Long Noncoding RNA Urothelial Carcinoma-Associated 1 Promotes the Proliferation and Metastasis of Human Lung Tumor Cells by Regulating MicroRNA-144

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Long noncoding RNA urothelial carcinoma-associated 1 (lncRNA UCA1) has gained more attention in recent years due to its oncogenic roles in various cancers. MicroRNA-144 (miR-144) participates in the regulation of the growth of many cancer cells. This study investigated the interaction between lncRNA UCA1 and miR-144 in lung cancer cells. The potential downstream protein of miR-144 was also assessed. Our results found that lncRNA UCA1 was highly expressed in human lung cancer A549, H517, H4006, H1299, and H1650 cells compared to normal embryonic lung WI-38 and HEL-1 cells. Knockdown of lncRNA UCA1 significantly inhibited lung cancer A549 cell viability, migration, invasion, and cell cycle progression, but promoted cell apoptosis. Besides, we found that lncRNA UCA1 was bound to miR-144. miR-144 participated in the regulation effects of lncRNA UCA1 on A549 cell viability, migration, invasion, cell cycle transition, and cell apoptosis. In addition, Pre-B-cell leukemia homeobox 3 (PBX3) was found to be a direct target gene of miR-144. Overexpression of PBX3 promoted A549 cell proliferation and metastasis. Suppression of PBX3 had an opposite effect.

Key words: Lung cancer; Long noncoding RNA urothelial carcinoma-associated 1 (lncRNA UCA1); MicroRNA-144 (miRNA-144); Pre-B-cell leukemia homeobox 3 (PBX3); Cell proliferation; Cell metastasis

INTRODUCTION

Lung cancer is the most common malignant cancer worldwide, which is characterized by the uncontrolled cell proliferation in lung tissue and accounts for 1.8 million new diagnosed people and 1.2 million deaths per year[1]. According to the different histological features, lung cancer can be classified as small cell lung carcinoma (SCLC, roughly 20% of cases) or non-small cell lung carcinoma (NSCLC, roughly 80% of cases)[2]. Despite remarkable improvements in the therapy of lung cancer in recent years, the mortality rate remains high. Novel therapeutic strategies and new molecular targets are urgently needed.

Long noncoding RNAs (lncRNAs), a class of noncoding RNAs in eukaryotic cells with more than 200 bases, have been demonstrated to play critical roles in many biological processes including gene regulation, cell proliferation, and cell differentiation under normal conditions and in human diseases[3]. Numerous research has reported that lncRNAs act as tumor suppressors or oncogenes in the development and progression of various cancers, including lung cancer[4-6]. For example, lncRNA MIR31HG overexpression significantly enhanced the gefitinib resistance of NSCLC cells by regulating the epidermal growth factor receptor (EGFR)/phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT) signaling pathway[7]. lncRNA LINC 01186 inhibited lung cancer cell migration and invasion by regulating the transforming growth factor β (TGF-β)/mothers against decapentaplegic 3 (SMAD3) pathways[8].

Urothelial carcinoma-associated 1 (UCA1) is a recently identified lncRNA and has gained more attention in recent years because of its critical roles in the growth of different cancer cells[9]. Wang et al. reported that upregulation of lncRNA UCA1 contributed to the progression of lung cancer[10]. The experimental study from Cheng et al. demonstrated that lncRNA UCA1 promoted non-T790M cells' acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) by activating the AKT/mammalian target of rapamycin (mTOR) pathway in EGFR-mutant NSCLC[11].

MicroRNA-144 (miR-144) has been found to participate in the proliferation and metastasis of many cancer cells[12-14]. Chen et al. revealed that miR-144 inhibited lung cancer cell proliferation but promoted apoptosis by targeting p53-induced glycolysis and apoptosis regulator (TIGAR)[15]. Moreover, Zha et al. showed that miR-144-zinc finger X-chromosomal protein (ZFX) pathway was involved in the growth regulation of NSCLC[16]. However, little information is available about the interaction between lncRNA UCA1 and miR-144 in the regulation of lung cancer cell proliferation and metastasis.

In this study, we analyzed the interaction between lncRNA UCA1 and miR-144 in the regulation of lung cancer cell viability, migration, invasion, cycle transition, and apoptosis. The possible downstream protein of miR-144 was also investigated. These findings will be helpful for understanding the critical roles of lncRNA UCA1 and miRNA-144 in lung cancer cells, and may provide a new therapeutic strategy for lung cancer therapy.

MATERIALS AND METHODS

Cell Culture

Human normal embryonic lung WI-38 and HEL-1 cells, human lung cancer A549, H517, H4006, H1229, and H1650 cells, and human embryonic kidney HEK293 cells were all purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT, USA) and 1% penicillin–streptomycin mixture (Hyclone Laboratories). Cultures were maintained in an incubator with 5% CO_2 at 37°C.

Quantitative Reverse Transcription PCR (qRT-PCR)

Total RNAs in cells were extracted using Trizol reagent (Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. The expression levels of UCA1 were tested using real-time polymerase chain reaction (PCR) analysis with the One Step SYBR® PrimeScript® PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, P.R. China). Taqman MicroRNA Reverse Transcription Kit and Taqman Universal Master Mix II (Applied Biosystems, Foster, CA, USA) along with the Taqman MicroRNA Assay of miR-144 and U6 short nuclear RNA (U6 snRNA; Applied Biosystems) were used for detection of the expression levels of miR-144 in cells. RNA PCR Kit (AMV) Ver.3.0 (TaKaRa Biotechnology) was performed to test the expression of Pre-B-cell leukemia homeobox 3 (PBX3) expressions. The RNA expression rate was quantified by relative quantification ($2^{-\Delta\Delta Ct}$) method[17], and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or U6 snRNA expression acted as the internal control in this study.

Cell Transfection

Short-hairpin RNA (shRNA) directed against human lncRNA UCA1 was ligated into the U6/GFP/Neo plasmid (GenePharma, Shanghai, P.R. China) and was referred to as sh-UCA1. The full-length Pre-B-cell leukemia homeobox 3 (PBX3) sequences and shRNA against PBX3 were constructed in Pex-2 and U6/GFP/Neo plasmid (Gene Pharma) to analyze the PBX3 functions, and they were referred to as pEX-PBX3 and sh-PBX3. Cell transfection was performed using Lipofectamine 3000 reagent (Life Technologies Corporation) as stated by the manufacturer's instructions.

The plasmid carrying a nontargeting sequence acted as the negative control (NC). The stably transfected cells were selected by the culture medium containing 0.5 mg/ml of G418 (Sigma-Aldrich). After 4 weeks, G418-resistant cell clones were established. miR-144 mimic, inhibitors, and their respective NC were synthesized (Life Technologies Corporation) and transfected into related cells. The highest transfection efficiency generally occurred after 48 h transfection, so 72 h posttransfection was considered as the harvest time in these experiments.

Cell Viability Assay

Trypan blue exclusion assay was used to determine A549 cell viability. Briefly, 1×10^5 cells were cultured in duplicate in 60-mm dishes. After the relevant treatment and the indicated time periods, cells were washed with phosphate-buffered saline (PBS) and stained with 0.4% trypan blue solution. The cell viability (%) was determined by the number of nonstained cells/total cell number × 100%.

Cell Apoptosis Assay

Cell apoptosis was evaluated using propidium iodide (PI) and fluorescein isothiocynate (FITC)-conjugated Annexin-V staining (Beijing Biosea Biotechnology, Beijing, P.R. China) according to the manufacturer's instructions. Briefly, A549 cells were washed with PBS three times and then fixed in 70% ethanol. After that, the fixed cells were washed twice with PBS and stained in PI/FITC-Annexin-V in the presence of 50 μg/ml RNase (Sigma-Aldrich), incubated for 1 h at room temperature in the dark, and then immediately subjected to flow cytometry analysis using the fluorescence-activated cell sorter (FACS) CAN system (Beckman Coulter, Fullerton, CA, USA). Data were analyzed using FlowJo software (Tree Star, Inc., Ashland, OR, USA).

Cell Migration and Invasion Assay

Cell migration was evaluated using a modified two-chamber migration assay with a pore size of 8 mm (Millipore, Bedford, MA, USA). After the relevant treatment, A549 cells were plated in the upper compartment of a

24-well Transwell culture chamber with 200 ml of serum-free medium, and 600 ml of complete medium was added to the lower compartment. Then cells were cultured at 37°C for 48 h and fixated with methanol. Nontraversed cells on the upper compartment were removed by a cotton swab carefully. Meanwhile, the traversed cells on the lower side of the filter were stained by 0.1% crystal violet solution and counted under a microscope (Nikon, Japan). Data were presented as the average number of cells from five randomly chosen fields.

Cell invasion was performed using 24-well Millicell Hanging Cell Culture inserts with 8-mm polyethylene terephthalate (PET) membranes (Millipore) according to the manufacturer's instructions, which is similar to the migration assay except that the Transwell membrane was precoated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA).

Reporter Vector Constructs and Luciferase Reporter Assay

Bioinformatics was used to identify potential binding partners for UCA1 and subsequently miR-144. The fragment of UCA1 containing the predicted miR-144 binding site was amplified by PCR and cloned into a pmirGlO Dual-luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA) to form the reporter vector UCA1-wild type (UCA1-wt). To mutate the putative binding site of miR-144 in the UCA1, the sequence of the putative binding site was changed and named as UCA1-mutated type (UCA1-mt). After that, the vector and miR-144 mimics were cotransfected into HEK 293 cells, and the Dual-Luciferase Reporter Assay System (Promega) was performed to detect the luciferase activity.

The construction process of PBX3-wt and PBX3-mt reporter vectors was similar to that of the UCA1-Wt and UCA1-mt. PBX3-wt and PBX3-mt reporter vectors and miR-144 mimic were cotransfected into HEK 293 cells, and the relative luciferase activities were measured by the Dual-Luciferase Reporter Assay System (Promega).

Western Blotting Assay

Western blotting assay was established using Bio-Rad Bis-Tris Gel system and performed as previously described[18]. Total proteins in the cells were electrophoresed on polyacrylamide gels, and the gels were transferred onto polyvinylidene difluoride (PDVF) membranes (Millipore) that were incubated with relevant antibodies. Anti-B-cell lymphoma-2 (Bcl-2; sc-7382), anti-Bcl2-associated X (Bax; sc-7480), anti-caspase 3 (sc-7272), anti-caspase 9 (sc-56076), anti-proliferating cell nuclear antigen (PCNA; sc-25280), and anti-GAPDH (sc-47724) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-cyclin A (ab181591), anti-cyclin E1

(ab33911), anti-cyclin D1 (ab134175), anti-cyclin-dependent kinase 2 (CDK2; ab32147), anti-CDK4 (ab137675), and anti-PBX3 (ab109173) were purchased from Abcam Biotechnology (Cambridge, MA, USA). Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (ab6788, ab6721, Abcam Biotechnology) for 1 h at room temperature. The protein signals were detected by enhanced chemiluminescence Western blotting substrate (Pierce, Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

All the above experiments were repeated three times. Values were expressed as mean ± standard deviation (SD). Graphpad 6.0 software (Graphpad, San Diego, CA, USA) were used for statistical analysis. Differences between two groups were calculated using Student's t-test, and differences between more than three groups were calculated using one-way analysis of variance (ANOVA), and a value of $p < 0.05$ was considered to be significantly different.

RESULTS

lncRNA UCA1 Was Highly Expressed in Lung Cancer Cells

The expression levels of lncRNA UCA1 in WI-38, HEL-1, A549, H157, H4006, H1299, and H1650 cells were measured by qRT-PCR. As shown in Figure 1,
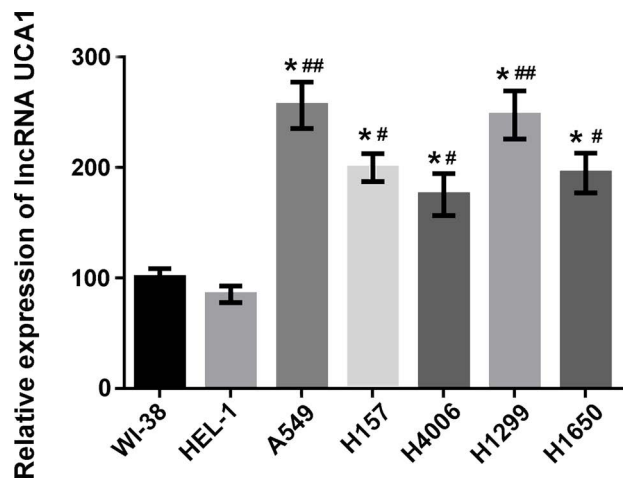

Figure 1. Long noncoding RNA (lncRNA) urothelial carcinoma-associated 1 (UCA1) was highly expressed in lung cancer cell lines. The expression levels of lncRNA UCA1 in human normal embryonic lung WI-38, HEL-1 cells, and lung cancer A549, H157, H4006, H1299, and H1650 cells were detected by qualitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay. Data were expressed as means ± standard deviation (SD) of three independent experiments. *$p < 0.05$ versus WI-38 cells; #$p < 0.05$ or ##$p < 0.01$ versus HEL-1 cells.

lncRNA UCA1 was highly expressed in lung cancer A549, H157, H4006, H1299, and H1650 cells compared to that in human normal embryonic lung WI-38 and HEL-1 cells ($p < 0.05$ or $p < 0.01$). A549 cells had the highest expression of lncRNA UCA1. This result indicated that the upregulated expression of lncRNA UCA1 might be involved in the cancerous development of normal lung cells.

Knockdown of lncRNA UCA1 Inhibited Cell Viability, Migration, Invasion, and Cell Cycle Progression, but Promoted Cell Apoptosis

To reveal the functional roles of lncRNA UCA1 in lung cancer cell proliferation and metastasis, sh-UCA1 was transfected into A549 cells. Results showed that after transfection with sh-UCA1 for 48 h, the expression levels
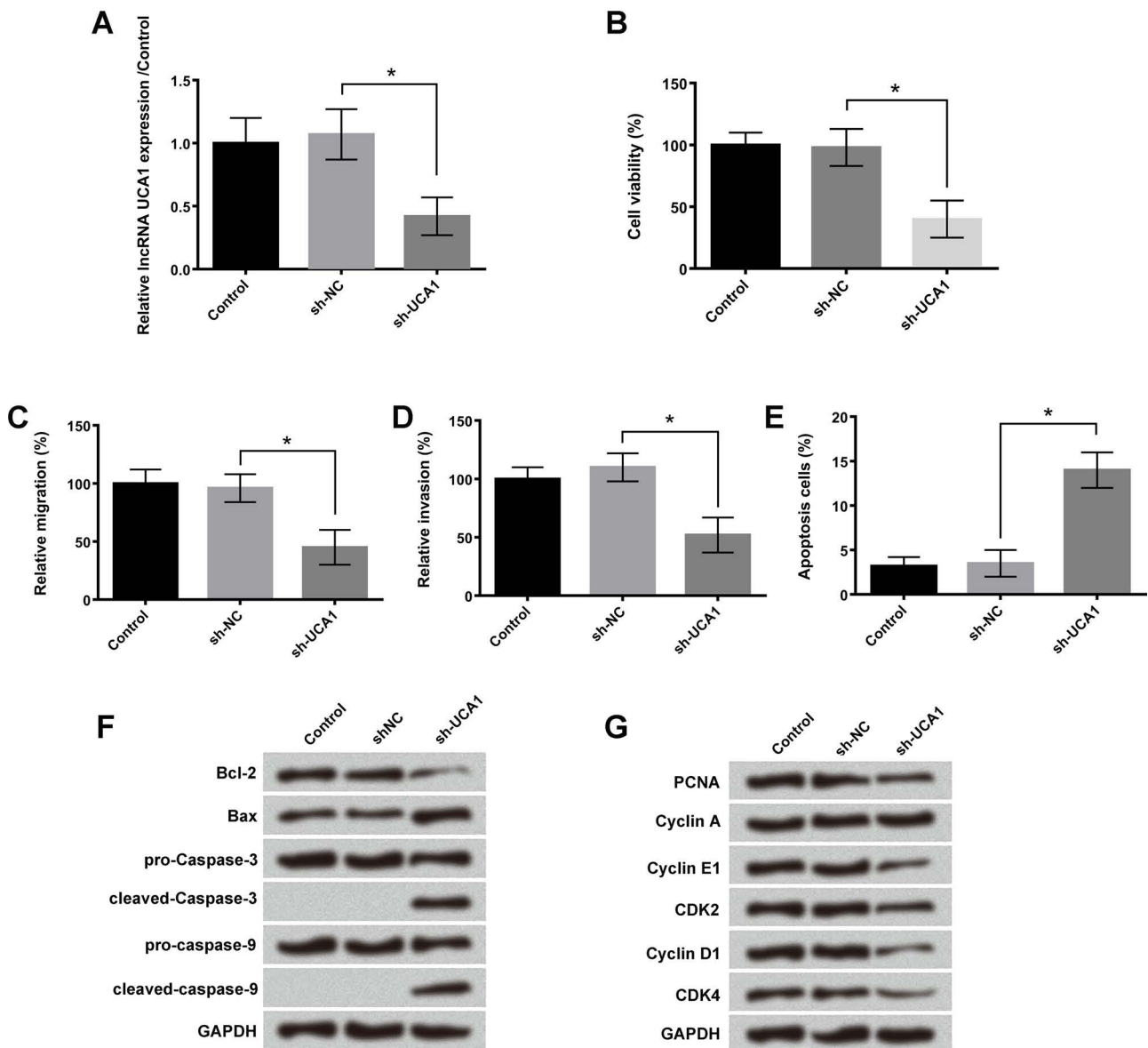
Figure 2. Knockdown of lncRNA UCA1 inhibited cell viability, migration, invasion, and cell cycle progression, but promoted cell apoptosis. After transfection with short hairpin RNA (sh)-UCA1 and negative control (NC) in A549 cells, the (A) expression levels of lncRNA UCA1, (B) cell viability, (C) relative migration, (D) relative invasion, (E) cell apoptosis, (F) expression levels of B-cell lymphoma-2 (Bcl-2), Bcl2-associated X (Bax), caspase 3, caspase 9, and (G) expression levels of proliferating cell nuclear antigen (PCNA), cyclin A, cyclin E1, cyclin D1, cyclin-dependent kinase (CDK2), and CDK4 were measured by qRT-PCR assay, trypan blue exclusion assay, Transwell assay, flow cytometry analysis, and Western blotting, respectively. Data were expressed as means±SD of three independent experiments. *$p < 0.05$.

of lncRNA UCA1 in A549 cells were significantly reduced ($p<0.05$) (Fig. 2A). Knockdown of lncRNA UCA1 remarkably inhibited A549 cell viability, migration, and invasion ($p<0.05$) (Fig. 2B–D). In addition, the number of apoptotic cells in the sh-UCA1 transfection group was significantly increased ($p<0.05$) (Fig. 2E). Western blotting assay revealed that the expression levels of cleaved caspase 3, cleaved caspase 9, and proapoptosis protein Bax were significantly increased, while the expression level of antiapoptosis protein Bcl-2 was decreased after sh-UCA1 treatment (Fig. 2F). Moreover, downregulation of UCA1 also remarkably inhibited the expression levels of PCNA, cyclin E1, cyclin D1, CDK2, and CDK4, which exert important roles in the G_1 and S phase of cell cycle progression (Fig. 2G). The above experiments suggested that lncRNA UCA1 was involved in the regulation of A549 cell proliferation, migration, invasion, cycle transition, and apoptosis.

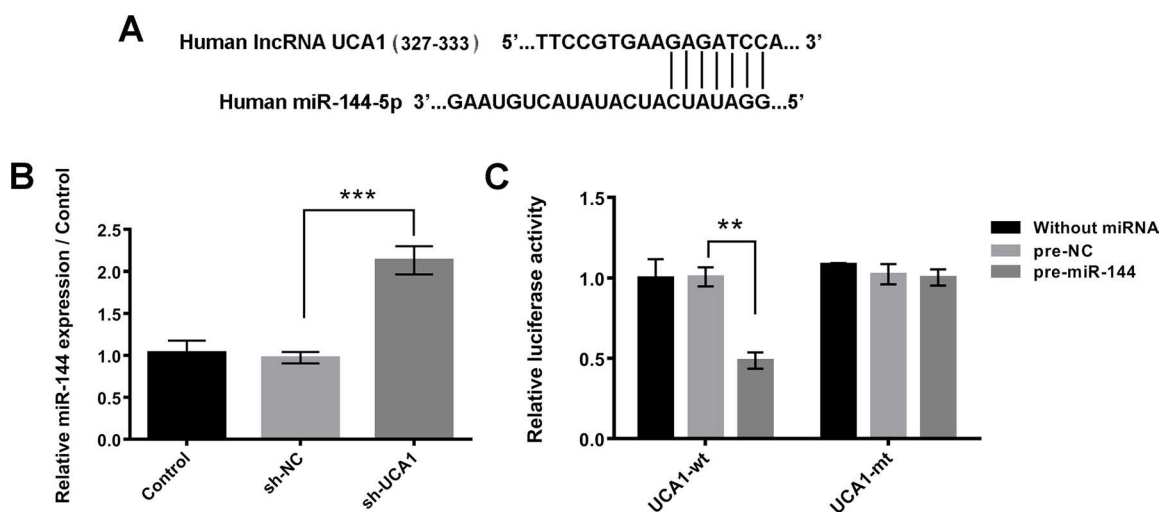### lncRNA UCA1 Bound to miR-144 in A549 Cells

Bioinformatics analysis found that lncRNA UCA1 was predicted to bind to miR-144. The potential binding sequences are shown in Figure 3A. Moreover, the relative miR-144 expression level in A549 cells after sh-UCA1 transfection was detected using qRT-PCR assay. Results showed that the expression levels of miR-144 and lncRNA UCA1 had clear negative correlation effects ($p<0.001$) (Fig. 3B). These results were further confirmed by dual luciferase reporter assay, which demonstrated that cotransfection with miR-144 and lncRNA UCA1-wt notably reduced the relative luciferase activity ($p<0.01$) (Fig. 3C).

miR-144 Participated in the Effects of lncRNA UCA1 on Cell Viability, Migration, Invasion, Cell Cycle Transition, and Cell Apoptosis
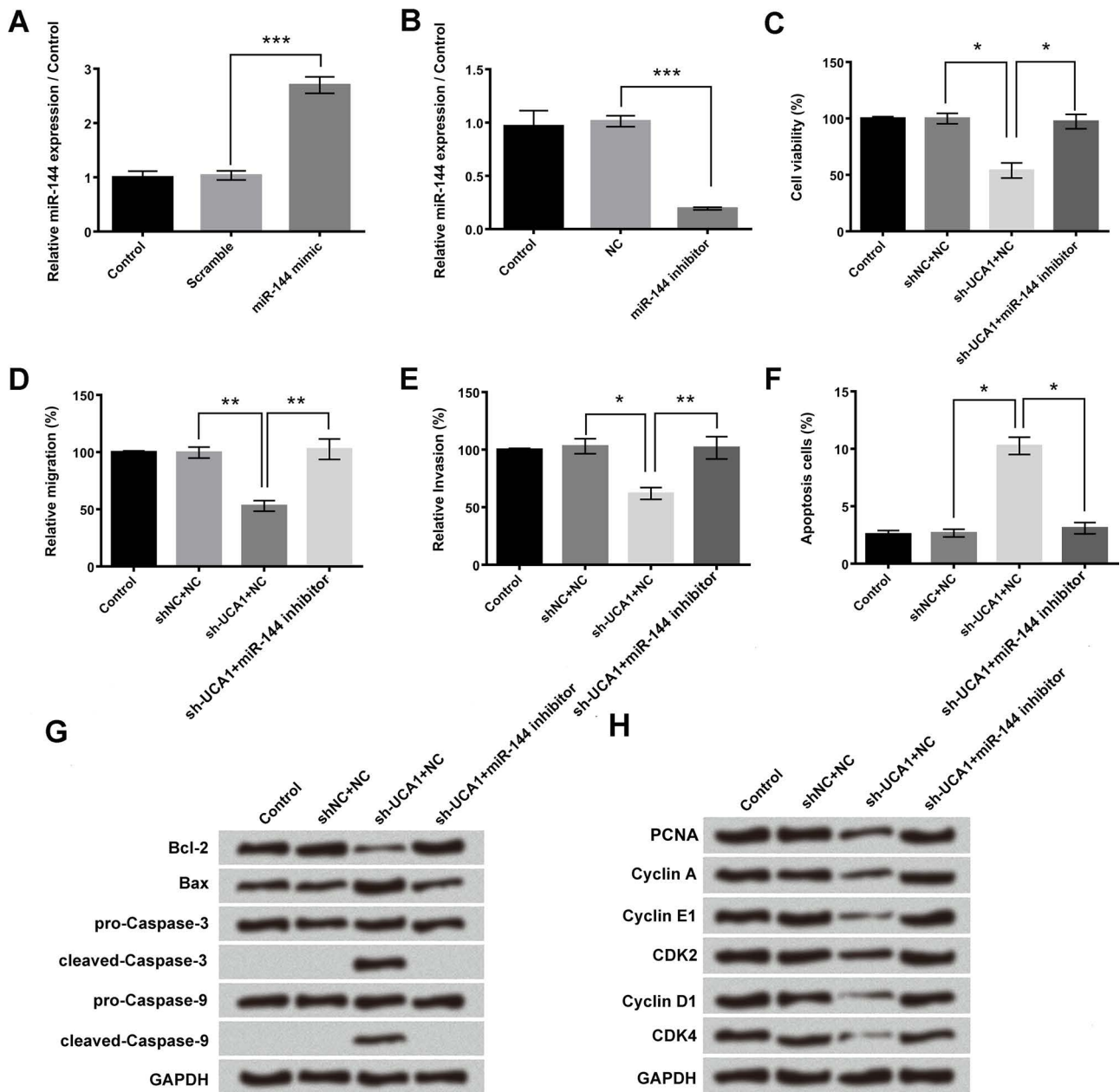
miR-144 mimic and miR-144 inhibitor were transfected into A549 cells, respectively, to clarify the regulatory effects between lncRNA UCA1 and miR-144. Transfection with miR-144 mimic significantly increased the relative miR-144 expression, and transfection with miR-144 inhibitor remarkably decreased the relative miR-144 expression in A549 cells ($p<0.001$) (Fig. 4A and B). Cotransfection with sh-UCA1 and miR-144 inhibitor significantly upregulated A549 cell viability, migration, and invasion compared to single sh-UCA1 transfection ($p<0.05$ or $p<0.01$) (Fig. 4C–E). The number of apoptotic cells and the protein expression levels of Bax, cleaved caspase 3, and cleaved caspase 9 after cotransfection of sh-UCA1 and miR-144 inhibitor were also remarkably decreased compared to single sh-UCA1 transfection ($p<0.05$) (Fig. 4F and G). Moreover, Figure 4H showed that cotransfection with sh-UCA1 and miR-144 inhibitor obviously enhanced the expression levels of PCNA, cyclin A, cyclin E1, cyclin D1, CDK2, and CDK4 compared to the single sh-UCA1 transfection group. These results indicated that knockdown of lncRNA UCA1 inhibited A549 cell proliferation, migration, invasion, and cell cycle transition through upregulating miR-144.

PBX3 Was a Direct Target Gene of miR-144

miR-144 was predicted to reversely bind to the 3′-untranslated region (3′-UTR) of PBX3 (Fig. 5A). In addition, the relative mRNA expressions of PBX3 after
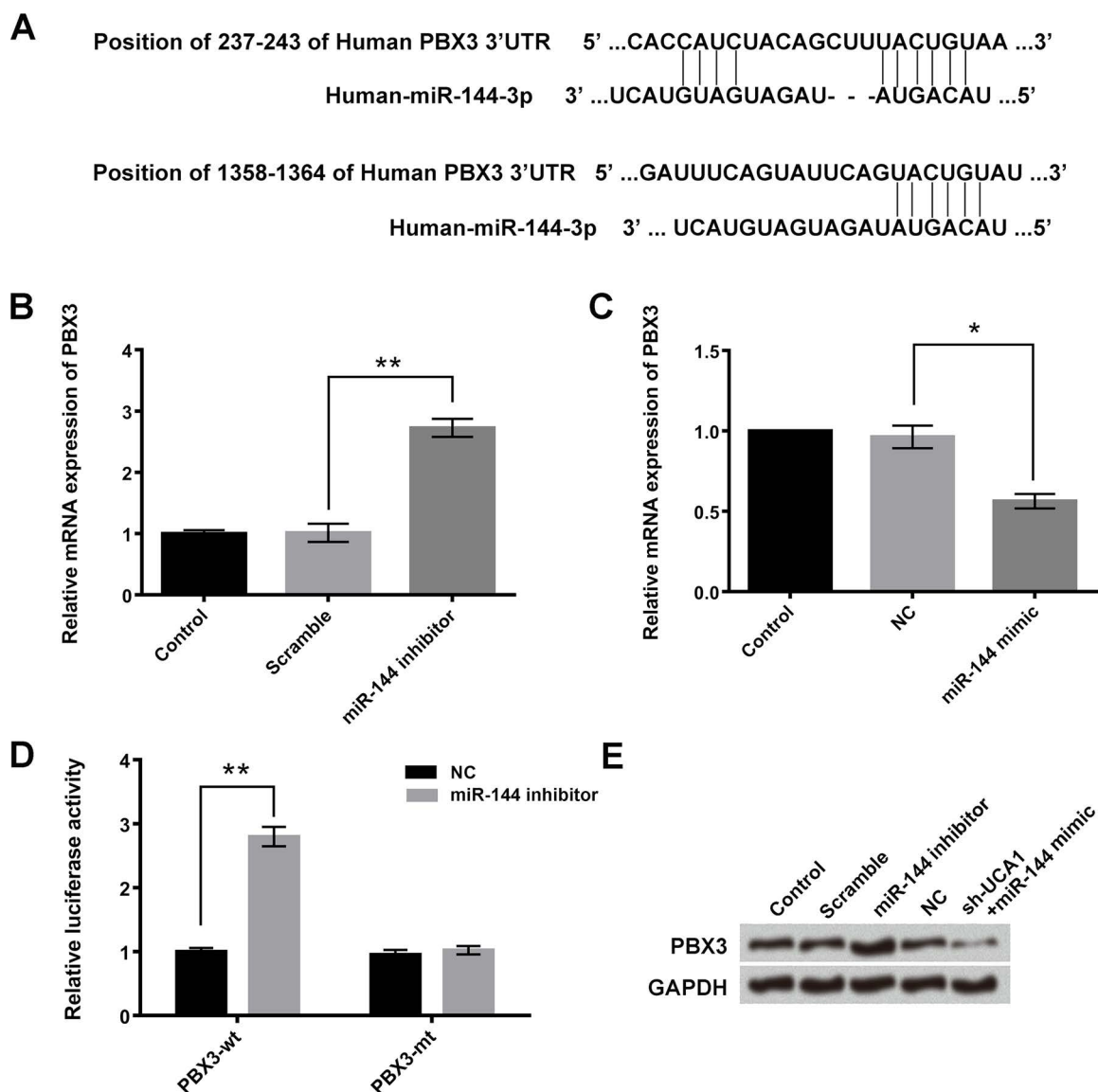

Figure 3. lncRNA UCA1 bound to miR-144 in A549 cells. (A) Bioinformatics analysis was used to predict the potential binding sequences between lncRNA UCA1 and miR-144. (B) After transfection with sh-UCA1 or negative control in A549 cells, the expression levels of miR-144 were detected by qRT-PCR assay. (C) Relative luciferase activities were measured after cotransfection with pre-miR-144 or its negative control (pre-NC) and UCA1-wild type (UCA1-wt) or UCA1-mutated type (UCA1-mt). Data were expressed as means ± SD of three independent experiments. **$p<0.01$, ***$p<0.001$.

Figure 4. miR-144 participated in the regulation of lncRNA UCA1 on cell viability, migration, invasion, cell cycle transition, and cell apoptosis. After transfection with miR-144 mimic or miR-144 inhibitor, the (A, B) expression levels of miR-144, (C) cell viability, (D) relative migration, (E) relative invasion, (F) cell apoptosis, (G) expression levels of Bcl-2, Bax, caspase 3, caspase 9, and (H) expression levels of PCNA, cyclin A, cyclin E1, cyclin D1, CDK2, CDK4 were measured by qRT-PCR assay, trypan blue exclusion assay, Transwell assay, flow cytometry analysis, and Western blotting, respectively. Data were expressed as means ± SD of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

transfection with miR-144 inhibitor or miR-144 mimic were detected by qRT-PCR assay. As shown in Figure 5B and C, the mRNA expression of PBX3 was upregulated after transfection with miR-144 inhibitor and downregulated after transfection with miR-144 mimic ($p < 0.05$ or $p < 0.01$). Dual luciferase reporter assay indicated that

cotransfection with miR-144 inhibitor and PBX3-wt significantly increased the relative luciferase activity ($p < 0.01$) (Fig. 5D). These results were further verified by Western blotting, which revealed that the protein expression levels of PBX3 were increased in the miR-144 inhibitor transfection group and decreased in the

A

Position of 237-243 of Human PBX3 3'UTR   5' ...CACCAUCUACAGCUUUACUGUAA ...3'
                                                        | | | | | | | | |
Human-miR-144-3p   3' ...UCAUGUAGUAGAU- - -AUGACAU ...5'

Position of 1358-1364 of Human PBX3 3'UTR   5' ...GAUUUCAGUAUUCAGUACUGUAU ...3'
                                                          | | | | | |
Human-miR-144-3p   3' ... UCAUGUAGUAGAUAUGACAU ...5'

B

Relative mRNA expression of PBX3

** (Scramble vs miR-144 inhibitor)

Control, Scramble, miR-144 inhibitor

C

Relative mRNA expression of PBX3

* (NC vs miR-144 mimic)

Control, NC, miR-144 mimic

D

Relative luciferase activity

■ NC
■ miR-144 inhibitor

** (PBX3-wt)

PBX3-wt, PBX3-mt

E

Control, Scramble, miR-144 inhibitor, NC, sh-UCA1 +miR-144 mimic

PBX3
GAPDH

Figure 5. Pre-B-cell leukemia homeobox 3 (PBX3) was a target gene of miR-144. (A) Bioinformatics analysis was used to predict the potential binding sequences between miR-144 and PBX3. (B, C) After transfection with miR-144 mimic or miR-144 inhibitor, the relative mRNA expressions of PBX3 in A549 cells were detected by qRT-PCR assay. (D) Relative luciferase activities were measured after cotransfection with miR-144 or their negative control (NC) and PBX3-wt or PBX3-mt. (E) Western blotting was performed to evaluate the protein expression levels of PBX3 after transfection miR-144 mimic or sh-UCA1+miR-144 inhibitor. Data were expressed as means±SD of three independent experiments. $*p<0.05$, $**p<0.01$.

cotransfection of sh-UCA1 and miR-144 mimic group (Fig. 5E).

PBX3 Overexpression Promoted A549 Cell Viability, Migration, Invasion, and Cell Cycle Transition, but Inhibited Cell Apoptosis

pEX-PBX3 and sh-PBX3 were used to increase or decrease the mRNA expression of PBX3 in A549 cells ($p<0.001$) (Fig. 6A and B). Results in Figure 6C–F indicated that overexpression of PBX3 significantly enhanced

the A549 cell viability, migration, and invasion, but inhibited cell apoptosis ($p<0.05$, $p<0.01$, or $p<0.001$). Downregulation of PBX3 had an opposite effect ($p<0.05$). Western blotting assay presented that sh-PBX3 treatment upregulated the expression levels of Bax, cleaved caspase 3, and cleaved caspase 9, and decreased the expression level of Bcl-2 (Fig. 6G). Moreover, the expression of PCNA, cyclin A, cyclin E1, cyclin D1, CDK2, and CDK4 in A549 cells were also enhanced after transfection of pEX–PBX3 and decreased after transfection of sh-PBX3
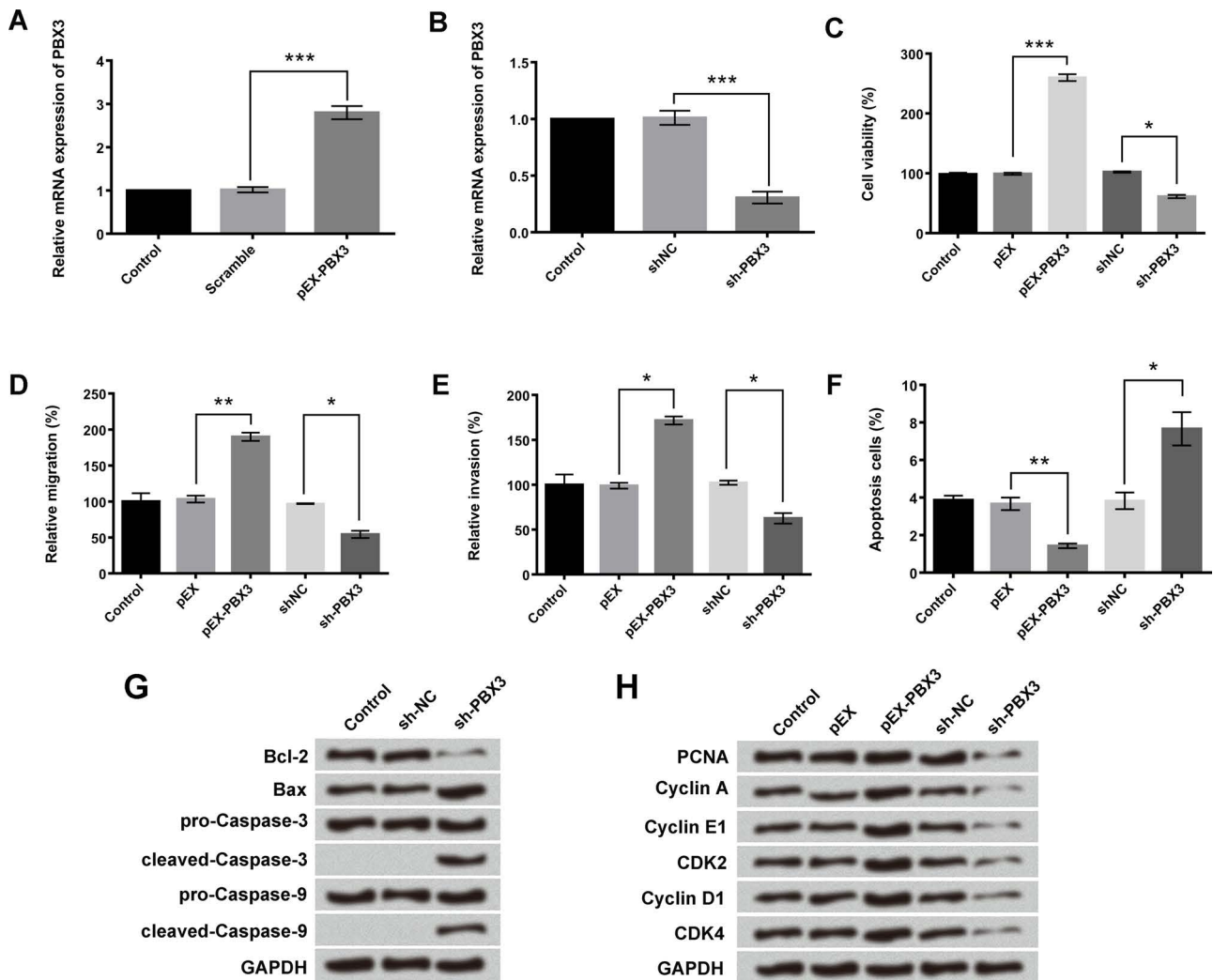
Figure 6. Different expressions of PBX3 have an influence on A549 cell viability, migration, invasion, apoptosis, and cycle transition. A549 cells were transfected with pEX-PBX3 or sh-PBX3. (A, B) mRNA expression of PBX3, (C) viability, (D) migration, (E) invasion, (F) apoptosis, (G) protein expression of Bcl-2, Bax, caspase 3, and caspase 9, (H) protein expression of PCNA, cyclin A, cyclin E1, cyclin D1, CDK2, and CDK4 was analyzed using qRT-PCR assay, trypan blue exclusion assay, Transwell assay, flow cytometry analysis, and Western blotting, respectively. Data were expressed as means±SD of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

(Fig. 6H). These results implied that PBX3 participated in the process of A549 cell proliferation, migration, invasion, cell cycle transition, and cell apoptosis.

DISCUSSION

The different expression levels of lncRNAs in eukaryotic cells were closely related to the occurrence and development of various cancers. Researchers have made great efforts to elucidate the internal molecular mechanism of lncRNAs in cancer progression and aim to find out effective therapeutic targets for cancer therapy. In this study, we investigated the functional roles of lncRNA UCA1 in lung cancer cell growth. The interaction between lncRNA UCA1 and miRNA-144 in the regulation of lung cancer cell viability, migration, invasion, cycle transition, and apoptosis was also analyzed. Our results found that the expression levels of lncRNA UCA1 in lung cancer cells were higher than in human normal lung embryonic cells. Knockdown of lncRNA UCA1 by transfection with sh-UCA1 remarkably decreased lung cancer A549 cell viability, migration, invasion, and cell cycle transition, but promoted cell apoptosis. Further experiments showed that lncRNA UCA1 bound to miR-144 in A549 cells. PBX3 was a target gene under miR-144 regulation, which was

involved in the regulation effects of miR-144 on A549 cell proliferation and metastasis.

lncRNA UCA1 does not encode specific proteins, but regulates gene expression on various levels in tumor cells[19]. Numerous studies have demonstrated the oncogenic roles of lncRNA UCA1 in tumor cells due to their effects on tumor cell growth, differentiation, and metastasis[19,20]. miR-144 has been shown to participate in the regulation of various cancer cell growth, including in lung cancer cells[12,15]. In the present study, we found that lncRNA UCA1 was highly expressed in lung cancer A549, H157, H4006, H1299, and H1650 cells compared to that in human normal embryonic lung WI-38 and HEL-1 cells. After transfection with sh-UCA1, knockdown of lncRNA UCA1 inhibited lung cancer A549 cell viability, migration, invasion, and cell cycle transition, but enhanced the rate of cell apoptosis. Further bioinformatics analysis found that lncRNA UCA1 bound to miR-144 in A549 cells. qRT-PCR identified that the expression levels of miR-144 and lncRNA UCA1 had clear negative correction effects. Dual luciferase reporter assay confirmed that cotransfection with lncRNA UCA1-wt and miR-144 significantly reduced the relative luciferase activity. Cotransfection with sh-UCA1 and miR-144 inhibitor remarkably reversed the inhibition of cell viability, migration, invasion, and cell cycle transition induced by single sh-UCA1. These findings revealed the pivotal roles of lncRNA UCA1 in regulating lung cancer A549 cell growth and metastasis, and miR-144 participated in the effects of lncRNA UCA1 on cell viability, migration, invasion, apoptosis, and cell cycle transition. Moreover, Nie et al. demonstrated that the lncRNA UCA1 exerted oncogenic functions in lung cancer A549 cells by targeting miR-193a-3p[21]. These results suggested that lncRNA UCA1 could promote the growth of lung cancer cells by regulating multiple miRNAs.

To further understand the potential mechanism of miR-144 on A549 cell growth, we determined that PBX3 was the direct target gene of miR-144 as evidenced by the results of bioinformatics analysis, dual luciferase activity assay, and the negative relationship of miR-144 and PBX3 in mRNA and protein levels. PBX3 is a member of the human PBX family and functions as a transcription factor in human cells[22]. Ramberg et al. reported that PBX3 was posttranscriptionally regulated by androgen in prostate cancer cells and acted as a biomarker for aggressive prostate cancer[23,24]. Dickson et al. identified that class I homeobox (HOX) A/PBX3 participated in the regulation of chemotherapy sensitivity on cytogenetically normal acute myeloid leukemia cells[25]. In this research, the cell viability, migration, and invasion of A549 cells were all remarkably increased after upregulation of PBX3 and decreased after PBX3 downregulation, further confirming the important regulatory roles of PBX3 in the growth and metastasis of lung cancer A549 cells. These results were consistent with a previous experimental study, which demonstrated that miR-144-3p inhibited gastric cancer SGC-7901 cell growth by suppression of the process of epithelial-to-mesenchymal transition (EMT) through targeting PBX3[26].

lncRNAs have been found to play critical regulatory roles in pathological processes of various diseases, including lung cancer[27,28]. Different lncRNAs may exhibit different tumor-suppressive or tumor-promoting functions because of their wide regulatory effects on several gene expressions in various cancers[29]. They worked together to form an intricate regulatory network. In our experiments, we found that the higher expression of lncRNA UCA1 in lung cancer cells promoted lung cancer A549 cell proliferation and metastasis. miR-144 was negatively regulated by lncRNA UCA1. PBX3 was a target gene of miR-144. We also realized that the lncRNA UCA1/miR-144/PBX3 pathway was only a very simple regulatory pathway in lung cancer cells. More deep-level and more complex regulatory channels are waiting for researchers to explore them.

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