

Long noncoding RNA UCA1 promotes cell proliferation, migration and invasion of human leukemia cells via sponging miR-126

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Abstract. – **OBJECTIVE:** Acute myeloid leukemia (AML) is a bone marrow malignancy. Long non-coding RNA (lncRNA) urothelial carcinoma-associated 1 (UCA1) plays an important role in several cancers. However, the role of lncRNA UCA1 in AML remained unclear.

MATERIALS AND METHODS: lncRNA UCA1 expressions in different cell lines were determined by RT-PCR. In human myelogenous leukemia (ML) cell lines K562 and HL60, effects of lncRNA UCA1 knockdown on cell viability, migration, invasion, and apoptosis were assessed, respectively. Binding effects between lncRNA UCA1 and microRNA (miR)-126, and between miR-126 and RAC1 3'UTR were detected by RT-PCR and luciferase activity assay. Involvements of miR-126 and RAC1 in lncRNA UCA1-mediated cell bioactivities were assessed.

RESULTS: We found that lncRNA UCA1 was upregulated in ML cell lines. Knockdown of lncRNA UCA1 inhibited cell viability, migration, invasion, and prompted apoptosis of ML cells in vitro. lncRNA UCA1 could bind with miR-126 and downregulate miR-126 expression. Simultaneously, the anti-growth and anti-metastasis actions of lncRNA UCA1 knockdown on ML cells were reversed by miR-126 suppression. RAC1 was a target gene of miR-126, and the anti-ML actions of miR-126 were abolished by RAC1 overexpression. Moreover, PI3K/AKT and JAK/STAT signaling pathways were blocked by miR-126 overexpression while were activated by RAC1 overexpression.

CONCLUSIONS: Taken together, this study elucidates a novel UCA1-miR-126-RAC1 regulatory network in ML cells, which may provide the feasibility for use lncRNA-based therapy in AML treatment.

Key Words:

Leukemia, Long noncoding RNA UCA1, microRNA-126, RAC1.

Introduction

Leukemia, also known as blood cancer, is a malignant disease caused by the hematopoi-

etic stem cell abnormalities. A large number of leukemia cells accumulate in bone marrow and other hematopoietic tissues, and infiltrate organs and tissues to inhibit the normal hematopoiesis, leading to clinical manifestations of anemia, bleeding, infection, and organ infiltration symptoms¹. Acute myeloid leukemia (AML) is a bone marrow malignancy with abnormal proliferation of bone marrow stromal cells. In AML, the rapid proliferation of abnormal myeloid cells in bone marrow affect the production of normal hematopoietic cells². AML is the most common acute leukemia affecting adults and its morbidity rises dramatically with age. Studies have shown that ionizing radiation and benzene are the most important environmental factors causing AML³. While combined chemotherapy is currently the main treatment to induce the remission of AML. Although complete remission rate for AML is near 70% with combination induction and consolidation chemotherapy, most patients will relapse or die from the treatment complications⁴. In addition to hematopoietic stem cell transplantation, there is no more effective treatment for AML. Even so, lacking of correctly matched stem cells, high difficulty of successful matching, and the high cost are still challenges for AML therapy⁵. Therefore, it is imperative to find drugs with specific targets in AML and detect the relevant mechanisms of regulation.

Long non-coding RNAs (lncRNAs), identified as non-protein-coding RNAs with a long length of over 200 nucleotides (nt), were previously regarded as “noise” in the genome⁶. Currently, studies regarding lncRNAs suggested that they were involved in many biological actions including cell growth, proliferation, etc. via regulating gene expressions⁷. Abnormally expressed lncRNAs were found in multiple cancers, and some of them were identified to be used as diagnostic indicators^{8,9}. lncRNA

urothelial carcinoma-associated 1 (UCA1) has been shown to play an important role in the progression of several cancers and others inflammatory diseases. Several scholars have reported the highly expressed lncRNA UCA1 in cancers, including breast cancer, colorectal cancer, bladder cancer, liver cancer, etc. Other researches also displayed that the expression level of lncRNA UCA1 was significantly increased in osteosarcoma and epilepsy patients^{10,11}. These reports suggested that lncRNA UCA1 might serve as a biomarker for cancers diagnosis¹²⁻¹⁶. However, the mechanism by which lncRNA UCA1 involved in tumor cell growth regulation is still unclear. To our best of knowledge, up to now, only one literature has mentioned the oncogenic activities of lncRNA UCA1 in AML¹⁷. More efforts are required to cross-check the role of lncRNA UCA1 in myelogenous leukemia (ML) cells, and to reveal the underlying mechanisms of actions.

In the present investigation, we measured the expression of lncRNA UCA1 in ML cells and detected its role in the growth of ML cells *in vitro*. Mechanism analysis revealed that lncRNA UCA1 might function as an endogenous sponge for microRNA (miR)-126, in having miR-126 exhausted and thus preventing Ras-related C3 botulinum toxin substrate 1 (RAC1) from degradation by miR-126. Our present results may provide evidence for the importance of UCA1-miR-126-RAC1 regulatory network in ML cells, shedding new light on the treatment for AML.

Materials and Methods

Cell Culture

Human chronic ML cell line K562, human AML cell line HL60, human osteosarcoma cell line MG63, human osteoblast cell line hFOB1.19 and human embryonic kidney cells HEK293 were all purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), and were cultured in the Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) in a humidified atmosphere with 5% CO₂ at 37°C. Subcultures were performed by using 0.05% trypsin solution (Sigma-Aldrich, St. Louis, MO, USA) and stable cultured cells were used for subsequent experiments.

RNA Extraction and Real-time Quantitative PCR (RT-PCR)

Total RNA of cells was extracted by using TRIzol reagent (Life Technologies Co., Carlsbad, CA, USA) according to the manufacturer's instructions and samples were purified by RNeasy Mini kit (Qiagen, Hilden, Germany). The One Step SYBR[®] PrimeScript[®] PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China) was used for the RT-PCR analysis to measure the expression levels of lncRNA UCA1. For miRNA expression assessment, the extracted RNA was reversed by using TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). TaqMan Universal Master Mix II supplemented with the TaqMan MicroRNA Assay (Applied Biosystems, Foster City, CA, USA) were used to measure the expression levels of miR-126 in cells. For RAC1 mRNA, the RNA PCR Kit (AMV) Ver.3.0 (TaKaRa Biotechnology, Dalian, China) was used. Data were analyzed according to the classic 2^{-ΔΔCt} method and were normalized to GAPDH or U6 snRNA expressions in each sample group.

Transfection and Generation of Stably Transfected Cell Lines

To knockdown lncRNA UCA1 in cells, short-hairpin RNA (shRNA) directed against human lncRNA UCA1 was ligated into the U6/GFP/Neo plasmid (GenePharma, Shanghai, China) referred as sh-UCA1. The miR-126 mimic, inhibitor and their negative control (NC) were synthesized by Life Technologies (Corporation, MD, USA). For the analysis of RAC1 function, the full-length of RAC1 sequences and shRNA directed against RAC1 were constructed in pEX-2 and U6/GFP/Neo plasmids (GenePharma, Shanghai, China) as pEX-RAC1 or sh-RAC1, respectively. The plasmids carrying non-targeting sequences of sh-UCA1 and sh-RAC1 were used as negative controls (sh-NCs). All cell transfections were performed by using Lipofectamine 3000 reagent (Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. The stably transfected cells were selected from the culture medium containing 0.5 mg/ml G418 (Sigma-Aldrich, St. Louis, MO, USA). After approximately 4 weeks, G418-resistant cell clones were established and used for the next experiments.

Cell Viability Assay

Cell viability was determined by trypan blue exclusion. In brief, 1 × 10⁵ cells were seeded

in duplicate in 60-mm dishes and cultured in DMEM culture medium in a humidified atmosphere with 5% CO₂ at 37°C. After correlation administrations, stable cultured cells at the indicated time periods were washed, and live cell numbers were determined by using Trypan Blue Staining Cell Viability Assay Kit (Beyotime Biotechnology, Beijing, China). After staining with trypan blue for 5 min, cell viability was quantified by counting after taking a photograph under the microscope (Leica Microsystems, Wetzlar, Germany).

Migration and Invasion Assay

Cell migration and invasion were determined by Transwell assay. Cell migration was determined by using a modified two-chamber with a pore size of 8 μm (BD Biosciences, San Jose, CA, USA), and BioCoat™ Matrigel™ Invasion Chambers supplemented with matrix (BD Biosciences, San Jose, CA, USA) were used for invasion assay. Briefly, 5 × 10⁴ cells suspended in 200 μl serum-free DMEM medium were added to the upper compartment of the chamber, and 600 μl of complete medium was added to the lower compartment. Then, cells were incubated at 37°C for 48 h. After that, the non-traversed cells were removed from the upper surface of the upper compartment carefully with a cotton swab. The traversed cells were fixed with 100% methanol (Thermo Fisher Scientific, Waltham, MA, USA), stained by crystal violet (Beyotime Biotechnology, Shanghai, China) and been counted microscopically. The data were presented as the average number of cells attached to the bottom surface from five randomly chosen fields.

Apoptosis Assay

Cell apoptosis analysis was performed by using Annexin V-FITC/propidium iodide (PI) Apoptosis Detection Kit (Shanghai Kaifang Biotechnology, Shanghai, China) followed by flow cytometry analysis. In brief, cells were seeded in 6-well plates (1 × 10⁵ cells/well). After transfection, cells were washed in phosphate buffered saline (PBS, Sigma-Aldrich, St. Louis, MO, USA) and were stained by 100 μl Annexin V and 4 μl of PI, that has been diluted 1:10 in 1 × Annexin V binding buffer in the presence of 50 μg/ml RNase A (Sigma-Aldrich, St. Louis, MO, USA). The stained cells were incubated for 1 h at room temperature in the dark. Treated cells were washed twice by cold PBS; flow cytometry analysis was performed by FACScan (Beckman Coulter, Full-

erton, CA, USA) to differentiate apoptotic cells (Annexin-V-positive and PI-negative) from necrotic cells (Annexin-V- and PI-positive). The data were analyzed using FlowJo software 10 (Tree Star, Ashland, OR, USA).

Western Blotting

The proteins were extracted from corresponding treated cells by using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Basel, Switzerland). Samples were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Western blot system was established by using Bio-Rad Bis-Tris Gel system (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Primary antibodies were prepared in 5% blocking buffer at a dilution of 1:1000 as follows: B cell lymphoma 2 (Bcl-2, sc509); Bax (sc20067); pro-Caspase 3 (sc7272); pro-Caspase 9 (sc17784); RAC1 (sc217); Janus kinase (JAK, sc135225); signal transducers and activators of transcription (STAT1, sc34524); p-STAT1 (sc8394); STAT2 (sc136079); p-STAT2 (sc21689) (Santa Cruz Biotechnology, Santa Cruz, CA, USA); Cleaved Caspase 3 (ab2302); Cleaved Caspase 9 (ab2324); PI3K (ab86714); p-PI3K (ab182651); AKT (ab8805); p-AKT (ab38449), p-JAK(ab32101); and GAPDH (ab8245) as an internal control (Abcam, Cambridge, MA, USA). The polyvinylidene difluoride (PVDF) membranes were incubated with corresponding primary antibodies at 4°C overnight, followed by washing and incubation with horseradish peroxidase-labeled secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. Then, membranes carried blots and antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system, and 200 μl Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA) was added to cover the membranes surface. The signals were captured and the intensity of the bands was quantified by Image Lab™ Software (Bio-Rad Laboratories, Hercules, CA, USA).

Dual-luciferase Reporter Assay

The fragment of lncRNA UCA1 and 3'UTR of RAC1 containing the predicted miR-126 binding site was amplified by PCR, respectively. Then, the fragments were cloned into a pmirGLO Dual-luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA) to form the reporter vector UCA1-wild-type (UCA1-wt) or

RAC-wt. The putative binding sites of miR-126 in the lncRNA UCA1 or RAC1 3'UTR were replaced to form the mutate reporter vector referred as UCA1-mutate-type (UCA1-mt) or RAC-mt. The reporter vectors were co-transfected into cells with miR-126 mimic or miRNA negative control, respectively. The Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) was used to measure the luciferase activity according to the manufacturer's instructions.

Statistical Analysis

All samples were run in triplicate and experiments were repeated at least three times. All data from three independent experiments were expressed as mean \pm standard deviation (SD). Statistical analyses were performed using GraphPad statistical software (GraphPad Software, San Diego, CA, USA). The p -values were calculated using Student's t -test for two groups or one-way ANOVA followed by Bonferroni test for more than two groups. p -value of < 0.05 was considered as statistically significant.

Results

LncRNA UCA1 Was Highly Expressed in ML Cell Lines

Firstly, we detected the expressions of lncRNA UCA1 in several kinds of cell lines including K562, HL60, MG63, hFOB1.19, and HEK293. Results in Figure 1 showed that lncRNA UCA1 expression levels in human chronic ML cell line K562 and AML cell line HL60 were higher than those in other cell lines ($p < 0.01$). These data suggested the high expression of lncRNA UCA1 in ML cells.

LncRNA UCA1 Knockdown Inhibited Cell Viability, Migration, and Invasion, While Promoted Apoptosis of ML Cells

To explore the effects of lncRNA UCA1 on ML cells, two different sequences of shRNAs specific for lncRNA UCA1 (sh-UCA1 #1 and sh-UCA1 #2) were transfected into K562 and HL60 cells, respectively. RT-PCR analytical results in Figure 2A suggested that sh-UCA1 transfection significantly decreased the expressions of lncRNA UCA1 in both K562 and HL60 cells when compared to shNC transfection ($p < 0.05$, $p < 0.01$, or $p < 0.001$). Since sh-UCA1 #2 resulted in a more excellent silencing effect in lncRNA

UCA1 expression than sh-UCA1 #1, sh-UCA1 #2 was selected for use in the next determinations.

Cell viability assay results in Figure 2B showed that sh-UCA1 transfection significantly decreased cell viability of both K562 and HL60 cells compared with the shNC group ($p < 0.01$). In addition, migration and invasion assay results in Figure 2C and 2D showed that sh-UCA1 transfection significantly inhibited cell migration and invasion in both two cell lines when compared with shNC ($p < 0.05$). Meanwhile, in Figure 2E, flow cytometry analytical results showed that the apoptotic cell rates in sh-UCA1 transfected groups were increased in K562 and HL60 cell lines compared with shNC groups ($p < 0.05$ or $p < 0.01$). Western blot assay results showed that in the sh-UCA1 transfected groups, Bcl-2 expression was decreased, while expressions of Bax, cleaved Caspase 3, and cleaved Caspase 9 were all increased (Figure 2F), suggesting that cell apoptosis was promoted by sh-UCA1 transfection. These results revealed the anti-growth and anti-metastasis activities of lncRNA UCA1 knockdown on K562 and HL60 cells *in vitro*.

LncRNA UCA1 Worked as a Sponge for miR-126

We found that miR-126 expression was increased in lncRNA UCA1 knockdown cells when compared with that in shNC group ($p < 0.001$,

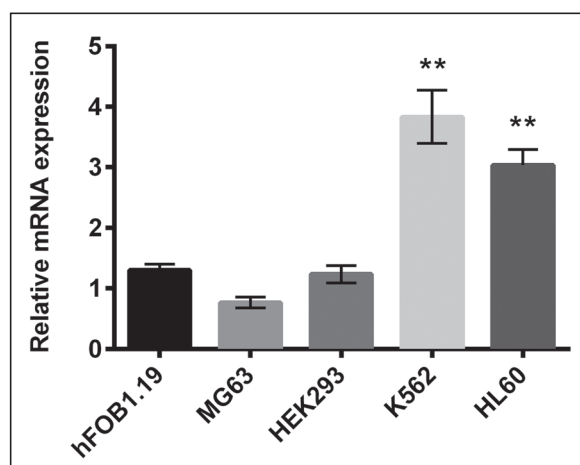


Figure 1. LncRNA UCA1 was highly expressed in human myelogenous leukemia (ML) cell lines. RT-PCR was performed to detect the expressions of lncRNA UCA1 in human chronic ML cell line K562, human acute ML cell line HL60, human osteosarcoma cell line MG63, human osteoblast cell line hFOB1.19, and human embryonic kidney cells HEK293. **, $p < 0.01$.

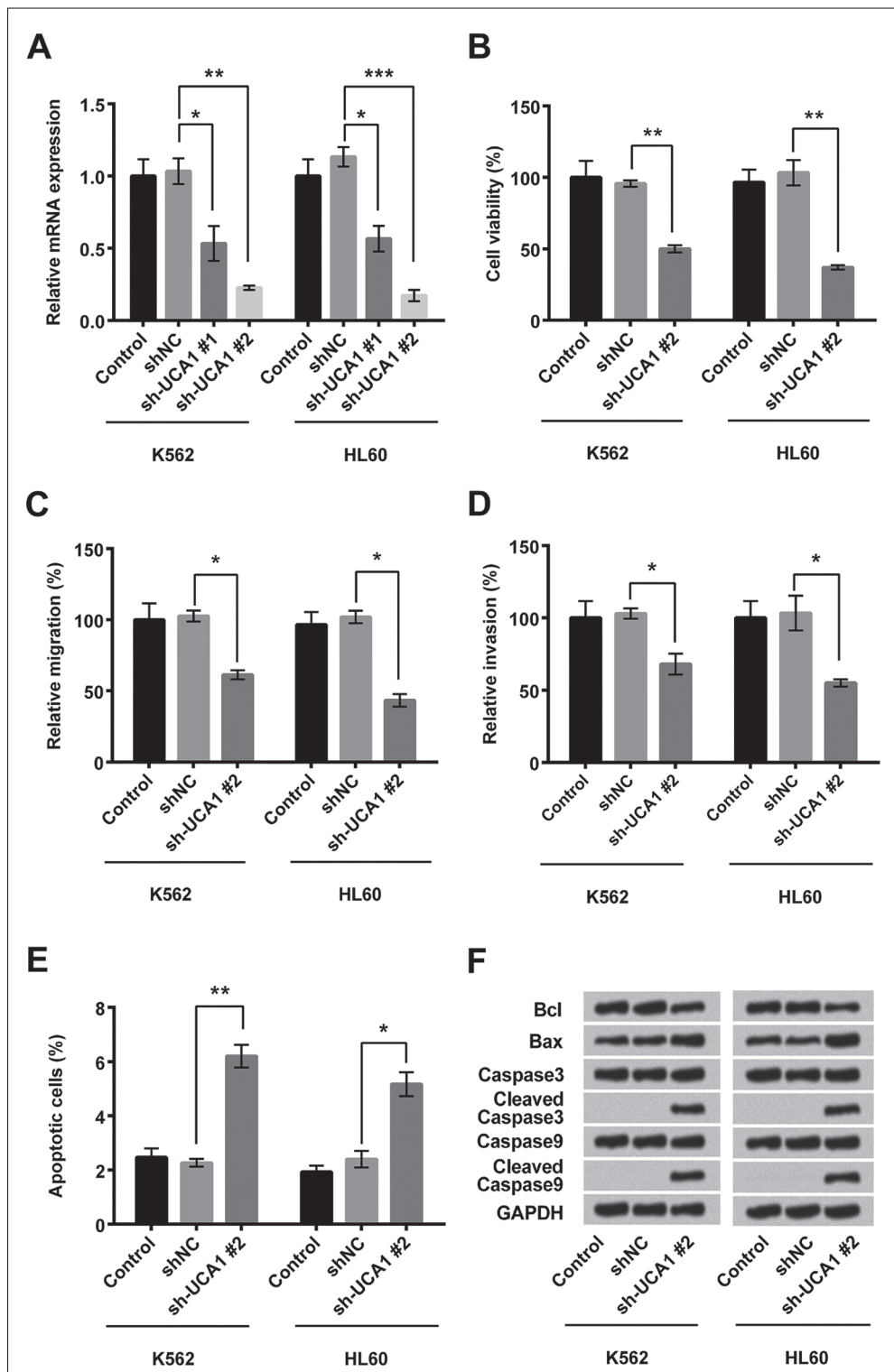


Figure 2. LncRNA UCA1 knockdown inhibited cell viability, migration, invasion and promoted apoptosis of myelogenous leukemia (ML) cells. K562 and HL60 cells were transfected with two different sequences of shRNAs specific for lncRNA UCA1 (sh-UCA1 #1 and sh-UCA1 #2) or a negative control (shNC). **(A)** Relative lncRNA UCA1 expressions after sh-UCA1 or shNC transfections were detected by RT-PCR. **(B)** Cell viability was detected by trypan blue exclusion. Relative **(C)** migration and **(D)** invasion of transfected cells were assessed by transwell assay. **(E)** Apoptotic cell rates were analyzed by flow cytometry. **(F)** Western blotting was performed to measure the expressions of apoptosis-related factors. GAPDH acted as internal control. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 3A). To assess the regulation between lncRNA UCA1 and miR-126, luciferase assay was performed. Results in Figure 3B showed that co-transfection with miR-126 mimic significantly decreased luciferase activity of lncRNA UCA1-wt group ($p < 0.01$), while there was no significant effect on the lncRNA UCA1-mt groups. These results suggested that lncRNA UCA1 and miR-126 might negatively regulate expressions of each other via binding effect.

lncRNA UCA1 Regulated the Cell Viability, Migration, Invasion, and Apoptosis of ML Cells Via Sponging miR-126

Next, we detected the involvement of miR-126 in lncRNA UCA1-mediated bioactivities in ML cells. To test this, lncRNA UCA1 and miR-126 were simultaneously silenced by transfection. Cell viability assay results in Figure 4A showed that cell viability of sh-UCA1 transfected cells was decreased compared with negative control ($p < 0.05$). But, miR-126 inhibitor transfection reversed this inhibitory effect that cell viability was elevated compared with sh-UCA1 transfection alone ($p < 0.05$). Migration and invasion assay results in Figure 4B and 4C showed that miR-126 inhibitor transfection increased the inhibited migration and invasion of K526 and HL60 cells ($p < 0.05$). Figure 4D showed that the apoptotic cell rates were increased after lncRNA UCA1 knock-down compared with negative control ($p < 0.05$), while were significantly decreased in miR-126

inhibitor co-transfected groups compared with sh-UCA1 transfection alone ($p < 0.05$). Western blotting results in Figure 4E suggested that sh-UCA1 transfection decreased expression of Bcl-2, whereas co-transfection with miR-126 inhibitor increased Bcl-2 expression in both K562 and HL60 cells. Expressions of Bax, cleaved Caspase 3, and cleaved Caspase 9 were all decreased by miR-126 inhibitor compared with sh-UCA1. These data evidenced that knockdown of lncRNA UCA1 inhibited ML cells growth and metastasis might be via the upregulation of miR-126.

RAC1 Was a Direct Target of miR-126

As shown in Figure 5A, we found that after miR-126 mimic transfection, RAC1 expression was decreased compared with scramble control ($p < 0.05$), and miR-126 inhibitor transfection increased expression of RAC1 compared with negative control ($p < 0.05$). These findings displayed that the expression of miR-126 was negatively related to RAC1 mRNA expression. Western blot analysis results in Figure 5B showed that miR-126 negatively regulated protein expression level of RAC1. Then, we assessed the targeting effect of miR-126 on 3'UTR of RAC1. Luciferase activity assay results in Figure 5C showed that in RAC-wt group, miR-126 mimic significantly decreased luciferase activity compared with negative control ($p < 0.01$), while there was no significant effect on RAC1-mt group. These results suggested that RAC1 was a direct target of miR-126.

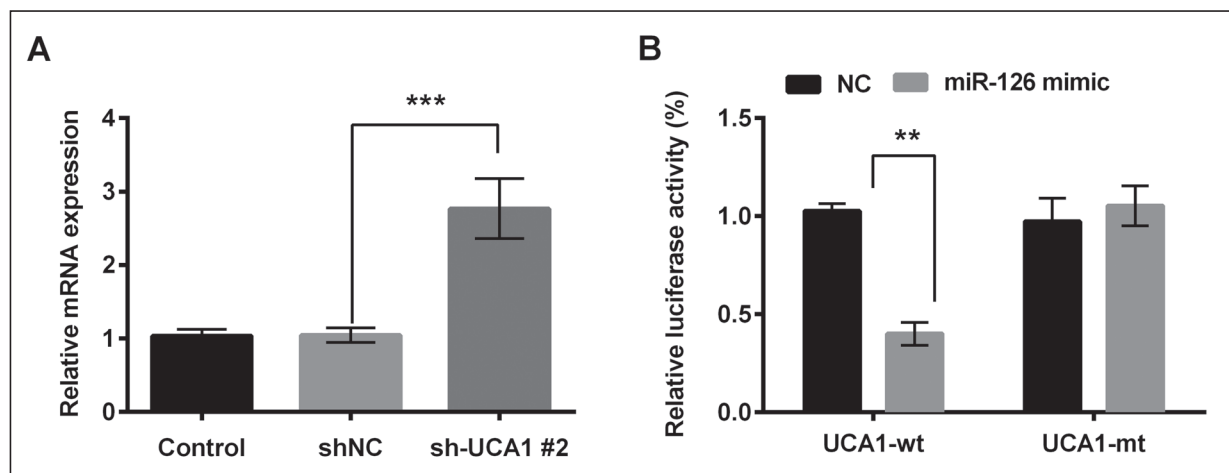
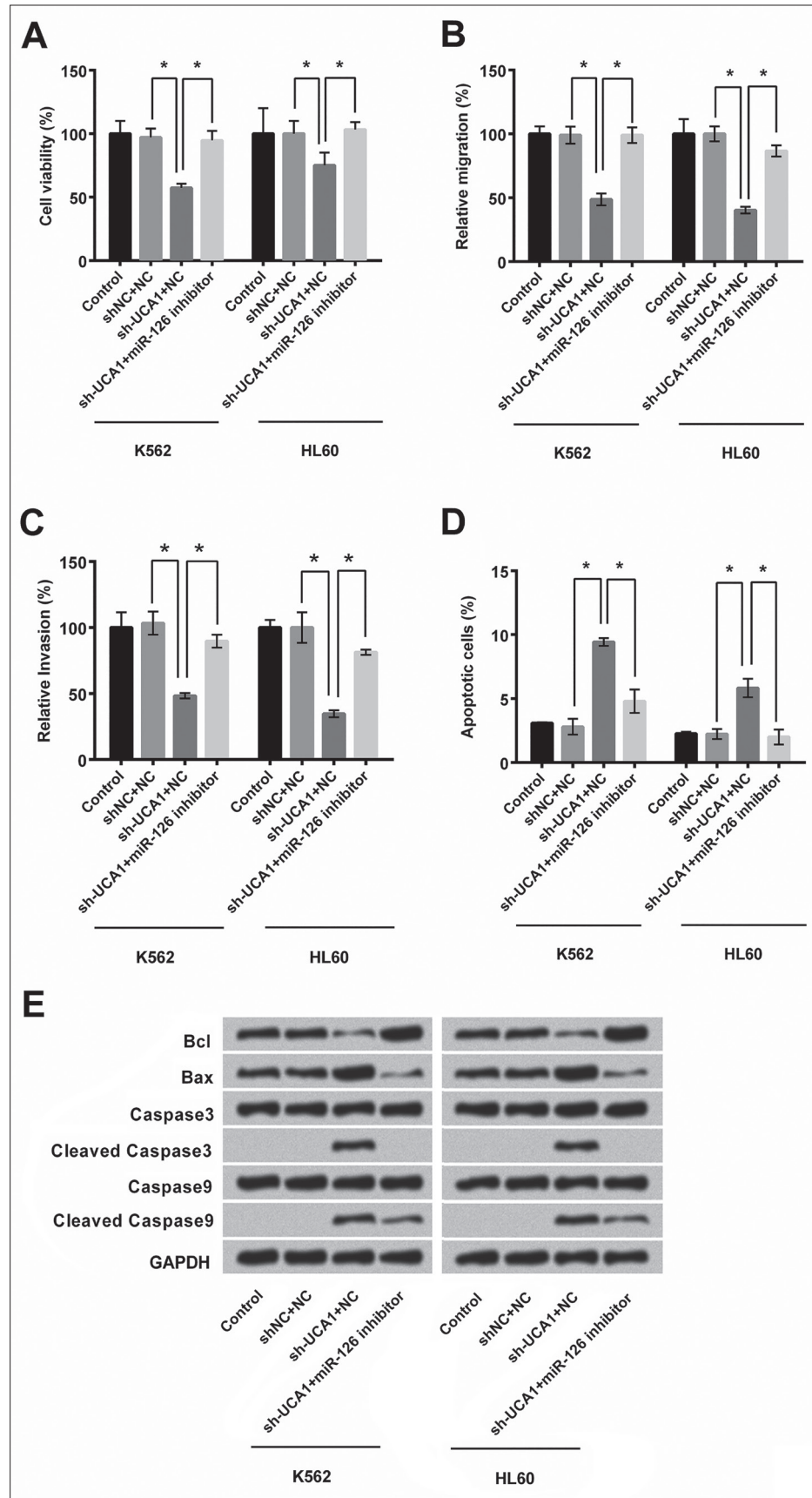


Figure 3. lncRNA UCA1 negatively regulated miR-126 expression via binding effect. **(A)** Relative miR-126 expressions after sh-UCA1 or shNC transfections were detected by RT-PCR. **(B)** Relative luciferase activity after co-transfected with UCA1-wild type (wt)/mutant type (mt) and miR-126 mimic was measured by Dual-luciferase assay. **, $p < 0.01$; ***, $p < 0.001$.

Figure 4. LncRNA UCA1 regulated the cell viability, migration, invasion, and apoptosis of myelogenous leukemia (ML) cells via sponging miR-126. K562 and HL60 cells were co-transfected with sh-UCA1, and miR-126 inhibitor. shNC and miR-126 negative control (NC) were transfected into cells as blank controls. **(A)** Cell viability was detected by trypan blue exclusion. Relative **(B)** migration and **(C)** invasion of transfected cells was assessed by transwell assay. **(D)** Apoptotic cell rates were analyzed by flow cytometry. **(E)** Western blotting was performed to measure expressions of apoptosis-related factors. GAPDH acted as internal control. *, $p < 0.05$.



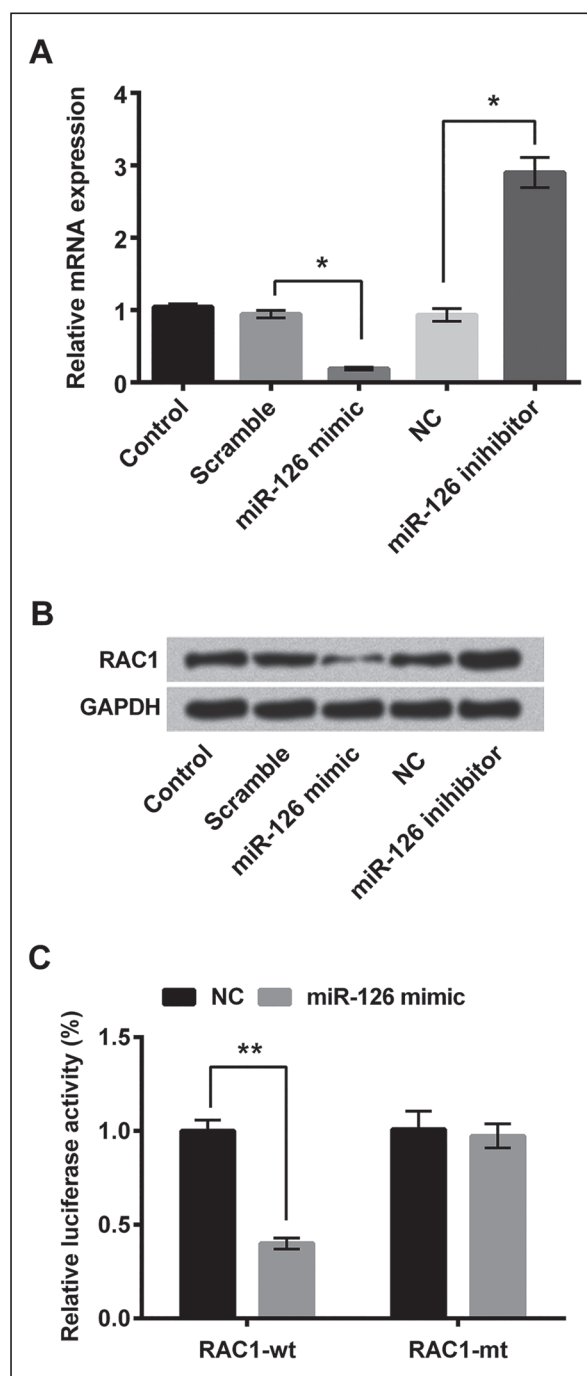


Figure 5. RAC1 was a direct target of miR-126. **(A)** Relative mRNA expression of RAC1 was detected by RT-PCR after transfections with miR-126 mimic or miR-126 inhibitor. Scramble control and miRNA negative control (NC) were transfected into cells as the black controls for miR-126 mimic and miR-126 inhibitor, respectively. **(B)** Western blotting was performed to measure protein expression of RAC1 after miR-transfection. GAPDH acted as internal control. **(C)** Relative luciferase activity after co-transfected with RAC1-wild type (wt)/mutant type (mt) and miR-126 mimic was measured by Dual-luciferase assay. *, $p < 0.05$; **, $p < 0.01$.

miR-126 Regulated the Cell Viability, Migration, Invasion, and Apoptosis of ML Cells Via Regulating RAC1 Expression

To assess the effect of RAC1 on leukemia cells *in vitro*, RAC1 was overexpressed in miR-126 mimic transfected cells. RT-PCR analytical results in Figure 6A showed that miR-126 mimic transfection decreased the mRNA expression of RAC1 compared with negative control ($p < 0.05$). While, in miR-126 mimic + pEX-RAC1 group, RAC1 was highly expressed in both K562 and HL60 cells compared with miR-126 mimic transfection alone ($p < 0.05$). We further examined whether RAC1 was involved in the regulatory effect of miR-126 on ML cells *in vitro*. Cell viability assay results in Figure 6B showed that miR-126 mimic transfection inhibited cells viability compared with negative control ($p < 0.05$), while co-transfection with pEX-RAC1 elevated cell viability compared with miR-126 mimic transfection alone ($p < 0.05$). Migration and invasion assay results in Figure 6C and 6D showed that overexpression of RAC1 in miR-126 mimic transfected cells promoted migration and invasion ($p < 0.05$). Meanwhile, flow cytometry analysis results in Figure 6E showed that overexpression of RAC1 in miR-126 mimic transfected cells inhibited cell apoptosis ($p < 0.001$ or $p < 0.05$). Western blotting results in Figure 6F showed that protein expression of Bcl-2 was increased by RAC1 overexpression, while Bax, cleaved Caspase 3, and cleaved Caspase 9 were all decreased after RAC1 overexpression. These results suggested that RAC1 was involved in the anti-growth and anti-metastasis effects of miR-126 on K562 and HL60 cells *in vitro*.

miR-126 Deactivated PI3K/AKT and JAK/STAT Signaling Pathways Via Negative Regulation of RAC1

The phosphorylation levels of main factors associated with PI3K/AKT and JAK/STAT signaling pathways were measured by Western blotting to explore the underlying mechanisms of which miR-126 exhibited anti-tumor actions. Results in Figure 7A showed that the expression levels of p-PI3K and p-AKT were all decreased in miR-126 mimic transfected cells, while overexpression of RAC1 restored these downregulations. Figure 7B showed that expressions of p-JAK, p-STAT1, and p-STAT2 were all decreased in miR-126 mimic transfected cells, and overexpression of RAC1 increased these phosphorylated factor expressions. These results

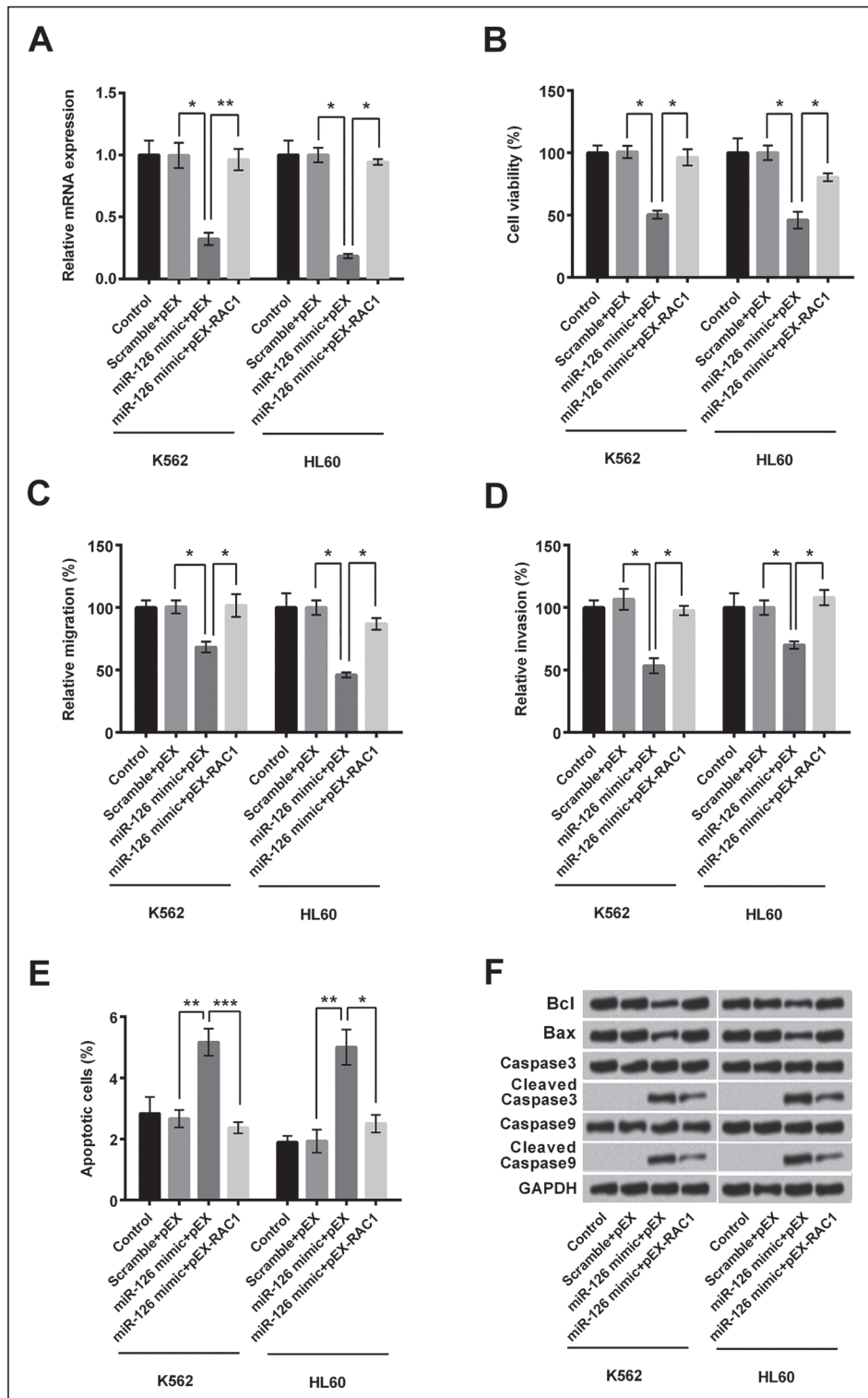


Figure 6. miR-126 regulated the cell viability, migration, invasion and apoptosis of myelogenous leukemia (ML) cells via regulating RAC1 expression. K562 and HL60 cells were co-transfected with miR-126 mimic and pEX-RAC1 overexpression vector. Scrambled oligonucleotide and the pEX empty vector were transfected as blank controls. **(A)** Relative mRNA expression level of RAC1 was detected by RT-PCR. **(B)** Cell viability was detected by trypan blue exclusion. Relative **(C)** migration and **(D)** invasion of transfected cells was assessed by transwell assay. **(E)** Apoptotic cell rates were analyzed by flow cytometry. **(F)** Western blotting was performed to measure expressions of apoptosis-related factors. GAPDH acted as internal control. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

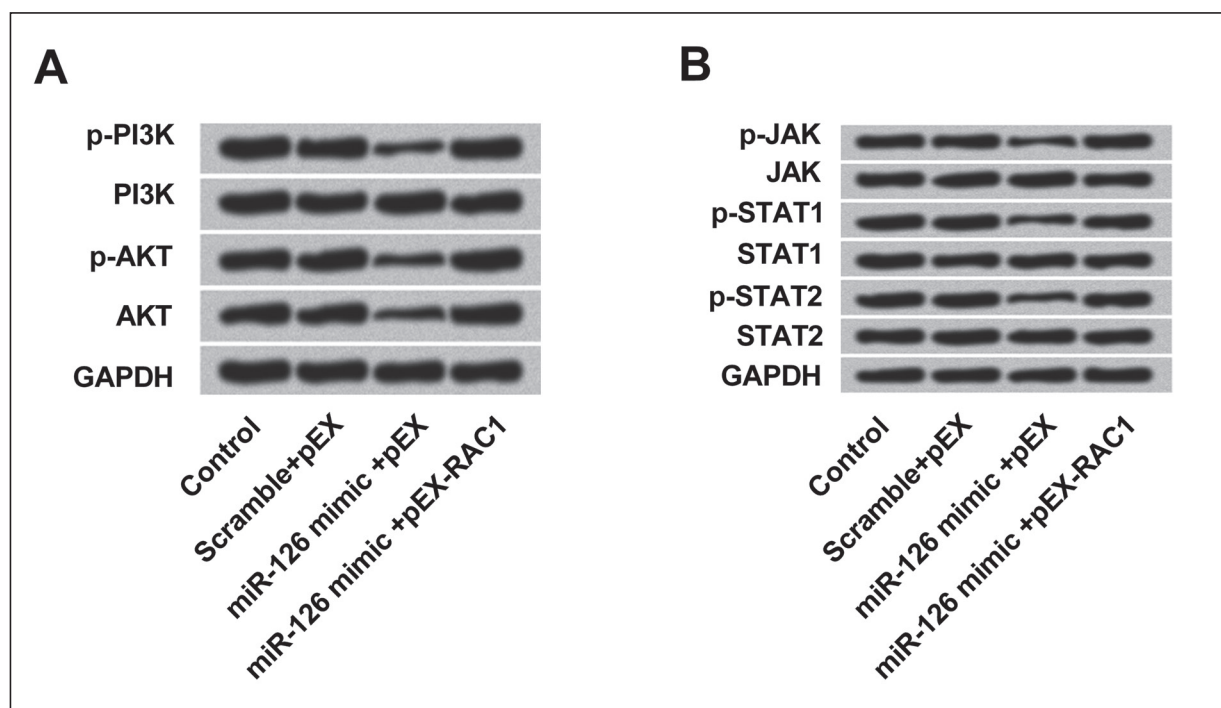


Figure 7. miR-126 deactivated PI3K/AKT and JAK/STAT signaling pathways via RAC1. K592 and HL60 cells were co-transfected with miR-126 mimic and pEX-RAC1. Scrambled oligonucleotide and the pEX empty vector were transfected as blank controls. Western blotting was performed to measure expressions of **(A)** PI3K, phosphorylated- (p-) PI3K, AKT, p-AKT, and **(B)** JAK, p-JAK, STAT1/2 and p-STAT1/2. GAPDH acted as internal control.

suggested that miR-126 repressed the activation of PI3K/AKT and JAK/STAT signaling pathways via regulating RAC1 expression.

Discussion

At present, chemotherapy is the main treatment for leukemia, while this method has many serious side effects. Clinical treatment of leukemia may fail due to treatment-related death and drug resistance¹⁸. Since the prognosis of AML is still poor¹⁹, more efforts are required to develop novel therapies. LncRNAs, which lack of protein-coding potential, can regulate protein-coding genes at epigenetic, transcriptional and post-transcriptional levels. They also play central roles in various of physiological processes⁷. In this study, the results demonstrated that lncRNA UCA1 was highly expressed in AML cell line HL60 as well as chronic ML cell line K562, suggesting the abnormal expression of lncRNA UCA1 in leukemia cells.

Increasing evidence suggested that a variety of lncRNAs are frequently aberrantly expressed

in cancers, exhibiting spatially and temporally regulated expression patterns²⁰. The aberrantly expressed lncRNAs are closely related to tumorigenesis, metastasis, prognosis, and diagnosis, and serve as oncogenes or and tumor suppressive genes^{21,22}. More efforts should be made to deeply clarify the biological and molecular mechanisms of lncRNAs in leukemia. In this work, we detected the regulatory effect of lncRNA UCA1 on the growth of ML cells by *in vitro* experiments. Our findings suggested that knockdown of lncRNA UCA1 inhibited cell viability and metastasis while prompted apoptosis of ML cells. The results demonstrated that lncRNA UCA1 could modulate the activities of ML cells, and lncRNA UCA1 has great potential to serve as a therapeutic target for the treatment of AML.

Although majority of lncRNAs have been shown to play important roles in human diseases, the precise molecular mechanisms of lncRNAs in modulating tumor growth remain largely unknown. We found that lncRNA UCA1 negatively regulated the expression of miR-126 via binding effect between each other. The

miRNAs are a class of small non-coding RNAs that bind to the 3'UTR of mRNAs, thereby inhibiting mRNAs translation or promoting mRNAs degradation²³. Mounting evidence showed that miRNAs play a central role in the regulation of cell development, differentiation, proliferation and apoptosis²⁴. lncRNAs have been found to act as miRNA sponges or miRNA inhibitors in having miRNAs exhausted, and modulated the expression of miRNA target genes at the level of post-transcriptional regulation²⁵. Researches have shown that thousands of lncRNAs possessed cell type-, tissue type-, developmental stage- and disease-specific expression patterns and localization, suggesting that individual lncRNA may be the potent natural miRNA sponges in certain conditions²⁶. In this report, we provided evidence of lncRNA UCA1 as a sponging molecular for miR-126, linking lncRNA-miRNA post-transcriptional regulation network in AML.

The miRNAs play an important role in hematopoietic differentiation and have been shown to be closely related to leukemia. Genome-wide analyses of miRNA expression have revealed signatures associated with selected cytogenetic and molecular subsets of AML²⁷. For instance, miR-34 expression was decreased, and miR-34 could inhibit the clonogenic expansion and metastasis of AML²⁸. It was found that expression of miR-126 was higher in hematopoietic stem cells derived from AML patients' bone marrow specimens than in healthy controls²⁹. But the researches regarding the exact role of miR-126 in AML were limited. This work found that miR-126 expression was negatively regulated by lncRNA UCA1 in ML cells. Then, miR-126 upregulation suppressed cell viability, migration, invasion and promoted cell apoptosis *in vitro*. Taken together, these data indicated that lncRNA UCA1 served as an endogenous sponge for miR-126, and miR-126 involved in lncRNA UCA1-mediated anti-AMI actions might be via binding with lncRNA UCA1.

To investigate whether lncRNA UCA1-induced the reduction of miR-126 was resulting in a derepression of its target genes, we particularly focused on the miR-126 target gene RAC1 for further studies. RAC1 is one family member of Rho-small guanosine triphosphates (GTPase), which is a pivotal signaling of biological processes, such as proliferation, apoptosis, survival, cell polarity, cell adhesion

and the plasticity of cell migration³⁰. It is not surprising that RAC1 plays important roles in tumor progression³¹. RAC1 has been implicated in CAF-mediated remodeling of tumor microenvironment in a manner that enhances cancer cell migration and invasion^{32,33}. In our studies, results demonstrated that RAC1 was a direct target of miR-126. The anti-growth and anti-metastasis actions of miR-126 in ML cells were alleviated or even abolished by RAC1 overexpression. Together with the interactions between lncRNA UCA1 and miR-126 which we discussed above, we herein briefly conclude that UCA1 exhibited oncogenic activities in AML might be via sponging miR-126, leaving RAC1 alone without being decay by miR-126. The present discoveries of lncRNA UCA1 have disclosed a new layer of complexity underlying the regulation of gene expression in AML.

In addition, results of the present paper further revealed that RAC1 could facilitate the activation of PI3K/AKT and JAK/STAT signaling pathways. Wang et al³⁴ demonstrated that RAC1/AKT signaling pathway was involved in the regulation of tumorigenicity. RAC1 has been implicated in the regulation of STAT activation, thereby contributing to inflammatory signaling³⁵. In this work, we elucidated that miR-126 contributed in anti-AML by targeting RAC1 and thus modulation of PI3K/AKT and JAK/STAT signaling pathways.

Conclusions

In summary, we highlighted lncRNA UCA1 as a potential biomarker for human AML. Functional analyses suggested pro-growth and pro-metastasis functions of lncRNA UCA1 in the progress of AML. Mechanism analyses revealed the oncogenic roles of lncRNA UCA1 might be realized by sponging miR-126, leaving RAC1 alone without being decay, and leading to the activation of PI3K/AKT and JAK/STAT signaling pathways. More efforts are needed to further elucidate the function and critical mechanisms of AML-specific lncRNAs in the progression of AML. More researches on lncRNA UCA1 might undoubtedly enhance our understanding of the pathogenesis and development of AML, and ultimately facilitate the lncRNA-directed diagnosis and therapy for this fatal disease.

Acknowledgements

Thanks for the help of Associate Professor Dong Yang, Blood Disease Hospital, Chinese Academy of Medical Sciences.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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