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LncRNA AB073614 promotes tumor migration and invasion by repressing CDKN1A in non-small cell lung cancer

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Abstract. – OBJECTIVE: Some researches have showed that long noncoding RNAs (lncRNAs) take part in varieties of biological behaviors during the tumor progression. This study aims to determine whether lncRNA AB073614 functioned in the metastasis of non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS: Real Time titative Polymerase Chain Reaction (R was used to detect AB073614 expression S-CLC tissues. Besides, wound healing assay transwell assay were conducted in NSCLC c Furthermore, the mechanism assays were p formed to identify how AB073 tioned i metastasis of NSCLC cells.

RESULTS: By comparing the ression level in adjacent tissues AB073 ression level in NSCLC sa was higher. Moreover, aft AB s knocked down, invasion an gration SCLC cells were inhibited. after AB073 as over-expressed, inv d migration NSCLC cells were p ted mRNA and protein expression le l of C was upregulated via knock n of AB073 hile mRNA and protein ession level of C 1A was down-regula via ov xpression of AB073614. Be-side e exp on of CDKN1A in NSCLC tis-sue n vely c lated to the expres-sion o 14.

CONC NS: results indicated that 614 c ce cell migration and cell inva n in NS hrough repressing CDKN1A, whi ight offe a potential therapeutic choice fo NSCLC.

Words:
noncoding RNA, AB073614, NSCLC, CDK-N1A.

Introduction

ung cancer, ich is one of the top causes umor-related ath globally, stills remain a h threat t public[1]. As the major sub-grou cer, non-small cell lung cancer (NSCLC) ccounts for almost 85% of all lung ncer cases[2]. Though tremendous advances have e to reduce the mortality of lung cancer, e p nosis of patients with lung cancer is far from satisfaction, and the 5-year survival rate of the cases remains only 16%[3]. The main management for NSCLC patients at an early stage is surgical resection combined with chemotherapy. Unfortunately, most of the patients eventually develop disease progression and require further intervention[4]. Metastasis is the leading cause of the high mortality in NSCLC. Thus, it's important to understand the molecular basis underlying the metastasis of NSCLC and improve the poor prognosis of patients with NSCLC.

Advances in human genome sequencing have revealed that non-coding RNAs (ncRNAs) account for almost 99% of total transcribed RNAs. Long noncoding RNAs (lncRNAs), defined as transcription longer than 200 nucleotides, is an important group of ncRNAs. LncRNAs have been indicated to be key regulators in numerous processes, including the development of diverse cancers. For example, by modulating SF1 and suppressing expression level of miR-184, lncRNA UCA1 accelerates cell proliferation and cisplatin resistance in oral squamous cell carcinoma[5]. Through targeting miR-221/SOCS3, lncRNA GAS5 suppresses cell proliferation, cell metastasis, and gemcitabine resistance in pancreatic

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cancer[6]. LncRNA RUNX1-IT1 act as an anti-oncogene in colorectal cancer by the inhibition of cell proliferation and cell migration which could function as a novel diagnostic biomarker[7]. Moreover, lncRNA ATB promotes cell migration and cell invasion in glioma by activating astrocytes through suppressing the expression of microRNA 2043p[8]. LncRNA AB073614 has recently been identified as a novel lncRNA in cancer tissues and cells. We aimed to determine whether LINP1 participates in the metastasis of NSCLC and the potential mechanism.

Patients and Methods

Patients and Sample Collection

In this research, 60 NSCLC patients were randomly selected and received surgery at Weinan Central Hospital of Shannxi Province. This study was approved by the Ethics Committee of Weinan Central Hospital of Shannxi Province. The signed written informed consents were obtained from all participants before the investigation.

Cell Culture and Cell Transfection

SPCA1, PC-9, H1975, and normal human bronchial epithelial cell16HBE (Shanghai Model Bank; Shanghai, China) were cultured in penicillin, Roswell Park Memorial Institute-1640 (RPMI-1640; Thermo Fisher Inc., Waltham, MA, USA) and 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA).

RNA Extraction and Real-time-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA from collected frozen tissue samples and cell lines with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was reverse-transcribed to complementary deoxyribose nucleic acids (cDNAs) through reverse Transcription Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). mRNA levels were quantified by SYBR Green real-time PCR and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the following primers: AB073614, forward: 5'-ATCTGCTCCTGGGTCTTAC-3' and reverse: 5'-TGGCTTGTCTGTTAGAGTC-3'; GAPDH, forward: 5'-CCAAAATCAGATGGCCAATGCTGG-3' and reverse: 5'-TGATGGGACTGTGGTCATTCA-3'. RT-qPCR was performed by the ABI 7500 system (Applied Biosystems, Foster City, CA, USA).

Cell Transfection

Lentivirus expressing short-hairpin RNA (shRNA) and lentivirus against [illegible] was provided by GenePharma (Shanghai, China). AB073614 shRNA (sh-AB073614) and the empty vector (control) were then used for the transfection in SPCA1 [illegible] cells using polybrene (GenePharma, Shanghai, China) when the density of cells reached 70%. AB073614 lentivirus (AB073614) and the empty vector (control) were then used for transfection in PC-9 cells using polybrene (Genepharma, Shanghai, China) when the density of cells reached

Western Blot Analysis

To investigate the relative protein expression, transfected cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed using lysis buffer. Reagent for coimmunoprecipitation assay (IP) was utilized to extract the protein from cells. Bicinchoninic acid (BCA) protein assay kit (Dalian, China) was chosen for quantifying protein concentrations. The target proteins were separated by Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis (SDS-PAGE). 5% fat-free milk was used to block non-specific protein interactions in Tris-Buffered Saline and Tween (TBST) which contains Tris-HCl (50 mM), NaCl (150 mM), and Tween 20 (0.05%) at 4°C for 1h. Then, they were incubated with antibodies after replaced to the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Abcam (Abcam, Cambridge, MA, USA) provided us with rabbit anti-GAPDH and rabbit anti-CDKN1A, as well as goat anti-rabbit secondary antibody. Enhanced chemiluminescence (ECL; Millipore, Billerica, MA, USA) was applied for the assessment of protein expression.

Wound Healing Assay

Empty vector, AB073614 shRNA or AB073614 lentivirus were transfected into NSCLC cells. These treated cells were cultured in 6-well plates and grew to about 90% confluent. Then, they were scratched by a sterile 10 μL pipette tip across the confluent cell layer and incubated in serum-free medium at 37°C in a humidified incubator containing 5% CO_2 for 24 h. Wound closure was captured using a light microscope (DFC500, Munich, Germany).

Transwell Assay

5 ×10^4 cells in 200 μL serum-free RPMI-1640 were transformed to top chamber of an 8 μm pore size insert (Corning, Corning, NY, USA) coated with or without 50 μg Matrigel (BD, Bedford, MA, USA). Serum-free medium was added into the upper chamber, and 10% FBS medium supplemented was then added into the lower chamber. After 48 h, the top surface of chambers was immersed for 10 min with by 100% precooling methanol after wiped by cotton swab. Then, they were stained in crystal violet for 30 min and inspected with the microscope (Olympus, Tokyo, Japan). Five fields were used to count the data for migration and invasion membrane.

Statistical Analysis

Statistical analysis was conducted by Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA). Statistical data was presented with GraphPad Prism software (La Jolla, CA, USA). Data were presented as mean ± SD (Standard Deviation). An independent-sample test was used to compare continuous data. The relative expression of mRNA was measured using the method of $2^{-\Delta\Delta CT}$. Result considered statistically significant at $p<0.0$

Results

Expression Level of AB 614 Elevated in NSCLC Sa es and

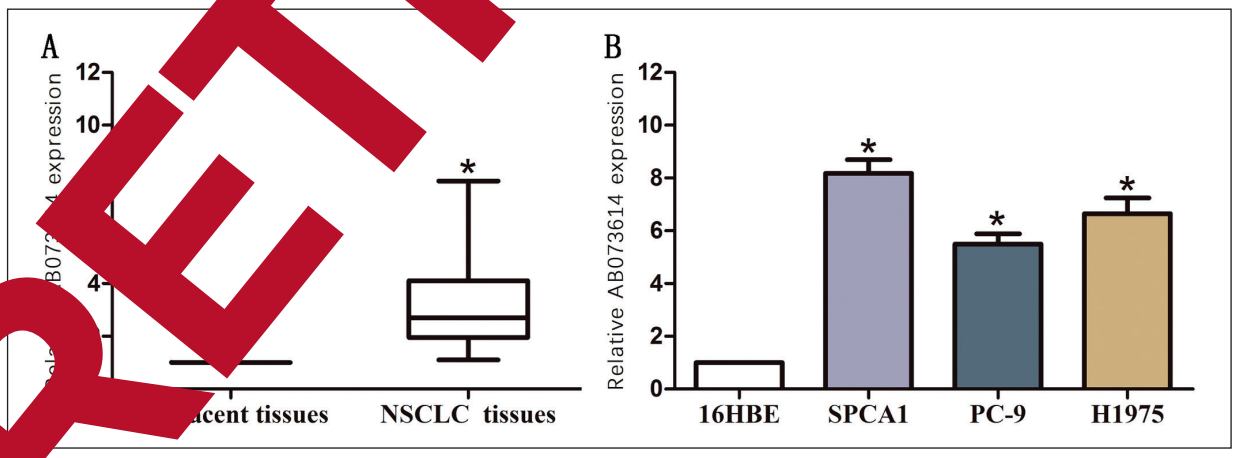
Firstly, the expression of A detected by performi RT-q 60 NSCLC patients' samples and 3 NSCLC cell lines. The result revealed that AB073614 was signif ntly upregulated in tumor tissue sam 1A). AB073614 expression in NSC cells wa remarkably higher when compa with that in 16HBE cell line (Figure 1B).

Knockdown of AB073 Inhibit NSCLC Cell Migratio

SPCA1 NSCLC c ine w sed for knockdown of A 3614. e AB073614 expression was dete RT-qP (Figure 2A). To ate the biologi role of AB073614 i SCLC cell n, we investigated 614 on NSC cell migration. As own in wound healing assay, the knockdown of AB inhibited SPCA1 cell migr compared w e negative control (F 2B). Meanwhile, e effects of knockdown of AB073614 on SPCA1 cell migration asured by t well assay were the same. results reve d that after AB073614 was k d down number of migrated SPCA1 NSC s decreased (Figure 3A).

Overexpression of AB073614 Promoted Cell Migration

PC NSCLC cell line was used for the overexpression of AB073614. The AB073614 expression was detected by RT-qPCR (Figure 2C). To evaluate the biological role of AB073614 in NSCLC cell migration, we investigated AB073614 on NSCLC cell migration. As shown in wound healing assay, the overexpression of AB073614 promoted the PC-9 cell migration compared with the neg-



ge 1. Expression levels of AB073614 were increased in NSCLC tissues and cell lines. ***A,*** AB073614 expression was s tly increased in the NSCLC tissues compared with adjacent tissues. ***B,*** Expression levels of AB073614 relative to GAP were determined in the human NSCLC cell lines and normal human bronchial epithelial cell (16HBE) by RT-qPCR. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

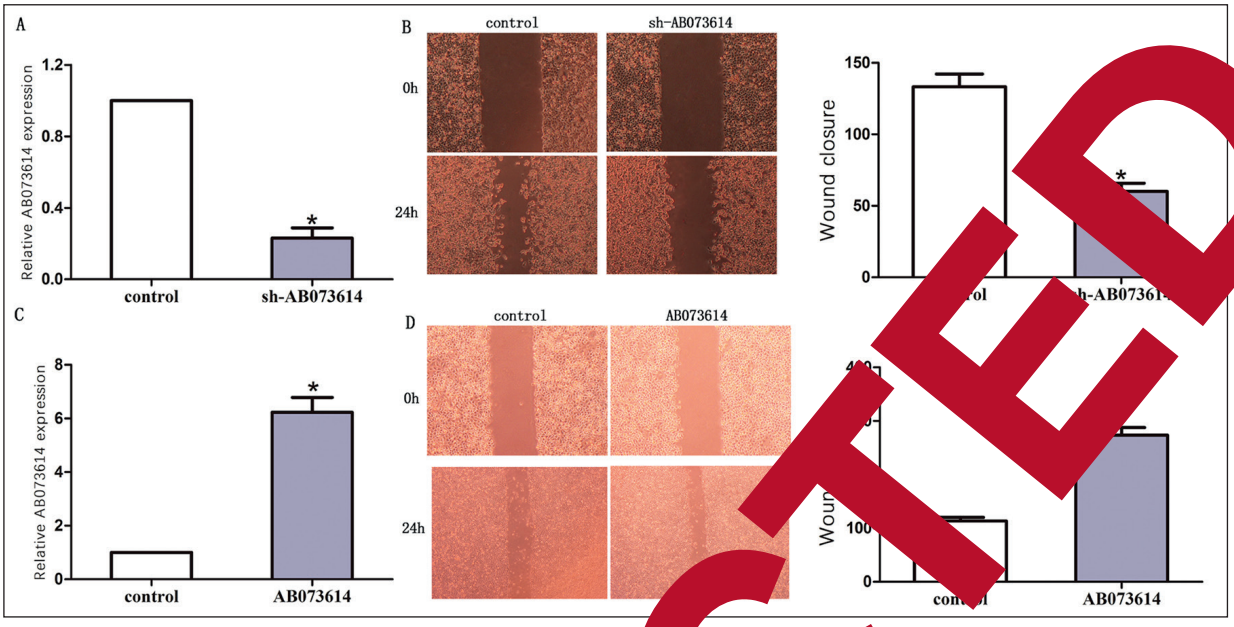


Figure 2. Wound healing assay showed that AB073614 pro[illegible] NSCLC cell [illegible]ation. ***A,*** AB073614 expression in SPCA1 NSCLC cells transduced with AB073614 shRNA(sh-AB[illegible]4) and the emp[illegible]ctor (control) was detected by RT-qPCR. GAPDH was used as an internal control. ***B,*** Wound heali[illegible]y showed [illegible]migrated length of SPCA1 NSCLC cells was significantly decreased *via* knockdown of AB073614. ***C,*** [illegible]on in PC-9 NSCLC cells transduced with AB073614 lentivirus (AB073614) and the empty vector (control) [illegible] by RT-qPCR. GAPDH was used as an internal control. ***D,*** Wound healing assay showed t[illegible]ted length of PC-9 NSCLC cells was significantly increased *via* overexpression of AB073614. The results represe[illegible]ree independent experiments (mean ± standard error of the mean). *$p<0.05$. **$p<0.01$.

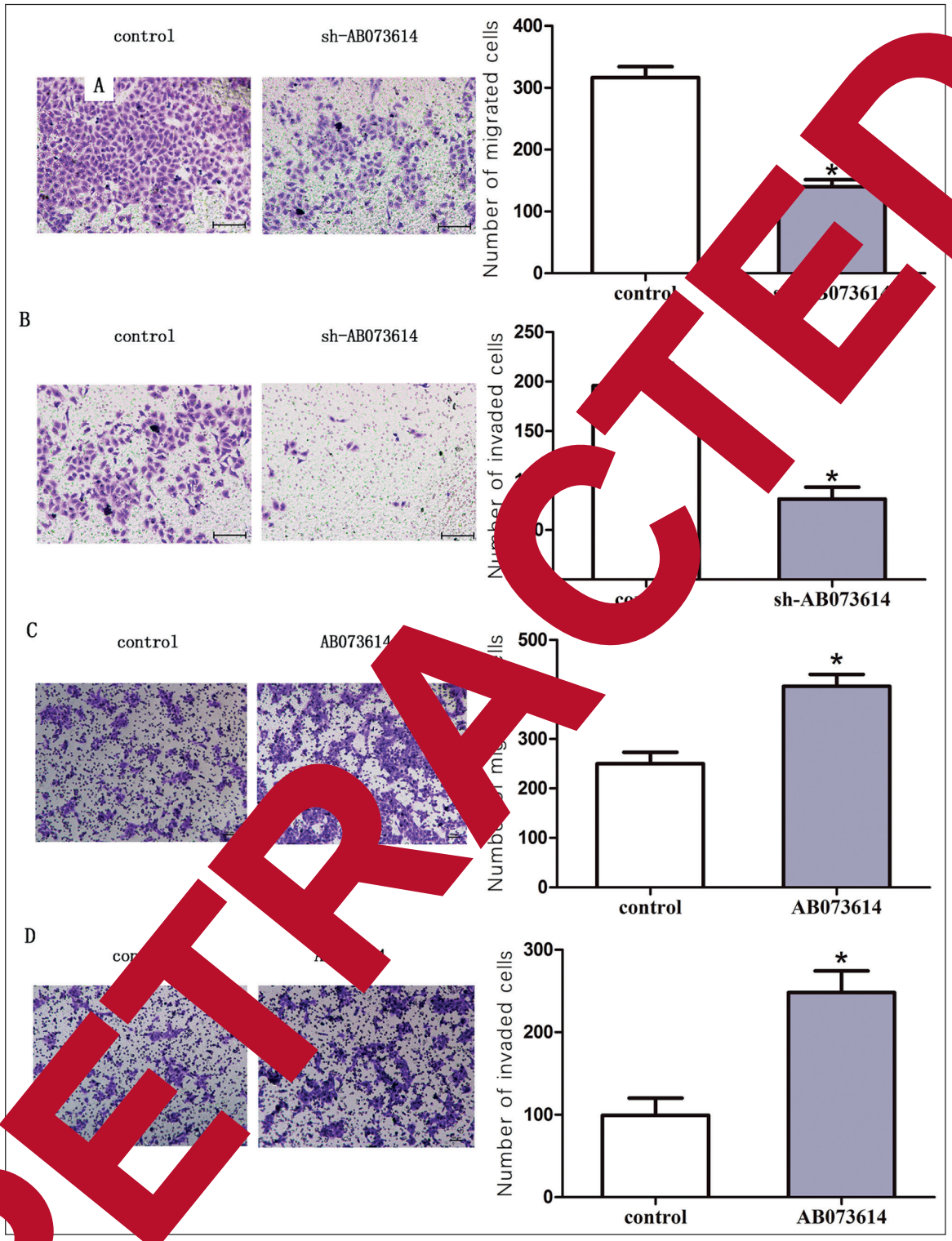
ative control group (Figure 2[illegible]hile, the effects of the overexpressi[illegible]f AB[illegible]614 on PC-9 cell migration mea[illegible]d by th[illegible]nswell assay were the same. T[illegible]ults after AB073614 was [illegible]rexp[illegible]the number of migrated PC-9 [illegible]LC cel[illegible] increased (Figure 3B).

AB073614 [illegible]motes NSCLC Cell Inva[illegible]n

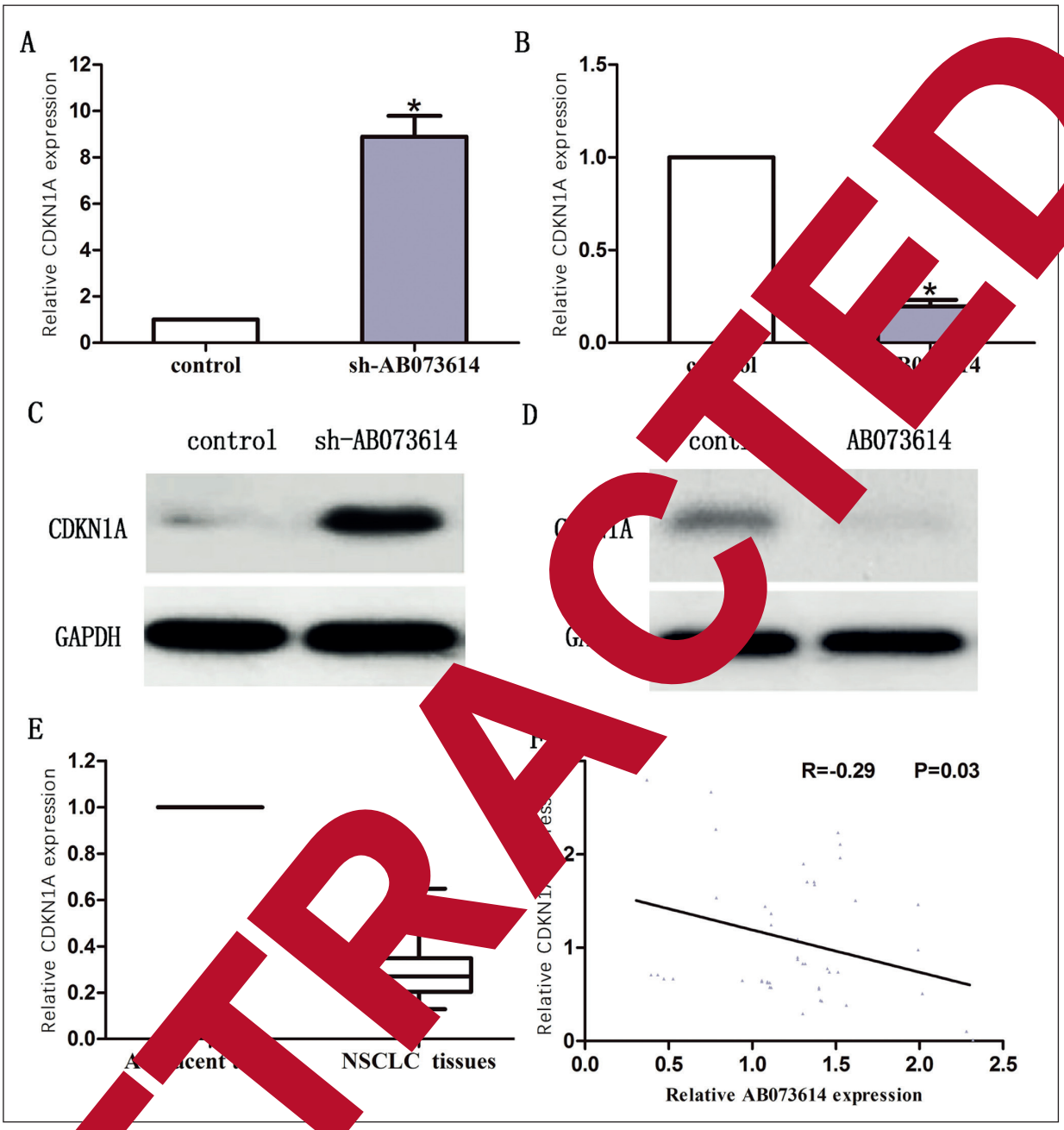
We n[illegible]valuated the actio[illegible]e of AB073614 in NS[illegible] cell in[illegible]ion. As shown in the transwell ass[illegible]ter A[illegible]3614 was knocked down, the cell[illegible]d[illegible]ity in [illegible]A1 NSCLC cells was repress[illegible]ure 3C[illegible]esides, after AB073614 [illegible]verexp[illegible]d[illegible] cell invaded ability in PC-9 [illegible]LC cel[illegible]promoted (Figure 3D).

AB[illegible]pressed CDKN1A [illegible]pression Directly

Previous studies showed that CDKN1A was [illegible]regulated in many tumors including NSCL[illegible]nd it was found to be regulated by many lncRNAs and mediated the function of lncRNAs in many malignant tumors. Then, to further verify the actual relationship between CDKN1A and AB073614, we further detected the CDKN1A expression of SPCA1 cells transfected with AB073614 shRNA (sh-AB073614) or the empty vector (control). The results of RT-qPCR showed that the expression level of CDKN1A was significantly higher in AB073614 shRNA (sh-AB073614) group in SPCA1NSCLC cells compared with that in empty vector (control) group (Figure 4A). And the expression level of CDKN1A was significantly lower in AB073614 lentivirus (AB073614) group in PC-9 NSCLC cells compared with that in empty vector (control) group (Figure 4B). Besides, the results of Western blot assay revealed that expression level of CDKN1A was significantly higher in AB073614 shRNA (sh-AB073614) group in NSCLC cells compared with that in empty vector (control) group (Figure 4C). And the expression level of CDKN1A was significantly lower in AB073614 lentivirus (AB073614) group in NSCLC cells compared with that in empty vector (control) group (Figure 4D). Furthermore, we found out



Fi assay showed that AB073614 promoted NSCLC cell migration and invasion. ***A,*** Transwell assay showed t grated cells was significantly decreased *via* knockdown of AB073614 in SPCA1 NSCLC cells (magnification,). ***B,*** Transwell assay showed that number of migrated cells was significantly increased *via* overexpression of AB073614 in NSCLC cells (magnification, 40X). ***C,*** Transwell assay showed that number of invaded cells was significantly decreased *v* down of AB073614 in SPCA1 NSCLC cells (magnification, 40X). ***D,*** Transwell assay showed that number of invaded cells significantly increased *via* overexpression of AB073614 in PC-9 NSCLC cells (magnification, 40X). The results represent the average of three independent experiments.



Figur AB0736 ppressed CDKN1A expression directly in NSCLC cells and tissues. ***A,*** RT-qPCR results showed that CDK expres as higher in AB073614 shRNA (sh-AB073614) compared with the empty vector (control). ***B,*** RT-qPCR resu ed CDKN1 pression was lower in AB073614 lentivirus (AB073614) compared with the empty vector (contro ern blot ts showed that CDKN1A expression was upregulated in AB073614 shRNA (sh-AB073614) pared emp tor (control). ***D,*** Western blot results showed that CDKN1A expression was downregulated in 14 len 3614) compared with the empty vector (control). ***E,*** CDKN1A was significantly downregulated in C tissue red with adjacent tissues. ***F,*** The linear correlation between the expression level of CDKN1A and AB 4 in NSCL issues. The results represent the average of three independent experiments. Data are presented as the me or of the mean. *$p<0.05$.

he expression of CDKN1A in NSCLC tissue s significantly lower when compared with that of adjacent tissues (Figure 4E). Correlation analysis demonstrated that CDKN1A expression level negatively correlated to AB073614 expression in NSCLC tissues (Figure 4F).

Discussion

Lung cancer (LC) ranks the second common cancer among human malignant tumors. 80% of lung cancer is NSCLC worldwide[9]. The incidence of NSCLC in China accounts for more than half of the whole world[10]. Although molecular targeted therapy is available for NSCLC patients, only a small part of patients benefits from those discovered driver mutations. Therefore, it is urgent to find more potential regulators and targets for treatment of NSCLC.

It has been reported in numerous researches that non-coding RNAs (ncRNAs) take part in varieties of biological behaviors during the tumor progression. Known as subgroups of ncRNAs family, long noncoding RNAs (lncRNAs) are non-coding RNA molecules which are longer than 200 bp and cannot be transcribed into protein. Moreover, it was found that lncRNAs are related to various kinds of cellular functions including carcinogenesis and metastasis.

In recent years, increasing studies have demonstrated that lncRNAs serve as imp[illegible] regulators in lung cancer initiation a[illegible] gression. For example, through regulating [illegible]R-488-HEY2 signal network, lncRNA PRN[illegible] functions as an oncogene in NSCLC and p[illegible] motes tumor progression[11]. The [illegible]expressio[illegible] of lncRNA-p21 suppresses ce[illegible]s in NS-CLC by directly decreasin[illegible] expr[illegible]n level of PUMA[12].

LncRNA AB073614 [illegible]new[illegible] cRNA in a few malig[illegible]t tu[illegible]luding NS-CLC. It has been [illegible]rted to p[illegible]ate in tumor developmen[illegible] metastasis. [illegible]stance, AB073614 pr[illegible]origenesis [illegible] ovarian cancer whic[illegible]s clos[illegible]lated to the poor prognosis [illegible] patients w[illegible]varian cancer[13]. By mod[illegible]ing the PI3K/A[illegible]ignaling pathway, [illegible]/3614[illegible]ys an important role in the reg[illegible]on of [illegible] proliferation and cell migration [illegible]l canc[illegible] Through regulating epithel[illegible]enchy[illegible] transition, AB073614 [illegible]tates [illegible]ro[illegible]ation and cell migration in [illegible]ma wh[illegible]icates a poor prognosis in case[illegible]ith gliom[illegible]. To date, there has not been an[illegible] correlation between lncRNA [illegible]/3614 [illegible]d NSCLC tumorigenesis. In this [illegible]earch, we figured out that the AB073614 [illegible]emarkably higher-expressed in NSCLC sa[illegible]. Besides, after AB073614 was knocked down, cell migration and cell invasion in NSCLC cell were inhibited. And after AB073614 was overexpressed, cell migration a[illegible]ell invasion in NSCLC cell were promo[illegible] results indicated that AB073614 m[illegible] act as a oncogene in NSCLC.

To further identify the under[illegible]echanism of how AB073614 affects NSCLC [illegible]umorigenesis and metastasis, w[illegible]edicted a[illegible]ke CDKN1A as the pote[illegible] binding pro[illegible] AB073614 by using [illegible]nforma[illegible]nalysis [illegible]d experimental verif[illegible]n. S[illegible] studi[illegible] have reported that CD[illegible]s anti-[illegible]static and anti-tumor[illegible]les i[illegible]riety [illegible]ancers. Cyclin-depe[illegible] kinase i[illegible]especially *CDKN1A* [illegible]is a canonic[illegible]lycomb target gene [illegible]tum[illegible]pressor[16]. For example, by suppressing *CDKN*[illegible]ositive feedback loop, EZH2[illegible]ols the pro[illegible]n of germinal center [illegible] and enables ce[illegible] cycle progression[17]. [illegible]-1 inhibits cell proliferation in breast tumor [illegible]egulating C[illegible]N1A transcription expression [illegible]h may prov[illegible]an attractive targeted therapy [illegible]DKN1A [illegible]ld enhance the response of cuta[illegible] to radiotherapy by manipulating Lang[illegible]ns cell survival and promoting Treg [illegible]ll generation[19]. In addition, CDKN1A gene is [illegible]tly correlated with prognosis of pa[illegible]nts [illegible]th gastric adenocarcinoma resection[20]. In our research, CDKN1A expression could be upregulated after knockdown of AB073614, while CDKN1A expression could be downregulated after overexpression of AB073614. Moreover, CDKN1A expression in NSCLC samples was negatively related to AB073614 expression. All the results above suggested that AB073614 might promote tumorigenesis of NSCLC *via* downregulating CDKN1A.

Conclusions

We showed that AB073614 was remarkably upregulated in patients with NSCLC. Besides, AB073614 could enhance migration and invasion of NSCLC cells through repressing CDKN1A. These findings suggest that AB073614 may contribute to therapy for NSCLC as a candidate target.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.
2) Zheng HE, Wang G, Song J, Liu Y, Li YM, Du WP. MicroRNA-495 inhibits the progression of non-small-cell lung cancer by targeting TCF4 and inactivating Wnt/beta-catenin pathway. Eur Rev Med Pharmacol Sci 2018; 22: 7750-7759.
3) Liu Z, Jiang L, Zhang G, Li S, Jiang X. MiR-24 promotes migration and invasion of non-small cell lung cancer by targeting ZNF367. J BUON 2018; 23: 1413-1419.
4) Yusen W, Xia W, Shengjun Y, Shaohui Z, Hongzhen Z. The expression and significance of tumor associated macrophages and CXCR4 in non-small cell lung cancer. J BUON 2018; 23: 398-402.
5) Fang Z, Zhao J, Xie W, Sun Q, Wang H, Qiao B. LncRNA UCA1 promotes proliferation and cisplatin resistance of oral squamous cell carcinoma by sunppressing miR-184 expression. Cancer Med 2017; 6: 2897-2908.
6) Liu B, Wu S, Ma J, Yan S, Xiao Z, Wan L, Zhang F, Shang M, Mao A. lncRNA GAS5 reverses EMT and tumor stem cell-mediated gemcitabine resistance and metastasis by targeting miR-221/SOCS3 in pancreatic cancer. Mol Ther Nucleic Acids 2018; 13: 472-482.
7) Shi J, Zhong X, Song Y, Wu Z, Gao P, Zh… J, Wang J, Liu J, Wang Z. Long non-codi… RUNX1-IT1 plays a tumour-suppressive … in colorectal cancer by inhibiting cell prolife… and migration. Cell Biochem Funct 2019; 37: … 20.
8) Bian EB, Chen EF, Xu YD, Yan… F, Ma CC, Wang HL, Zhao B. Exos… lncR… TB activates astrocytes that p… te glion… ell invasion. Int J Oncol 2019; … 13-721.
9) Rizou T, Perlikos F, … M, … opoulos S, Toump… I, Kr… Development of novel real-ti… T-qPCR … logies for quantification … e COL11A1 … general and C tran… evaluation … on-small cell lung … er sp… ns. J BUON 2018; 23: 1699-1710.
10) Chen… lmore CM, Hamm… S, Kim CF, Wong KK… n-small-cell lung ca… ers: a heteroge… set of … ases. Nat Rev Cancer 2014; 14: … 546.
11) Cheng D, Bao C, Zhang X, Lin X, Huang H, Zhao L. LncRNA PRNCR1 interacts with HEY2 to abolish miR-448-mediated growth inhibition i… cell lung cancer. Biomed Pharm… 107: 1540-1547.
12) Yang T, Zhang W, Wang L, Xia… Guo B, Gong Y, Liang X, Huang D, Li Q, Na… g Y, Shao J. Long intergenic noncoding … inhibits its apoptosis by decreas… PUMA e… n i… non-small cell lung ca… J Int Med … 1218616144.
13) Cheng Z, Guo J, C… Luo … ng W, Qu X. A long noncoding … promot… umorigenesis and … dic… ognosis … varian cancer. Onc… get 201… 5381-… 9.
14) Wang Y, … H, Xue J, Lia… hou X. LncRNA … regulates pr… ion and metasta… c… al cancer cel… via the PI3K/AKT … naling … y. Biomed Pharmacother 2017; 93: 1230-123…
15) … ng YM, Song Y… ockdown of long noncoding RNA AB073614 inhibits glioma cell proliferation and mi… ation via affecting epithelial-mesenchymal tra… on. Eur Rev Med Pharmacol Sci 2016; 20: 399… 02.
16) … chutina … knovich R, Geng H, Johnson NA, … elnick AM, Elemento O. EZH2-me… netic silencing in germinal center B cells contributes to proliferation and lymphomagenesis. Blood 2010; 116: 5247-5255.
17) … n W, Rivas MA, Calvo FM, Teater M, Purwada A, … edmond D, Shen H, Challman MF, Elemento O, Singh A, Melnick AM. EZH2 enables germinal centre formation through epigenetic silencing of CDKN1A and an Rb-E2F1 feedback loop. Nat Commun 2017; 8: 877.
18) Bianco S, Jangal M, Garneau D, Gevry N. LRH-1 controls proliferation in breast tumor cells by regulating CDKN1A gene expression. Oncogene 2015; 34: 4509-4518.
19) Price JG, Idoyaga J, Salmon H, Hogstad B, Bigarella CL, Ghaffari S, Leboeuf M, Merad M. CDKN1A regulates Langerhans cell survival and promotes Treg cell generation upon exposure to ionizing irradiation. Nat Immunol 2015; 16: 1060-1068.
20) Nie X, Wang X, Lin Y, Yu Y, Liu W, Xie F, Wang X, Tan J, Huang Q. SNP rs1059234 in CDKN1A gene correlates with prognosis in resected gastric adenocarcinoma. Clin Lab 2016; 62: 409-416.