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Original Articles

LncRNA-*UCA1* exerts oncogenic functions in non-small cell lung cancer by targeting miR-193a-3p

Wei Nie [a,1], Hui-juan Ge [b,1], Xiao-qun Yang [b,1], Xiangjie Sun [b], Hai Huang [a], Xia Tao [c], Wan-sheng Chen [c,*], Bing Li [a,**]

[a] Department of Respiratory Medicine, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
[b] Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai 200032, China
[c] Department of Pharmacy, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

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ABSTRACT

Recently, the long non-coding RNA (lncRNA) urothelial carcinoma-associated 1 (*UCA1*) has been identified as an oncogenic gene in multiple human tumor entitles, and dysregulation of *UCA1* was tightly linked to carcinogenesis and cancer progression. However, whether the aberrant expression of *UCA1* in non-small cell lung cancer (NSCLC) is associated with malignancy, metastasis or prognosis has not been characterized. In this study, we found that *UCA1* was upregulated in NSCLC tissues. Higher expression of *UCA1* led to a significantly poorer survival time, and multivariate analysis revealed that *UCA1* was an independent risk factor of prognosis. *UCA1* overexpression enhanced, whereas *UCA1* silencing impaired the proliferation and colony formation of NSCLC cells. Moreover, mechanistic investigations showed that *UCA1* upregulated the expression of miR-193a-3p target gene ERBB4 through competitively 'spongeing' miR-193a-3p. Overall, we concluded that *UCA1* functions as an oncogene in NSCLC, acting mechanistically by upregulating ERBB4 in part through 'spongeing' miR-193a-3p.

Introduction

Lung cancer is one of the most common tumors and the leading cause of cancer-related mortality worldwide, with about 1.8 million new cases each year [1]. Although the majority of the cases occur in the less developed regions, the disease remains as the most common cancer in China [2]. Non-small cell lung cancer (NSCLC) ranks the majority of diagnosed lung cancer cases. Despite the emergence of new adjuvant chemotherapy regimens and targeted biologic agents, as well as significant progress in understanding the pathophysiological mechanisms in NSCLC, the 5-year survival rates and recurrence rate remain dismal. It is of paramount importance to understand the underlying pathological mechanisms contributing to NSCLC for developing novel diagnostic biomarkers and therapeutic strategies and improving the prognosis of NSCLC [3–5].

Protein-coding genes account for only ~2% of the human genome, whereas the vast majority of transcripts are non-coding RNAs (ncRNAs), including long ncRNAs (lncRNAs). Mounting evidence has shown that lncRNAs are important factors in cancer. Human *urothelial carcinoma associated 1* (*UCA1*), an lncRNA which was first identified in human bladder carcinoma [6], was previously shown to dysregulate and exert its oncogenic activity in cancer [7–11]. In 2014, Li et al. reported that *UCA1* promotes glycolysis through the mTOR-STAT3/microRNA143 pathway [12], bringing a speculation that *UCA1* exerted its oncogenic functions as competitive endogenous RNAs and mediated the bioavailability of miRNAs on their targets, which was subsequently confirmed by researches in hepatocellular carcinoma [13]. However, whether the aberrant expression of *UCA1* in NSCLC is associated with malignancy or prognosis remains unknown, and the mechanism through which *UCA1* exerts its oncogenic activity needs to be illustrated.

We hypothesized that lncRNA *UCA1* contributes to NSCLC progression by functioning as miRNA sponge. We found that *UCA1* expression was increased in NSCLC tissues and is associated with poor prognosis. Further experiments showed that overexpression of *UCA1* functioned as competitive endogenous RNAs (ceRNAs) and promoted the cell proliferation and colony formation, leading to upregulation of the ERBB4 protein modulated by *UCA1*-targeting miR-193a-3p. Taken together, this study pointed out that the upregulation of *UCA1* promoted the proliferation of NSCLC cells in part through functioning as miR-193a-3p sponge.

* Corresponding author. Tel.: +86 021 81871349; fax: +86 021 81886181.
E-mail address: chenws126@126.com (W. Chen).
** Corresponding author. Tel.: +86 021 81885322.
E-mail address: bing_li1962@163.com (B. Li).
[1] These authors contributed equally to this work.

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Materials and methods

Patients

Between 2008 and 2010, a total of 112 fresh NSCLC tissues and paired adjacent normal lung tissues from patients undergoing resection were collected from Shanghai Changzheng Hospital. All patients were followed up by phone calls or clinic visits every 6 months until cancer related death or study end. None of the patients received preoperative chemotherapy or radiotherapy. Detailed information about demography, clinical characteristics and histopathology was collected retrospectively for all patients. The histological diagnosis and grade of tumor were defined through the evaluation of H&E tissue sections, according to the classification guidelines of the World Health Organization [14]. All patients were staged based on the International Association for the Study of Lung Cancer (IASLC) Tumor-Node-Metastasis (TNM) classification, 7th edition [15]. Informed consent was obtained from all patients. This study was reviewed and approved by the Ethics Committee of Shanghai Changzheng Hospital.

Cell culture

The human lung cancer-derived cell lines A549, H1299, H446, H460, and NCI-H1650, and one cultured human lung epithelial cell (BEAS-2B) were obtained from the Chinese Academy of Sciences Cell Bank. All cells were grown in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco BRL, Gaithersburg, MD, USA) and were maintained in a 37 °C incubator with a humidified atmosphere containing 5% CO_2.

RNA extraction and real-time PCR

Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was generated using the Reverse Transcription System Kit (Takara, Dalian, China). For mRNA and lncRNA analyses, real-time PCR was performed as previously described [16]. Expressions of mRNA and lncRNA were normalized with β-actin. The primer sequences can be found in Supplementary Table S1. For miRNA analysis, real-time PCR was performed as above using TaqMan miRNA assays according to the manufacturer's instructions (Applied Biosystems). The real-time PCR reactions were performed in triplicate. The relative levels of gene expression were represented as $\Delta Ct = Ct_{gene} - Ct_{reference}$, and the fold change of gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

Plasmid construction

The complementary DNA encoding *UCA1* was PCR-amplified inserted into pcDNA3.1 vector (Invitrogen) or subcloned into pHBLV-IRES-ZsGreen-PGK-puro vector (Hansheng, China). *UCA1* constructs with mutations at the putative miR-193a-3p-binding site were generated using the appropriate primers; the product was inserted upstream of the firefly luciferase gene in pGL3-Basic (Promega, Madison, WI). The resulting vectors were sequenced and named *UCA1*-WT and *UCA1*-Mut. siRNA of *ERBB4* was obtained from Cell Signaling Technology. Primers for subcloning and plasmid construction were listed in Supplementary Table S1.

Ectopic expression and gene silencing of UCA1 in cells

Transfections were performed using the Lipofectamine 3000 kit (Invitrogen) according to the manufacturer's instructions. The siRNAs to *UCA1* (si*UCA1*) and scrambled siRNA (siNC) were purchased from Transheep (Transheep, Shanghai, China). The siRNAs used in this study are detailed in Supplementary Table S1. Virus particles were harvested 48 hours after contrasfecting pHBLV-IRES-ZsGreen-PGK-puro constructs with the packaging plasmid ps-PAX2 and the envelope plasmid pMD2G into HEK-293FT cells using Lipofectamine 3000 Reagent (Life Technologies, USA) according to the manufacture's instructions. A549 and H1299 cells were infected with recombinant lentivirus-transducing units (pHBLV-UCA1 or pHBLV-NC) plus 6 μg/mL polybrene (Sigma, USA). After 48-72 hours incubation, cells were harvested for RNA and protein extraction, or be resuspended for the following assays. Stable infecting cells were maintained with 2.5 μg/mL of puromycin (Sigma, USA). MiR-193a-3p miRNA mimics and miR negative control were purchased from Life Technologies (Ambion, Grand Island, NY, USA) and introduced into cells at a final concentration of 50 nM. The transfected cells were harvested 48 hours after transfection.

Cell proliferation assay

The cell proliferation was assessed using the Cell Counting Kit-8 (Dojindo, Tokyo, Japan) according to the manufacturer's protocol. Briefly, a total of approximately 5×10^3 NSCLC cells were plated in 96-well plates, treated with 10 ml/well of Cell Counting Kit-8 solution during the last 4 h of culture, and the cell proliferation curves were plotted using the 450 nm absorbance at each time point. All experiments were performed in triplicate.

Colony formation assay

Eight hundred stable *UCA1*-overexpressing or -knockdown cells were plated into 6-well plates and incubated in RPMI-1640 with 10% FBS at 37 °C. Fourteen days later, the cells were fixed and stained with 0.1% crystal violet. The number of colonies, defined as >50 cells/colony, was counted.

RNA immunoprecipitation (RIP)

A549 cells were used to perform RIP experiments using Ago2 antibody (Cell Signaling) and the Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA, USA) according to the manufacturer's instructions. After the antibody was recovered using protein A + G beads, qRT-PCR was performed to detect *UCA1* and miRNAs in the precipitates.

Luciferase reporter assay

Transient transfection of the lncRNA-*UCA1* reporter plasmid and the internal control *Renilla* luciferase plasmid was carried out with the appropriate plasmids using Lipofectamine 3000 (Invitrogen). The relative luciferase activity was normalized to *Renilla* luciferase activity 48 h after transfection, and luciferase activity was measured using a dual-luciferase reporter gene assay system (Promega, Madison, WI, USA).

Western blot analysis

Total cell lysates were prepared with sample buffer and boiled at 95 °C for 5 min. The samples were transferred to SDS–PAGE at 80 V for 3 h and then transferred to PVDF membranes for another 2 h. After incubation with specific antibodies for ERBB4 (Santa Cruz Biotechnology sc-8050, Santa Cruz, CA, USA) or β-actin (Sigma-Aldrich, Saint Louis, MO, USA) at 4 °C overnight, the membranes then were washed by 1% TBST for three times, incubated with secondary antibodies for 1 h and detected by chemiluminescence.

Statistical analysis

Significant between-group differences were estimated using Student's t-test, Wilcoxon signed-rank test, and Pearson's chi-square test, as appropriate. Pearson correlation analysis was used to estimate the relationship between the expression level of *UCA1* and miR-139a-3p. Overall survival (OS) curves were calculated with the Kaplan–Meier method and were analyzed with the log-rank test. The univariate and multivariate Cox proportional hazards models were utilized to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the outcome. All *P* values were two-sided and obtained using the SPSS 20.0 software package (SPSS, Chicago, IL, USA). Differences were defined as statistically significant for p-values < 0.05.

Results

UCA1 *is increased in human NSCLC tissues and is associated with poor prognosis*

To explore whether *UCA1* is detectable and altered in NSCLC, we first examined the expression of *UCA1* in 112 pairs of NSCLC tissues and paired adjacent normal lung tissues. As presented in Fig. 1A, elevated expression of *UCA1* was observed in tumor tissues compared with adjacent normal tissues ($p < 0.001$). We then measured *UCA1* expression level in five human NSCLC cell lines (A549, H1299, H446, H460, and NCI-H1650) and cultured human lung epithelial cells (BEAS-2B). As shown in Fig. 1B, *UCA1* expression was significantly higher in all five NSCLC cell lines compared with that in human lung epithelial cells (all $p < 0.05$).

To assess the correlation of *UCA1* expression with clinicopathologic characteristics, the expression levels of *UCA1* in tumor tissues were categorized as low (n = 73) or high (n = 39) in relation to the Youden index (Fig. 1C). Correlation regression analysis showed that high expression of *UCA1* was significantly correlated with tumor size ($p = 0.014$) and TNM stage ($p = 0.042$) (Table 1). Then we applied overall survival (OS) curves that were plotted according to *UCA1* expression level by the Kaplan–Meier method to investigate whether the *UCA1* expression level was correlated with the outcome of NSCLC patients. As shown in Fig. 1D, high *UCA1* expression in NSCLC tissues is significantly associated with worse OS ($p = 0.005$, log-rank test). Univariate Cox proportional hazards analysis of OS revealed that the

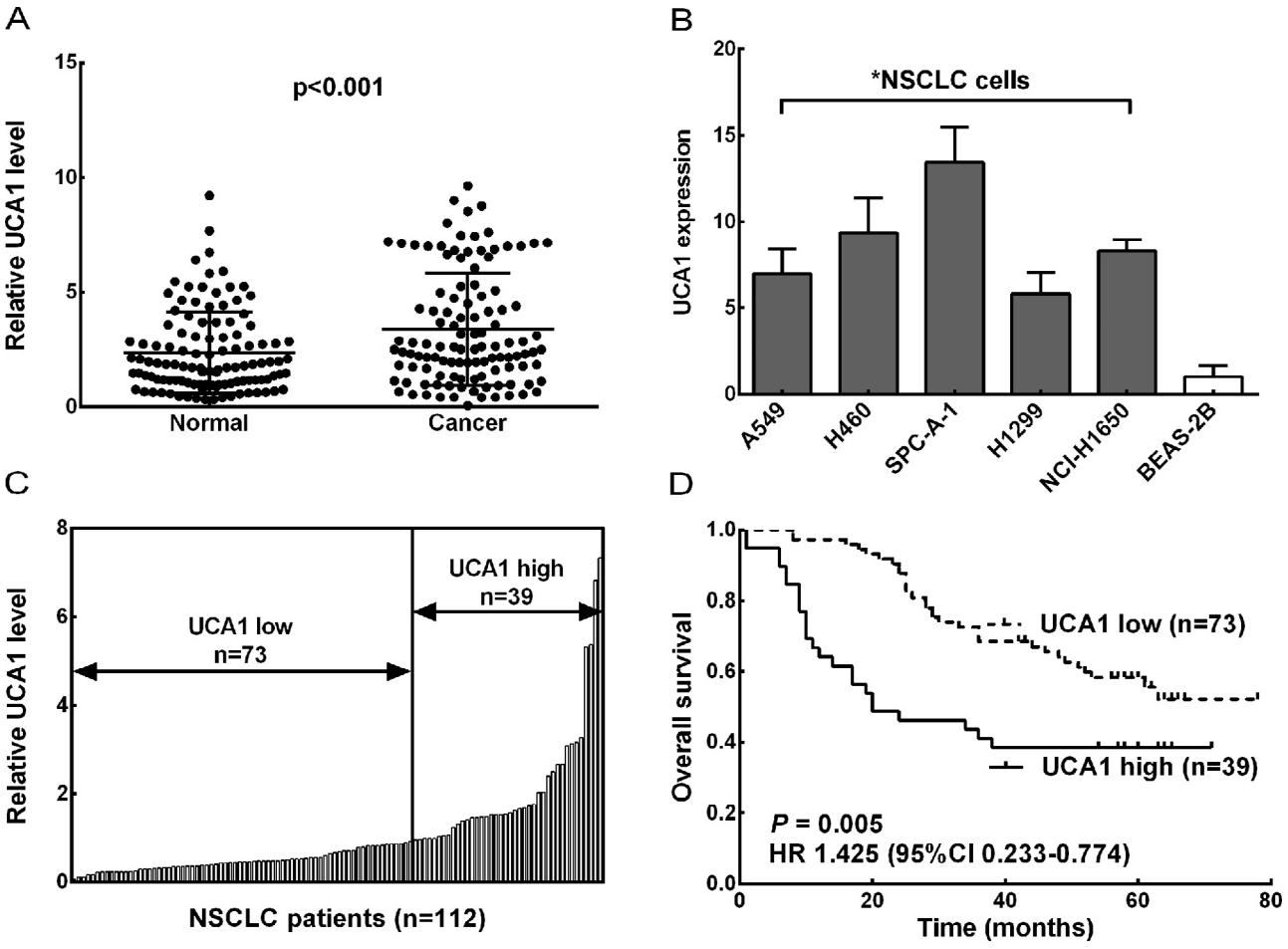

Fig. 1. *UCA1* expression in NSCLC and its association with patients' prognosis. (A) Expression of *UCA1* in 112 pairs of NSCLC tissues and paired adjacent normal lung tissues. (B) Expression of *UCA1* in five NSCLC cell lines and one cultured human lung epithelial cells. Data are presented as mean ± standard error based on at least three independent experiments. *p < 0.05. (C) The total of 112 NSCLC patients included in this study were divided into low (n = 73) or high (n = 39) *UCA1* group in relation to the Youden index. (D) The Kaplan–Meier overall survival curves by *UCA1* levels. Patients with elevated *UCA1* expression showed reduced survival times compared with patients with low levels of *UCA1* expression (log-rank test; p = 0.005).

relative level of *UCA1* expression (p = 0.005), T stage (p = 0.009), N stage (p = 0.000), and TNM stage (p = 0.000) were prognostic indicators, and multivariate analysis showed that *UCA1* expression was an independent prognostic indicator for OS in patients with NSCLC (p = 0.014) in addition to TNM stage (p = 0.001) (Table 2). These results indicate that *UCA1* may play a pivotal role in the development and progression of human NSCLC.

Table 1
Association between *UCA1* expression and clinicopathological characteristics.

Characteristics	No. of patients (n = 112)	Low expression (n = 73)	High expression (n = 39)	*P* value
Age (years)				0.506
≤60	42 (37.5%)	29 (69.0%)	13 (30.9%)	
>60	70 (62.5%)	44 (62.9%)	26 (37.1%)	
Gender				0.180
Male	67 (59.8%)	47 (70.1%)	20 (29.9%)	
Female	45 (40.2%)	26 (57.8%)	19 (42.2%)	
Smoking status				0.976
No	40 (35.7%)	26 (65.0%)	14 (35.0%)	
Yes	72 (64.3%)	47 (65.2%)	25 (34.7%)	
Tumor size				0.026*
≤3 cm	73 (65.2%)	53 (72.6%)	20 (27.4%)	
>3 cm	39 (33.0%)	20 (51.3%)	19 (48.7%)	
N stage				0.439
N0	77 (68.8%)	52 (67.5%)	25 (32.5%)	
N1~3	35 (31.3%)	21 (60.0%)	14 (40.0%)	
TNM stage				0.034*
I + II	90 (80.4%)	63 (70.0%)	27 (30.0%)	
III	22 (19.6%)	10 (45.5%)	12 (54.5%)	

TNM, tumor-node-metastasis staging system. *$P < 0.05$.

UCA1 *promotes the proliferation of NSCLC cells in NSCLC cell lines*

To investigate whether *UCA1* has a role in the pathogenesis of NSCLC, A549 and H1299 cells were selected as research

Table 2
Univariate and multivariate analyses of different prognostic factors for OS in 112 patients with NSCLC.

Prognostic factors	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age (≤60/>60)	1.201	0.254–1.434	0.612			
Gender (male/female)	0.371	0.103–1.108	0.112			
Smoking status (no/yes)	0.983	0.775–1.246	0.887			
Tumor size (≤3 cm/>3 cm)	1.208	0.793–1.987	0.388			
N stage (N0/N1~3)	1.839	1.379–2.453	0.000*			
TNM stage (I + II/III)	2.096	1.535–2.862	0.000*	1.720	1.227–2.409	0.002*
UCA1 (low/high)	1.639	1.268–2.119	0.005*	1.409	1.077–1.844	0.014*

HR, hazard ratio; CI, confidence interval. *$P < 0.05$.

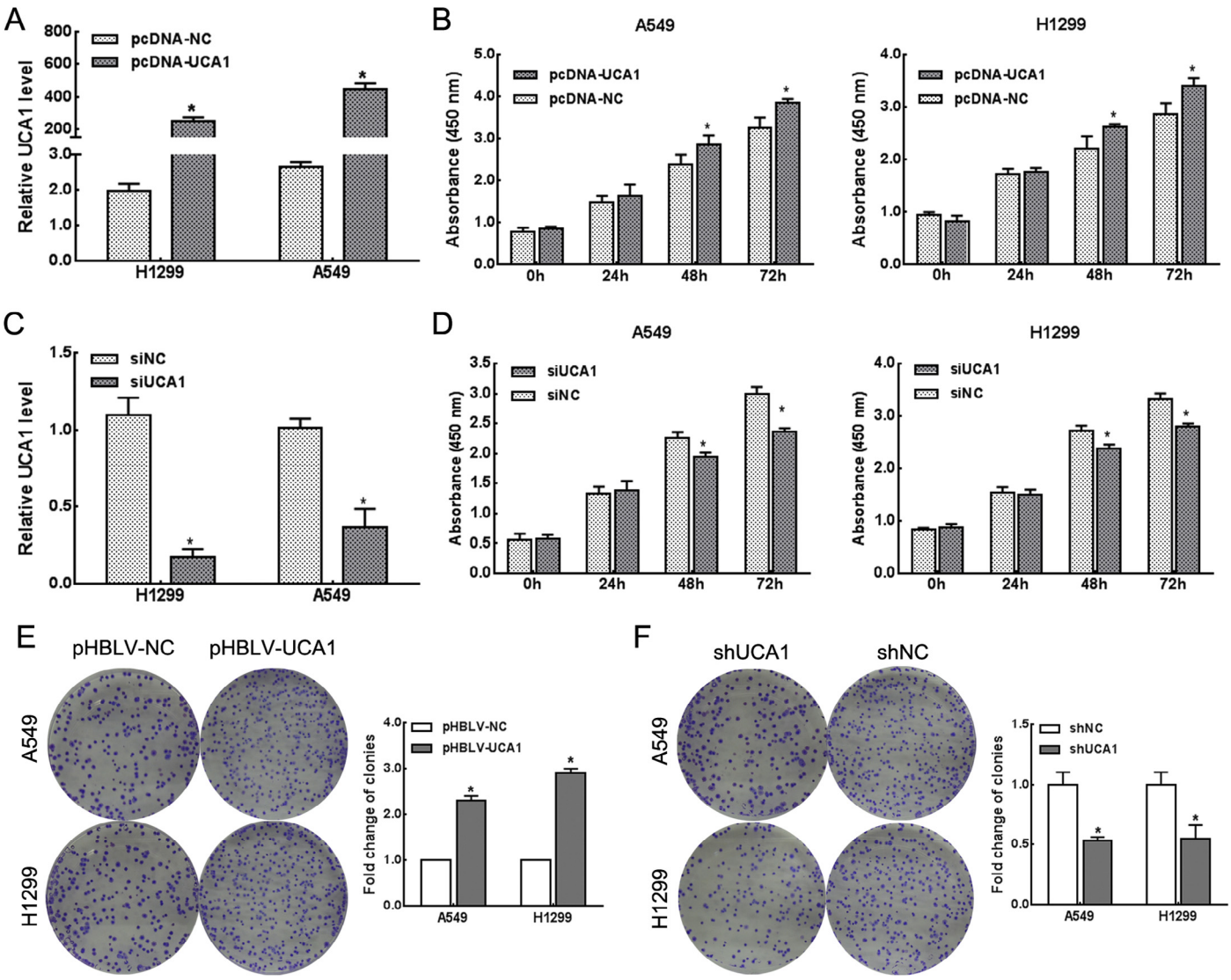

Fig. 2. *UCA1* promotes the proliferation and colony formation of NSCLC cells. (A) Overexpression of *UCA1* in A549 and H1299 cell lines analyzed by qRT-PCR. (B) The overexpression of *UCA1* significantly promoted A549 (left) and H1299 (right) cell proliferation as measured using the CCK-8 assay. (C) Knockdown of *UCA1* in A549 and H1299 cell lines analyzed by qRT-PCR. (D) The down-regulation of *UCA1* inhibited A549 (left) and H1299 (right) cell growth as measured using the CCK-8 assay. (E) Colony numbers of *UCA1*-overexpressing A549 and H1299 cells were significantly more than those transfected with NC. (F) Colony numbers of A549 and H1299 cells transfected with sh*UCA1* were significantly less than those transfected with shRNA-NC. $^{*}p < 0.05$.

representatives of NSCLC cells in the following studies; both cells were ectopically expressed and silenced of *UCA1*. The expression levels of *UCA1* were significantly increased in pcDNA-*UCA1* transfecting cells compared with the pcDNA-NC transfecting counterparts (Fig. 2A), while the *UCA1* was decreased in the cells transfected with si*UCA1* (Fig. 2C). We then conducted the CCK-8 cell counting assays and colony formation assay to investigate the influence of *UCA1* on tumor cell proliferation. The CCK8 assay showed that the tumor cell growth of the *UCA1*-overexpressing A549 and H1299 cells was significantly accelerated compared with that of the controls (Fig. 2B), while knockdown of *UCA1* by siRNA effectively inhibited the cell proliferation (Fig. 2D). The colony formation assay visualized the cell growth 14 days after transfection; colony numbers of *UCA1*-overexpressing A549 and H1299 cells-*UCA1* were significantly more than those transfected with NC (Fig. 2E), while cells transfected with sh*UCA1* were significantly less than those transfected with shRNA-NC (Fig. 2F). These results suggested that *UCA1* promoted cell proliferation in NSCLC *in vitro*.

UCA1 *acts as a molecular sponge for miR-193a-3p*

Recently, *UCA1* have been reported to be involved in tumor progression by functioning as ceRNAs to specific miRNAs. To examine whether *UCA1* has a similar mechanism in NSCLC, we predicted miRNA target sites using the online microRNA-target program (http://www.microRNA.org, http://www.mircode.org) and found out 10 common miRNAs with relevant binding sites in *UCA1* in both programs (Supplementary Fig. S1A). The expression levels of these miRNAs were measured in pcDNA-*UCA1* treated A549 and H1299 cells by qRT-PCR. In comparison with those of pcDNA-NC treatment groups, miR-135a, miR-18a, miR-143, and miR-193a-3p expressions showed a sharp decrease in both pcDNA-*UCA1* transfected A549 and H1299 cells (Supplementary Fig. S2B).

It is well defined that miRNA exerts its function by binding to Ago2, a core component of the RNA-induced silencing complex (RISC) complex that is required for miRNA-mediated gene silencing, and potential microRNA targets can be isolated from this complex after

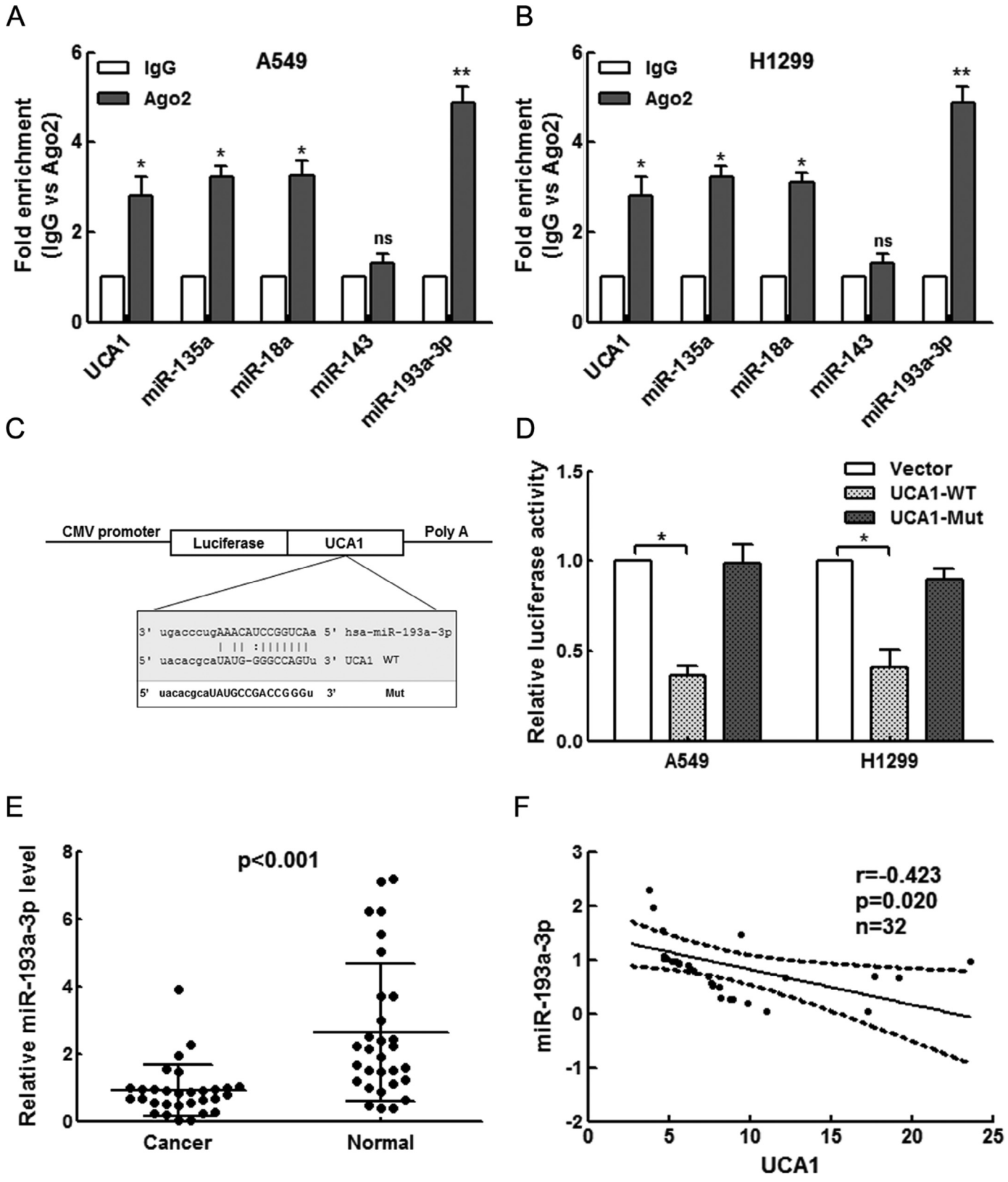

Fig. 3. *UCA1* acts as a molecular sponge for miR-193a-3p. (A)The association between *UCA1*, miRNAs and Ago2 was ascertained by analyzing A549 cell lysates by RNA immunoprecipitation with an Ago2 antibody. (B) The association between *UCA1*, miRNAs and Ago2 was ascertained by analyzing H1299 cell lysates by RNA immunoprecipitation with an Ago2 antibody. (C) Putative miR-193a-3p-binding sequence of *UCA1* RNA. Mutation was generated on the *UCA1* RNA sequence in the complementary site for the seed region of miR-193a-3p. (D) A549 and H1299 cells were transfected with vector, wild-type *UCA1* (*UCA1*-WT) or mutant *UCA1* (*UCA1*-Mut) with a mutation of the miRNA binding site; 48 h after transfection, the luciferase activity was measured using a dual-luciferase reporter gene assay system. *$p < 0.05$. (E) Expression of miR-193a-3p in 32 pairs of NSCLC tissues and paired adjacent normal lung tissues. (F) The correlation between *UCA1* and miR-193a-3p level was measured in 32 NSCLC tissues using Pearson correlation analysis. *UCA1* and miRNAs were detected by qRT-PCR. *$p < 0.05$. ns, not significant.

Ago2 co-immunoprecipitation [17]. To assess whether *UCA1* associates with RISC complex, we detected miR-135a, miR-18a, miR-143, and miR-193a-3p as well as *UCA1* in the Ago2 pellet. As shown in Fig. 3A, miR-135a, miR-18a and *UCA1* were enriched approximately 3-fold, whereas miR-193a-3p was enriched by greater than 5-fold. These results indicate that *UCA1* may play a role in deregulation of miR-193a-3p. For further confirmation, we constructed luciferase reporters containing *UCA1*, which contains wild-type (WT) or mutated-type (Mut) miR-193a-3p binding sites, for target investigation. We found that the corresponding *UCA1*-Mut construct no longer suppressed miR-193a-3p (Fig. 3D), supporting that miR-193a-3p is a *UCA1*-targeting miRNA. We next measured the expression levels of miR-193a-3p in 32 NSCLC tissues randomly selected from the same set of 112 patients shown in Fig. 1A. It was found that the expression level of miR-193a-3p was significantly lower in NSCLC tissues compared to that of ANT (Fig. 3E), and miR-193a-3p level was significantly negatively correlated with *UCA1* level (Fig. 3F).

MiR-193a-3p reverses the promoting effect of UCA1 *on the growth of NSCLC cells*

To dissect the importance of miR-193a-3p binding in *UCA1*-promoting NSCLC progression, we ectopically expressed miR-193a-3p in stable *UCA1* overexpressing A549 and H1299 cells, and by using CCK-8 assays, we found that the overexpression of miR-193a-3p attenuated the promoting proliferation effect of *UCA1* (Fig. 4A), which was also observed in colony formation assay (Fig. 4B). These results showed that *UCA1* promotes tumor cell growth in part via competitively binding miR-193a-3p.

UCA1 *modulated expression of endogenous miR-193a-3p targets ERBB4*

It has been found that miR-193a-3p functions as a tumor suppressor in human NSCLC by downregulating ERBB4 [18,19]. To determine whether *UCA1* regulates NSCLC progression by affecting miR-193a-3p targets, we evaluated the effect of *UCA1* on ERBB4. After transfection with the *UCA1*-WT in A549 and H1299 cells, the expression of ERBB4 was obviously increased in protein but not mRNA levels, while *UCA1*-Mut had no significantly effects on ERBB4 expression (Fig. 5A and B). In addition, transfection of the si*UCA1* in A549 and H1299 cells obviously decreased the expression of ERBB4 protein but not mRNA levels (Fig. 5C and D). Furthermore, consistent with the overexpression of miR-193a-3p or silence of *UCA1*, silence of *ERBB4* has similar effects on cell proliferation and colony formation of NSCLC cell lines (Fig. S2). Taken together, these results showed that *UCA1* eliminates the repression on ERBB4 induced by miR-193a-3p and exerts oncogenic functions by modulating miR-193a-3p/ERBB4.

Discussion

In spite of the encouraging advances in new cytotoxic drugs and targeted biologic agents of NSCLC, the prognosis for patients with advanced cancer remains dismal. Hence, it is of great importance to illustrate the underlying mechanisms on the formation and development of NSCLC. Long non-coding RNAs (lncRNAs) are 200–10,000 nucleotide long non-coding RNAs that control gene expression at epigenetic, transcriptional and post-transcriptional levels. It has been proven that lncRNAs can negatively or positively affect the expression of the coding gene by multiple mechanisms, such as transcriptional interference, inducing chromatin remodeling, modulating alternatively splicing patterns, etc. [20]. Currently, the critical functions of lncRNAs have been established in regulating almost all physiological and pathological processes: cell growth, differentiation and development, cell cycle and apoptosis by as yet unknown control mechanism. LncRNAs also play essential roles in human malignancies and function as tumor suppressors or oncogenes [21–24]. Thus, discovery of new lncRNAs will probably change the landscape of cancer genetics.

Significantly different lncRNA profiles can serve as phenotypic signatures for different cancers for their exploitation in cancer prognostics and therapeutics. In the present study, using a cohort of NSCLC patients, we determined that the *UCA1* up-regulation confers poorer clinical outcome. Our data indicated that *UCA1* might be an attractive biomarker for risk prognostication and that NSCLC patients with *UCA1* over-expression should receive appropriate adjuvant radiochemotherapy after lesion resection. Collectively, the results suggest that clinical-oriented research on lncRNAs in NSCLC should be undertaken and further research should be designed to discover more tumor-related lncRNAs as candidate prognostic biomarkers and therapy targets.

A growing volume of literature has proposed that lncRNAs can act as ceRNAs, abrogating the endogenous suppressive effect of these miRNAs on their targeted transcripts. For example, a maternally

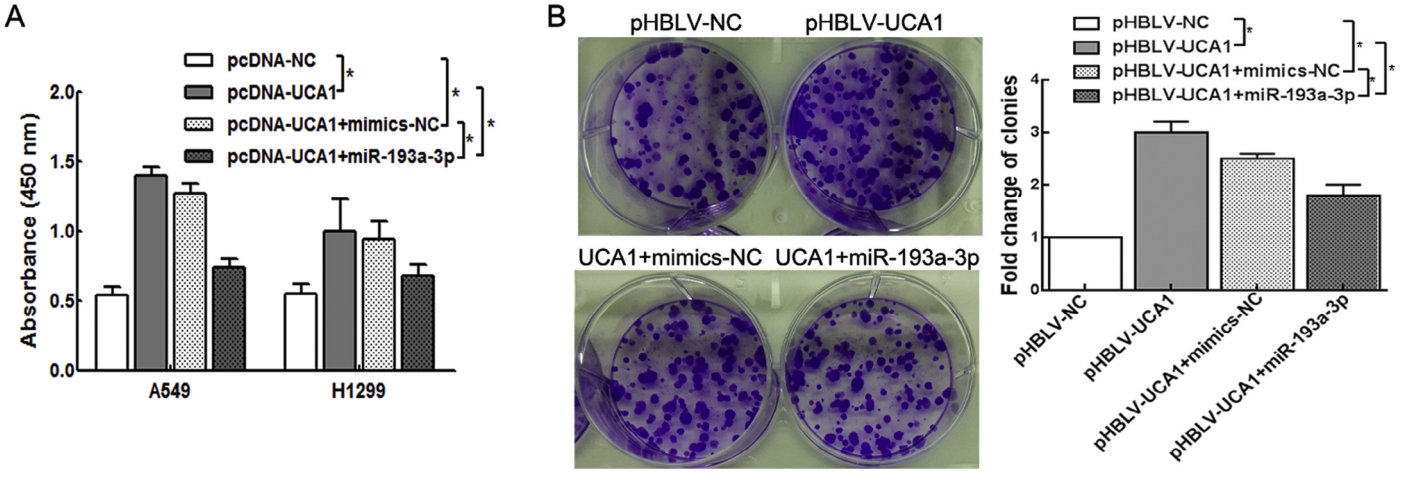

Fig. 4. *UCA1* promotes NSCLC cell growth by competitively binding miR-193a-3p. (A) The introduction of miR-193a-3p mimics attenuated the promoting proliferation effect of *UCA1* in A549 and H1299 cells. Cell number was determined by the CCK-8 assay, and the relative number of cells to 0 h is presented. (B) The introduction of miR-193a-3p mimics attenuated the promoting colony formation effect of *UCA1* in A549 cells. All values are presented as mean ± standard error based on at least three independent experiments. $^{*}p < 0.05$.

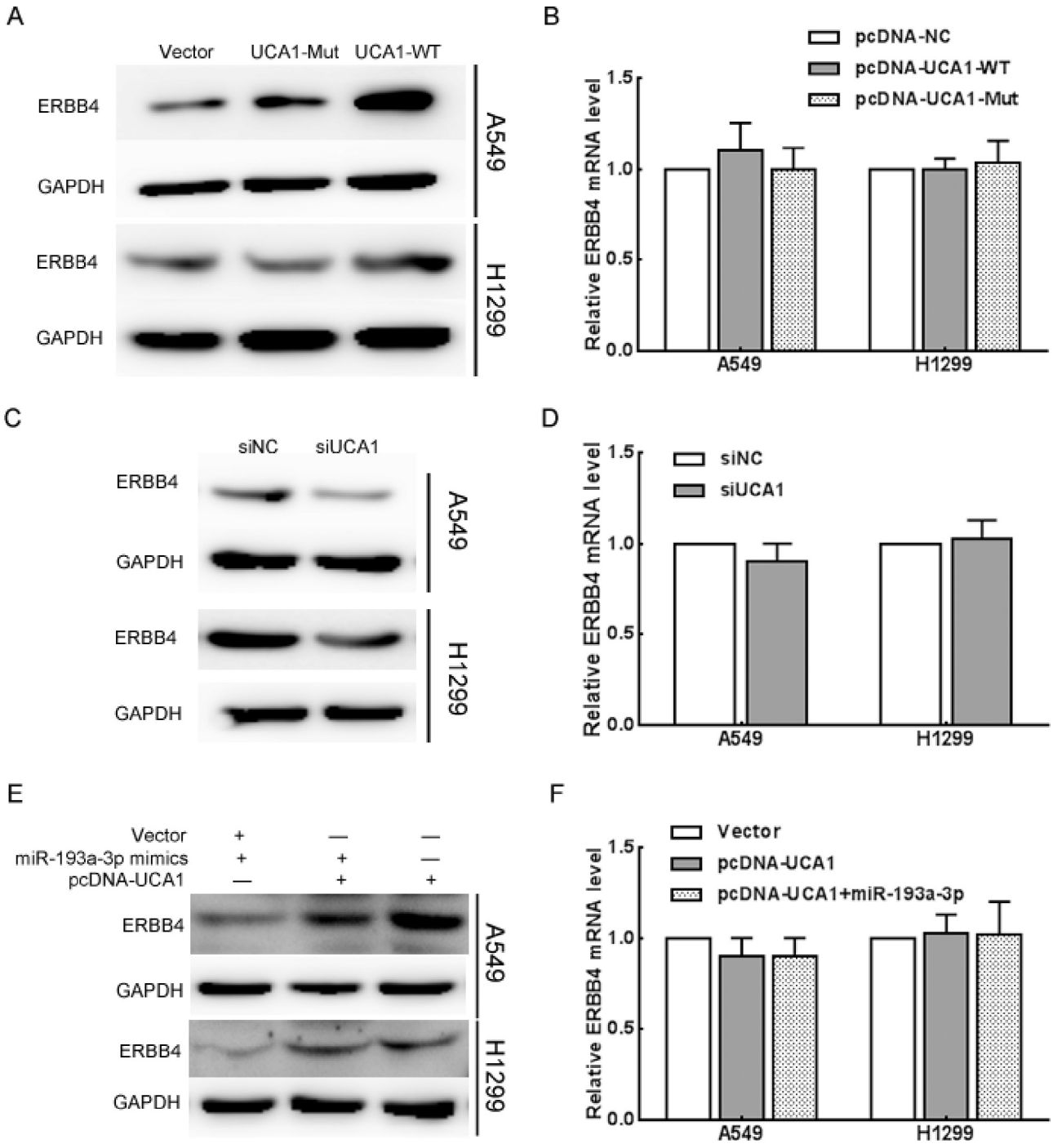

Fig. 5. *UCA1* modulated expression of endogenous miR-193a-3p targets ERBB4. (A) After transfected with Vector, *UCA1*-Mut, or *UCA1*-WT, the protein level of ERBB4 in A549 and H1299 cells was examined by western blot. (B) After transfected with Vector, *UCA1*-Mut, or *UCA1*-WT, the mRNA level of ERBB4 in A549 and H1299 cells was examined by qRT-PCR. (C) After transfected with siNC or si*UCA1*, the protein level of ERBB4 in A549 and H1299 cells was examined by western blot. (D) After transfected with siNC or si*UCA1*, the mRNA level of ERBB4 in A549 and H1299 cells was examined by qRT-PCR. (E) After transfected with Vector, pcDNA-*UCA1*, or pcDNA-*UCA1* combined with miR-193a-3p mimics, the protein level of ERBB4 in A549 and H1299 cells was examined by western blot. (F) After transfected with Vector, pcDNA-*UCA1*, or pcDNA-*UCA1* combined with miR-193a-3p mimics, the mRNA level of ERBB4 in A549 and H1299 cells was examined by qRT-PCR. $^{*}p < 0.05$.

expressed imprinted lncRNA, *H19*, acts as an endogenous sponge of miRNA let-7, which can directly bind to let-7 and regulate its target genes, *ZEB1* and *ZEB2*, during epithelial to mesenchymal transition (EMT) progression of colorectal cancer [25]. In the present study, we identified *UCA1* as an oncogenic player and revealed a previously unknown mechanism involving *UCA1* and miRNAs in NSCLC biology. By *in vitro* gain-/loss-of-function studies, *UCA1* were confirmed to be involved in a miRNA related regulatory network of NSCLC cell growth. Our finding that *UCA1* promoted cancer cell proliferation revealed several important aspects. Herein, *UCA1* was identified to be significantly upregulated in NSCLC patient tissues and cell lines, indicating the pathological and clinical implication of *UCA1* in NSCLC. In accordance with this, overexpression of *UCA1* remarkably promotes *in vitro* cell proliferation and colony formation. Moreover, knockdown of *UCA1* negatively regulated cell growth. Finally, subsequent mechanism study demonstrated that *UCA1*

functioned as a miRNA sponge and abolished the endogenous suppressive effect of miR-193a-3p on ERBB4, and the effects of *UCA1* on proliferation are overcome by the transfection of miR-193a-3p mimics.

Consistent with our findings, a recent study verified that *UCA1* is associated with miR-143 and modulated the expression of miR-143 targets, HK2, which provides powerful evidence that *UCA1* positively regulates gene expression at the post-transcriptional level [12]. Moreover, based on the bioinformatics analysis combined with *in vitro* and *in vivo* function experiments [13], *UCA1* was found to contribute to progression of hepatocellular carcinoma by acting as an endogenous sponge by directly binding to miR-216b and activate FGFR1/ERK signaling pathway. Aside from miR-143 and miR-216b, it seems interesting to investigate whether *UCA1* might exert its function by interacting with other unknown miRNAs in human tumor entitles.

In summary, our studies indicate that *UCA1* expression may potentially predict the prognosis of NSCLC patients, and this possibility must be confirmed in future studies with large sample size. We applied mechanic analysis to reveal the involvement of *UCA1* in promoting NSCLC progression by functioning as miR-193a-3p sponge, and indicated a novel *UCA1*–miR-193a-3p–ERBB4 signaling pathway regulatory network in NSCLC. These findings indicate that *UCA1* is an important molecular marker for predicting prognosis and is an important target for NSCLC therapy, and would add the known cross-talk between established pathways.

Conflict of interest

None.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.canlet.2015.11.024.

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2015, CA Cancer J. Clin. 65 (2015) 5–29.
[2] W. Chen, R. Zheng, H. Zeng, S. Zhang, J. He, Annual report on status of cancer in China, 2011, Chin. J. Cancer Res. 27 (2015) 2–12.
[3] F. Janku, D.J. Stewart, R. Kurzrock, Targeted therapy in non-small-cell lung cancer – is it becoming a reality?, Nat. Rev. Clin. Oncol. 7 (2010) 401–414.
[4] X.L. Ma, Z.L. Xiao, L. Liu, X.X. Liu, W. Nie, P. Li, et al., Meta-analysis of circulating tumor cells as a prognostic marker in lung cancer, Asian Pac. J. Cancer Prev. 13 (2012) 1137–1144.
[5] M. Reck, D.F. Heigener, T. Mok, J.C. Soria, K.F. Rabe, Management of non-small-cell lung cancer: recent developments, Lancet 382 (2013) 709–719.
[6] X.S. Wang, Z. Zhang, H.C. Wang, J.L. Cai, Q.W. Xu, M.Q. Li, et al., Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma, Clin. Cancer Res. 12 (2006) 4851–4858.
[7] Y. Fan, B. Shen, M. Tan, X. Mu, Y. Qin, F. Zhang, et al., Long non-coding RNA UCA1 increases chemoresistance of bladder cancer cells by regulating Wnt signaling, FEBS J. 281 (2014) 1750–1758.
[8] X. Fu, Y. Liu, C. Zhuang, L. Liu, Z. Cai, W. Huang, Synthetic artificial microRNAs targeting UCA1-MALAT1 or c-Myc inhibit malignant phenotypes of bladder cancer cells T24 and 5637, Mol. Biosyst. 11 (2015) 1285–1289.
[9] T. Wang, J. Yuan, N. Feng, Y. Li, Z. Lin, Z. Jiang, et al., Hsa-miR-1 downregulates long non-coding RNA urothelial cancer associated 1 in bladder cancer, Tumour Biol. 35 (2014) 10075–10084.
[10] M. Xue, X. Li, Z. Li, W. Chen, Urothelial carcinoma associated 1 is a hypoxia-inducible factor-1alpha-targeted long noncoding RNA that enhances hypoxic bladder cancer cell proliferation, migration, and invasion, Tumour Biol. 35 (2014) 6901–6912.
[11] M. Xue, X. Li, W. Wu, S. Zhang, S. Wu, Z. Li, et al., Upregulation of long non-coding RNA urothelial carcinoma associated 1 by CCAAT/enhancer binding protein alpha contributes to bladder cancer cell growth and reduced apoptosis, Oncol. Rep. 31 (2014) 1993–2000.
[12] Z. Li, X. Li, S. Wu, M. Xue, W. Chen, Long non-coding RNA UCA1 promotes glycolysis by upregulating hexokinase 2 through the mTOR-STAT3/microRNA143 pathway, Cancer Sci. 105 (2014) 951–955.
[13] F. Wang, H.Q. Ying, B.S. He, Y.Q. Pan, Q.W. Deng, H.L. Sun, et al., Upregulated lncRNA-UCA1 contributes to progression of hepatocellular carcinoma through inhibition of miR-216b and activation of FGFR1/ERK signaling pathway, Oncotarget 6 (2015) 7899–7917.
[14] E. Brambilla, W.D. Travis, T.V. Colby, B. Corrin, Y. Shimosato, The new World Health Organization classification of lung tumours, Eur. Respir. J. 18 (2001) 1059–1068.
[15] P. Goldstraw, J. Crowley, K. Chansky, D.J. Giroux, P.A. Groome, R. Rami-Porta, et al., The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours, J. Thorac. Oncol. 2 (2007) 706–714.
[16] W. Nie, M.D. Xu, L. Gan, H. Huang, Q. Xiu, B. Li, Overexpression of stathmin 1 is a poor prognostic biomarker in non-small cell lung cancer, Lab. Invest. 95 (2015) 56–64.
[17] F.V. Karginov, C. Conaco, Z. Xuan, B.H. Schmidt, J.S. Parker, G. Mandel, et al., A biochemical approach to identifying microRNA targets, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 19291–19296.
[18] T. Yu, J. Li, M. Yan, L. Liu, H. Lin, F. Zhao, et al., MicroRNA-193a-3p and -5p suppress the metastasis of human non-small-cell lung cancer by downregulating the ERBB4/PIK3R3/mTOR/S6K2 signaling pathway, Oncogene 34 (2015) 413–423.
[19] H. Liang, M. Liu, X. Yan, Y. Zhou, W. Wang, X. Wang, et al., miR-193a-3p functions as a tumor suppressor in lung cancer by down-regulating ERBB4, J. Biol. Chem. 290 (2015) 926–940.
[20] X. Shi, M. Sun, H. Liu, Y. Yao, Y. Song, Long non-coding RNAs: a new frontier in the study of human diseases, Cancer Lett. 339 (2013) 159–166.
[21] P.M. Guenzl, D.P. Barlow, Macro lncRNAs: a new layer of cis-regulatory information in the mammalian genome, RNA Biol. 9 (2012) 731–741.
[22] C.H. Li, Y. Chen, Targeting long non-coding RNAs in cancers: progress and prospects, Int. J. Biochem. Cell Biol. 45 (2013) 1895–1910.
[23] P. Qi, X. Du, The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine, Mod. Pathol. 26 (2013) 155–165.
[24] M.D. Xu, P. Qi, X. Du, Long non-coding RNAs in colorectal cancer: implications for pathogenesis and clinical application, Mod. Pathol. 27 (2014) 1310–1320.
[25] W.C. Liang, W.M. Fu, C.W. Wong, Y. Wang, W.M. Wang, G.X. Hu, et al., The LncRNA H19 promotes epithelial to mesenchymal transition by functioning as MiRNA sponges in colorectal cancer, Oncotarget 6 (2015) 22513–22525.