Ahsa04060:Cytokine-cytokine receptor interaction	8	0.089
hsa04668:TNF signaling pathway	5	0.010

Differentially expressed genes ($p < 0.05$) that were used to run pathway analysis were selected by criteria: log2 ratio ≥ 1 or ≤ -1. The best (smallest p-value) 3000 differentially regulated genes were analyzed.

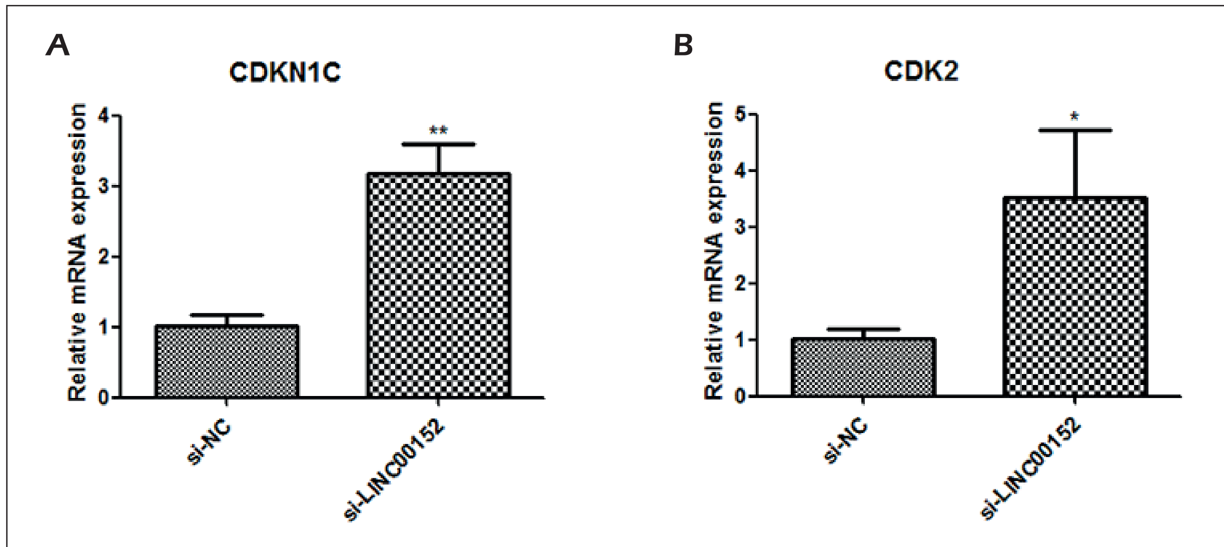

Figure 5. The expression of CDKN1C in LINC000152 knockdown SKOV3 cells. **A**, CDKN1C is up-regulated after inhibition of LINC000152; **B**, CDK2 is up-regulated after inhibition of LINC000152. Data are presented as Mean±SD, $*p < 0.05$, $**p < 0.01$.

arrest, namely prolonged G1 phase and shortened S phase. Various mechanisms behind the functions of LINC00152 were illustrated, such as epigenetic modifications, and lncRNA-micro Ribonuclease Acid (miRNA) and lncRNA-protein interactions[17,18]. Specifically, LINC00152 interacts with Thrombospondin 1 (THBS1) through miR-18a-5p[19] and acts as a competing endogenous Ribonucleic acid (ceRNA) to regulate Erb-B2 Receptor Tyrosine Kinase 4 (ERBB4)[18] through miR-193a-3p. Furthermore, LINC00152 activates several pathways, specifically Rapamycin (mTOR) pathway[13], and Phosphatidylinositol 3-Kinase (PI3K)/Protein Kinase B (AKT) signaling pathway[14]. In addition, LINC00152 could bind to the enhancer of Zeste Homologue 2 (EZH2) and silence p15 and p21 expression to promote cell cycle, facilitating cell proliferation[11,17]. It was also reported that LINC00152 triggers EMT programme[4]. Our study first utilized a high throughput method to investigate the mechanism regarding LINC00152's function. We found that the inhibition of LINC00152 in cancer cell caused increased expression of 149 mRNAs and decreased expression of 292 mRNAs. Based on GO analysis, these mRNAs closely relate to the structural constituent of muscle, nucleus, and cellular response to mechanical stimulus. Meanwhile, according to KEGG analysis, the most relevant pathway is the TNF signaling pathway. These results provide potential mechanisms of LINC00152 mediating ovarian cancer cell. Among the identified mRNAs, we noticed that CDKN1C expression decreased in ovarian cancer tissues compared to normal tissues and increased after LINC00152 silencing in SKOV3 cells comparing to the control cells, which was reversely correlated to the expression of LINC00152. CDKN1C is a cell

Table VI. Microarray result in both tissues and cells shows the expression of CDKN1C is in negatively correlated with that of LINC00152.

	Type	LINC00152	CDKN1C
FC (1/1a)	Cancer/control	71.73	-16.55
FC (5/5a)	Cancer/control	11.84	-14.66
FC (6,7,8/10,11,12)	si-LINC00152/si-NC	##	2.09

FC represents expression fold change, and a positive value represents a higher expression compared to control, while a negative value represents a lower one. For tissues, 1 and 5 represents cancer tissues, 1a and 5a represents adjacent normal counterparts. For cells, 6, 7 and 8 represents cells transfected with siRNA targeting LINC00152, and 10, 11 and 12 represents cells transfected with negative control siRNA. ##Means that the microarray only contains probes for mRNA.

cycle regulator and a cyclin-dependent kinase inhibitor. Cyclin-dependent kinase family members are often transcriptionally dysregulated in human cancer *via* promoter DNA methylation[20]. It has been reported[21] that CDKN1C is repressed in breast cancer cells mainly through histone modifications. MiR-34c can activate CDKN1C to act as a tumor suppressor in high grade serous ovarian cancer[22]. Our finding that CDKN1C expression is negatively correlated to that of LINC00152 suggests the role of LINC00152 in mediating cell cycle and proliferation through the regulation of CDKN1C expression. Further investigation is needed to elucidate the molecular mechanisms underlying the interplays of LINC00152 and cyclin-dependent kinase family with the focus on CDKN1C.

This report demonstrates the high expression of LINC00152 in ovarian cancer tissues, as well as LINC00152's influence on cell cycle and proliferation. Moreover, the transcriptional profile regulated by LINC00152 in SKOV3 cells is characterized. Studies of more clinical samples will further reveal the association between LINC00152 expression level and the patients' clinical features, including survival, tumor size, metastasis, and chemo-responses.

Conclusions

The development of methods for early diagnosis and effective treatment of ovarian cancer is paramount for improving the patient survival rate. This study has identified LINC00152 as a LncRNA overexpressed in ovarian cancer. LINC00152 also has a higher expression level in late-stage ovarian cancers comparing to early-stage ones. *In vitro* studies suggest that LINC00152 regulates cell cycle and cell proliferation in ovarian cancer cells. CDKN1C is a possible downstream target of LINC00152. Based on these data, LINC00152 may serve as a potential therapeutic target of ovarian cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

This work was supported by the grants from the National Natural Science Foundation of China (81572765, 31571403), Beijing Natural Science Foundation (2171001) and China Scholarship Council.

References

1) Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.
2) Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigo R. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012; 22: 1775-1789.
3) Isin M, Dalay N. LncRNAs and neoplasia. Clin Chim Acta 2015; 444: 280-288.
4) Zhao J, Liu Y, Zhang W, Zhou Z, Wu J, Cui P, Zhang Y, Huang G. Long non-coding RNA Linc00152 is involved in cell cycle arrest, apoptosis, epithelial to mesenchymal transition, cell migration and invasion in gastric cancer. Cell Cycle 2015; 14: 3112-3123.
5) Ozes AR, Miller DF, Ozes ON, Fang F, Liu Y, Matei D, Huang T, Nephew KP. NF-kappaB-HOTAIR axis links DNA damage response, chemoresistance and cellular senescence in ovarian cancer. Oncogene 2016; 35: 5350-5361.
6) Medrzycki M, Zhang Y, Zhang W, Cao K, Pan C, Lailler N, McDonald JF, Bouhassira EE, Fan Y. Histone h1.3 suppresses h19 noncoding RNA expression and cell growth of ovarian cancer cells. Cancer Res 2014; 74: 6463-6473.
7) Huang KC, Rao PH, Lau CC, Heard E, Ng SK, Brown C, Mok SC, Berkowitz RS, Ng SW. Relationship of XIST expression and responses of ovarian cancer to chemotherapy. Mol Cancer Ther 2002; 1: 769-776.
8) Guo C, Wang X, Chen LP, Li M, Li M, Hu YH, Ding WH, Wang X. Long non-coding RNA MALAT1 regulates ovarian cancer cell proliferation, migration and apoptosis through Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 3703-3712.
9) Yang Y, Jiang Y, Wan Y, Zhang L, Qiu J, Zhou S, Cheng W. UCA1 functions as a competing endogenous RNA to suppress epithelial ovarian cancer metastasis. Tumour Biol 2016; 37: 10633-10641.
10) Zhang J, Liu J, Xu X, Li L. Curcumin suppresses cisplatin resistance development partly via modulating extracellular vesicle-mediated transfer of MEG3 and miR-214 in ovarian cancer. Cancer Chemother Pharmacol 2017; 79: 479-487.
11) Neumann O, Kesselmeier M, Geffers R, Pellegrino R, Radlwimmer B, Hoffmann K, Ehemann V, Schemmer P, Schirmacher P, Lorenzo Bermejo J, Longerich T. Methylome analysis and integrative profiling of human HCCs identify novel protumorigenic factors. Hepatology 2012; 56: 1817-1827.
12) Zhou J, Zhi X, Wang L, Wang W, Li Z, Tang J, Wang J, Zhang Q, Xu Z. Linc00152 promotes prolifera-

tion in gastric cancer through the EGFR-dependent pathway. J Exp Clin Cancer Res 2015; 34: 135.

13) Ji J, Tang J, Deng L, Xie Y, Jiang R, Li G, Sun B. LINC00152 promotes proliferation in hepatocellular carcinoma by targeting EpCAM via the mTOR signaling pathway. Oncotarget 2015; 6: 42813-42824.

14) Cai Q, Wang ZQ, Wang SH, Li C, Zhu ZG, Quan ZW, Zhang WJ. Upregulation of long non-coding RNA LINC00152 by SP1 contributes to gallbladder cancer cell growth and tumor metastasis via PI3K/AKT pathway. Am J Transl Res 2016; 8: 4068-4081.

15) Wu Y, Tan C, Weng WW, Deng Y, Zhang QY, Yang XQ, Gan HL, Wang T, Zhang PP, Xu MD, Wang YQ, Wang CF. Long non-coding RNA Linc00152 is a positive prognostic factor for and demonstrates malignant biological behavior in clear cell renal cell carcinoma. Am J Cancer Res 2016; 6: 285-299.

16) Zhang YH, Fu J, Zhang ZJ, Ge CC, Yi Y. LncRNA-LINC00152 down-regulated by miR-376c-3p restricts viability and promotes apoptosis of colorectal cancer cells. Am J Transl Res 2016; 8: 5286-5297.

17) Chen WM, Huang MD, Sun DP, Kong R, Xu TP, Xia R, Zhang EB, Shu YQ. Long intergenic non-coding RNA 00152 promotes tumor cell cycle progression by binding to EZH2 and repressing p15 and p21 in gastric cancer. Oncotarget 2016; 7: 9773-9787.

18) Yue B, Cai D, Liu C, Fang C, Yan D. Linc00152 functions as a competing endogenous RNA to confer oxaliplatin resistance and holds prognostic values in colon cancer. Mol Ther 2016; 24: 2064-2077.

19) Eggert T, Wolter K, Ji J, Ma C, Yevsa T, Klotz S, Medina-Echeverz J, Longerich T, Forgues M, Reisinger F, Heikenwalder M, Wang XW, Zender L, Greten TF. Distinct functions of senescence-associated immune responses in liver tumor surveillance and tumor progression. Cancer Cell 2016; 30: 533-547.

20) Bachman KE, Park BH, Rhee I, Rajagopalan H, Herman JG, Baylin SB, Kinzler KW, Vogelstein B. Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene. Cancer Cell 2003; 3: 89-95.

21) Yang X, Karuturi RK, Sun F, Aau M, Yu K, Shao R, Miller LD, Tan PB, Yu Q. CDKN1C (p57) is a direct target of EZH2 and suppressed by multiple epigenetic mechanisms in breast cancer cells. PLoS One 2009; 4: e5011.

22) Yu Z, Kim J, He L, Creighton CJ, Gunaratne PH, Hawkins SM, Matzuk MM. Functional analysis of miR-34c as a putative tumor suppressor in high-grade serous ovarian cancer. Biol Reprod 2014; 91: 113.

23) Ma B, Wang J, Song Y, Gao P, Sun J, Chen X, Yang Y, Wang Z. Upregulated long intergenic noncoding RNA KRT18P55 acts as a novel biomarker for the progression of intestinal-type gastric cancer. Onco Targets Ther 2016; 9: 445-453.

24) Wei CC, Nie FQ, Jiang LL, Chen QN, Chen ZY, Chen X, Pan X, Liu ZL, Lu BB, Wang ZX. The pseudogene DUXAP10 promotes an aggressive phenotype through binding with LSD1 and repressing LATS2 and RRAD in non small cell lung cancer. Oncotarget 2017; 8: 5233-5246.

25) Lian Y, Xu Y, Xiao C, Xia R, Gong H, Yang P, Chen T, Wu D, Cai Z, Zhang J, Wang K. The pseudogene derived from long non-coding RNA DUXAP10 promotes colorectal cancer cell growth through epigenetically silencing of p21 and PTEN. Sci Rep 2017; 7: 7312.

26) Lv XY, Ma L, Chen JF, Yu R, Li Y, Yan ZJ, Cheng Y, Ma Q. Knockdown of DUXAP10 inhibits proliferation and promotes apoptosis in bladder cancer cells via PI3K/Akt/mTOR signaling pathway. Int J Oncol 2018; 52: 288-294.

27) Feng L, Houck J.R, Lohavanichbutr P, Chen C. Transcriptome analysis reveals differentially expressed lncRNAs between oral squamous cell carcinoma and healthy oral mucosa. Oncotarget 2017; 8: 31521-31531.

28) Ebrahimi-Fakhari D, Saffari A, Westenberger A, Klein C. The evolving spectrum of PRRT2-associated paroxysmal diseases. Brain 2015; 138: 3476-3495.

29) Liu H, Zhou T, Wang B, Li L, Ye D, Yu S. Identification and functional analysis of a potential key lncRNA involved in fat loss of cancer cachexia. J Cell Biochem 2018; 119: 1679-1688.