

Research Article

# LncRNA UCA1 elevates the resistance of human leukemia cells to daunorubicin by the PI3K/AKT pathway via sponging miR-613

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Acute leukemia is a hematological malignant tumor. Long non-coding RNA urothelial cancer-associated 1 (UCA1) is involved in the chemo-resistance of diverse cancers, but it is unclear whether UCA1 is associated with the sensitivity of acute leukemia cells to daunorubicin (DNR). DNR (100 nM) was selected for functional analysis. The viability, cell cycle progression, apoptosis, and invasion of treated acute leukemia cells (HL-60 and U-937) were evaluated by cell counting kit-8 (CCK-8) assay, flow cytometry assay, or transwell assay. Protein levels were detected with Western blot analysis. Expression patterns of UCA1 and miR-613 were assessed by quantitative real-time polymerase chain reaction (qRT-PCR). The relationship between UCA1 and microRNA-613 (miR-613) was verified by dual-luciferase reporter assay. We observed that UCA1 expression was elevated in HL-60 and U-937 cells. DNR constrained viability, cell cycle progression, invasion, and facilitated apoptosis of HL-60 and U-937 cells in a dose-dependent manner, but these impacts mediated by DNR were reverted after UCA1 overexpression. MiR-613 was down-regulated in HL-60 and U-937 cells, and UCA1 was verified as a miR-613 sponge. MiR-613 inhibitor reversed DNR treatment-mediated effects on viability, cell cycle progression, apoptosis, and invasion of HL-60 and U-937 cells, but these impacts mediated by miR-613 inhibitor were counteracted after UCA1 inhibition. Notably, the inactivation of the PI3K/AKT pathway caused by DNR treatment was reversed after miR-613 inhibitor introduction, but this influence mediated by miR-613 inhibitor was offset after UCA1 knockdown. In conclusion, UCA1 up-regulation facilitated the resistance of acute leukemia cells to DNR via the PI3K/AKT pathway by sponging miR-613.

## Introduction

Acute leukemia is a malignant clonal disease of hematopoietic stem/progenitor cells, which includes acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) [1]. The treatment of acute leukemia includes chemotherapy, immunotherapy, radiotherapy, targeted therapy, or stem cell therapy [2]. Although these treatments improve the prognosis of patients with acute leukemia, chemotherapy resistance remains a major problem [3,4].

Long non-coding RNAs (lncRNAs) are a kind of non-protein encoding RNAs that exert crucial regulatory roles in gene regulatory networks [5]. Studies have revealed that lncRNAs are involved in many pathological and physiological processes, such as tumorigenesis, organogenesis, cell lineage choice, and tissue homeostasis [6–9]. Increased researches have revealed that lncRNAs are associated with tumor chemo-resistance [10]. Long non-coding RNA urothelial cancer-associated 1 (UCA1) has been reported to be involved in the occurrence and progression of multiple tumors [11]. Mounting researches have

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pointed out that UCA1 participates in the chemo-resistance in a series of tumors. For instance, UCA1 exerts a promoting influence on cisplatin resistance in oral squamous cell cancer [12] and bladder cancer [13]. Also, UCA1 up-regulation elevates the resistance of ovarian cancer cells to paclitaxel [14]. Furthermore, UCA1 also facilitates the progression of chronic myeloid leukemia and AML [15,16]. Additionally, UCA1 is associated with Adriamycin resistance in pediatric AML [17]. Nevertheless, the mechanism by which UCA1 regulates daunorubicin (DNR) resistance in acute leukemia remains unclear.

MicroRNAs (miRNAs) are a large class of non-coding RNAs that generally bind to mRNAs to cause translation inhibition or degradation [18]. Researchers have reported that miRNAs play vital roles in intracellular transformation, benign and malignant states, and the progression of cancer [11,19]. MicroRNA-613 (miR-613) has been revealed to play a repressive role in a range of tumors, such as osteosarcoma [20], colorectal cancer [21], and glioma [22]. Moreover, miR-613 increase cisplatin sensitivity in gastric cancer [23]. However, the role of miR-613 in DNR resistance in acute leukemia is unclear.

Consequently, we aimed to survey the function and regulatory mechanism of UCA1 in DNR resistance in acute leukemia.

## Materials and methods

### Cell culture and treatment

Human bone marrow stromal cells HS-5 and two acute leukemia cell lines HL-60 and U-937 were bought from American Type Culture Collection (Manassas, VA, U.S.A.) and cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Thermo Fisher Scientific, Waltham, MA, U.S.A.) supplemented with fetal bovine serum (FBS, 10%, HyClone, Logan, UT, U.S.A.) and streptomycin/penicillin (1%, HyClone) in an incubator with 5% CO<sub>2</sub> at 37°C.

For DNR treatment, HL-60 and U-937 cells were cultured in a complete medium supplemented with different doses of DNR (0, 25, 50, and 100 nM) (Sigma, Louis, Missouri, U.S.A.). Moreover, DNR (100 nM)-treated HL-60 and U-937 cells were used for subsequent research.

### Cell transfection

The sequence of UCA1 was cloned into the empty pCDNA3.0 vector (pCDNA3.0-NC) (Invitrogen, Carlsbad, CA, U.S.A.) to construct the overexpression vector of UCA1 (pCDNA3.0-UCA1). Small interference RNA (siRNA) targeting UCA1 (si-UCA1) and its negative control (si-NC) were synthesized by GenePharma (Shanghai, China). MiR-613 mimic (miR-613), scrambled mimic control (miR-NC), miR-613 inhibitor (Anti-miR-613), and scrambled inhibitor control (Anti-miR-NC) were synthesized by GenePharma. Transient transfection was executed using HiPerFect Transfection Reagent (Qiagen, Hilden, Germany). Oligonucleotide sequences were displayed as shown below: si-NC (5'-GCGCGATAGCGCGAATATA-3'), si-UCA1 (5'-GGACAACAGUACACGCAUA-3'), miR-NC (5'-UUCUCCGAACGUGUCACGUTT-3'), miR-613 (5'-AGGAAUGUCCUUCUUGCC-3'), Anti-miR-NC (5'-AUCCGUAGGCGUUAGCCUAU-3'), and Anti-miR-613 (5'-GGCAAAGAAGGAACAUCCT-3').

### Cell counting kit-8 (CCK-8) assay

The viability of HL-60 and U-937 cells was evaluated by CCK-8 (Dojindo Laboratories, Tokyo, Japan). In short, HL-60 and U-937 cells ( $1 \times 10^4$ ) were cultured in RPMI-1640 medium supplemented with DNR for 24, 48, and 72 h. Then, the CCK-8 solution (10  $\mu$ l) was added to each well and incubated for 2 h. The color reaction at 450 nm was measured using Microplate Absorbance Reader (Thermo Fisher Scientific).

### Flow cytometry assay

The apoptosis of HL-60 and U-937 cells with or without DNR treatment was analyzed with the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (BD Biosciences, San Jose, CA, U.S.A.). In brief, HL-60 and U-937 cells were cultured in RPMI-1640 medium supplemented with DNR for 48 h. After washing, HL-60 and U-937 cells ( $3 \times 10^5$ ) were re-suspended in binding buffer (100  $\mu$ l). Then, the cells were stained with Annexin V-FITC (5  $\mu$ l) and PI (100  $\mu$ l) and incubated for 30 min in the dark. In the end, the cells were analyzed using the FACScan flow cytometry (BD Biosciences).

### Transwell assay

The invasion ability of HL-60 and U-937 cells with or without DNR treatment was assessed by transwell assay. In brief, the cell medium containing HL-60 and U-937 cells ( $1 \times 10^5$ ) were added to the top chamber (8  $\mu$ m, BD Biosciences)

coated with matrigel matrix (BD Biosciences). Contemporaneously, RPMI-1640 medium with FBS (10%) were added to the down chamber. After culture for 24 h, the cells in the lower chamber were counted by the CCK-8 assay.

## Western blot analysis

Collected HL-60 and U-937 cells were lysed in RIPA lysis buffer (Thermo Fisher Scientific) and then centrifuged for 10 min ( $13,000 \times g$ ,  $4^{\circ}\text{C}$ ). Subsequently, the supernatants were collected and quantified with a BCA Protein Assay Kit (Pierce, Holmdel, NJ, U.S.A.). About 30  $\mu\text{g}$  total protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (10%, SDS-PAGE). Afterward, the isolated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, U.S.A.) and then blocked with Tris Buffered Saline Tween (TBST) buffer with 5% skim milk. The PVDF membranes were then incubated with primary antibodies: anti-cyclin D1 (1:200, ab16663), anti-Vimentin (1:1000, ab137321), anti-Cleaved-caspase-3 (Cleaved-casp-3) (1:500, ab32042), anti-phosphatidylinositol 3-kinase (PI3K) (1:1000, ab32089), anti-phosphorylated PI3K (p-PI3K) (1:500, ab278545) anti-protein kinase B (AKT) (1:10,000, ab179463), anti-phosphorylated-AKT (p-AKT) (1:1000, ab192623), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:2500, ab9485). Next, the membranes were incubated with goat anti-rabbit or mouse IgG. GAPDH was regarded as a loading control. All antibodies used in the present study were obtained from Abcam (Cambridge, MA, U.S.A.). The bands were visualized through the ImageJ software of National Institutes of Health (Bethesda, MD, U.S.A.).

## Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of HL-60 and U-937 cells was extracted through TRIzol reagent (Thermo Fisher Scientific). About 1  $\mu\text{g}$  total RNA was reverse transcribed into complementary DNA using Moloney Murine Leukemia Virus (M-MLV) First Strand Kit (Life Technologies, Grand Island, NY, U.S.A.) or MiRNA Reverse Transcription kit (Life Technologies). qPCR was executed using 0.1  $\mu\text{g}$  complementary DNA and the SYBR Fast qPCR Mix (Thermo Fisher Scientific). The primer sequences were presented as below: UCA1: 5'-TCGGGTAACCTTACGGT-3' (F) and 5'-GGTCCATTGAGGCTGTAG-3' (R); GAPDH: 5'-GATTCCACCCATGGCAAATTC-3' (F) and 5'-TCGCTCCTGGAAGATGGTGAT-3' (R); miR-613: 5'-GCGCGAGGAATGTTTCCTTC-3' (F) and 5'-AGTGCAGGGTCCGAGGTATT-3' (R) as well as U6 small nuclear RNA (snRNA): 5'-CTCGCTTCGGCAGCAC-3' (F) and 5'-AACGCTTCACGAATTTGCGT-3' (R). GAPDH or U6 snRNA was used as an internal control for UCA1 and miR-613. The levels of UCA1 and miR-613 were calculated by the  $2^{-\Delta\Delta\text{Ct}}$  method.

## Dual-luciferase reporter assay

The potential binding sites between UCA1 and miR-613 were predicted by the starbase, LncBase and mircode databases. The sequences of wild-type (WT) UCA1 (with predicted miR-613 binding sites) and mutant (MUT) UCA1 were synthesized and inserted into the pGL3-control vector (Promega, Madison, WI, U.S.A.). After that, A luciferase reporter vector were cotransfected into HL-60 and U-937 cells together with miR-NC or miR-613. A dual-luciferase reporter assay kit (Promega) was employed to analyze the luciferase activities.

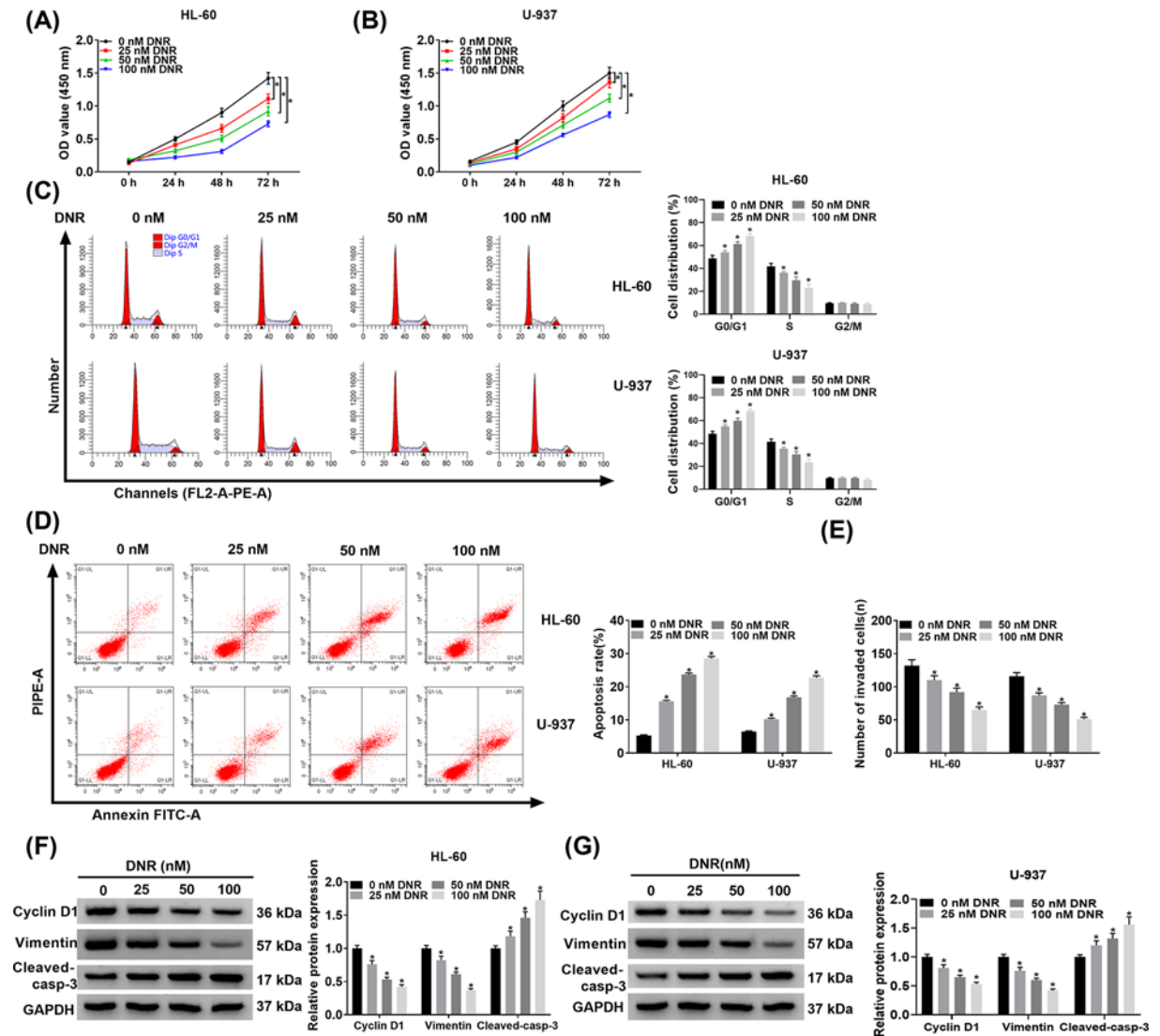
## Statistical analysis

Data derived from at least three independent experiments were presented as mean  $\pm$  standard deviation. GraphPad Prism 6.0 (GraphPad, San Diego, CA, U.S.A.) and SPSS 18.0 software (SPSS, Chicago, IL, U.S.A.) were utilized for statistical analysis.  $P < 0.05$  was deemed to indicate a statistically significant difference. The differences between two or among more groups were analyzed through Student's *t* test or one-way analysis of variance (ANOVA).

## Results

### DNR repressed viability, cell cycle progression, invasion, and triggered apoptosis of HL-60 and U-937 cells

To survey the effects of DNR on malignant behaviors of HL-60 and U-937 cells, we first performed CCK-8 assay to assess the viability of HL-60 and U-937 cells treated with different DNR doses (0, 25, 50, and 100 nM). Compared with the control group, DNR treatment suppressed the viability of HL-60 and U-937 cells with the enhancement of DNR dose (Figure 1A,B). Flow cytometry assay manifested that DNR treatment promoted cell cycle arrest and apoptosis of HL-60 and U-937 cells with the increase of DNR (Figure 1C,D). Transwell assay was then conducted and the results presented that DNR treatment caused a decrease in the invasion ability of HL-60 and U-937 cells



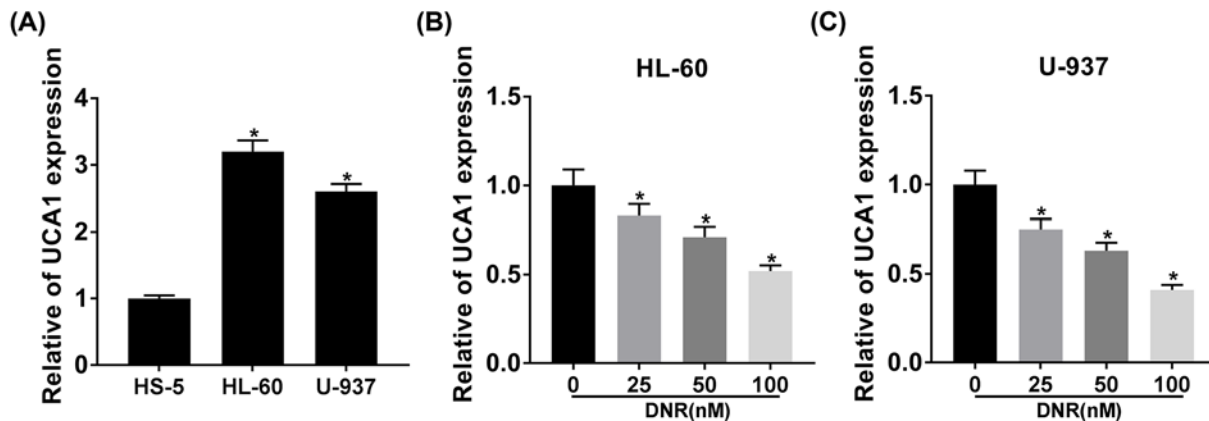
**Figure 1. Effects of DNR treatment on malignant behaviors of acute leukemia cells**

(A–F) HL-60 and U-937 cells were treated with different DNR doses (0, 25, 50, and 100 nM). (A and B) CCK-8 assay was carried out to analyze the viability of HL-60 and U-937 cells. (C and D) Flow cytometry assay was conducted to determine cell cycle progression and apoptosis of HL-60 and U-937 cells. (E) Transwell assay was employed to evaluate the invasion of HL-60 and U-937 cells. (F and G) The levels of Cyclin D1, Vimentin, and Cleaved-casp-3 in HL-60 and U-937 cells were detected through Western blot analysis; \* $P < 0.05$ .

in a dose-dependent manner (Figure 1E). Afterward, the levels of Cyclin D1 (proliferation-associated protein), Vimentin (migration-associated protein), and Cleaved-casp-3 (apoptosis-related protein) were detected and the results exhibited that DNR treatment decreased protein levels of Cyclin D1 and Vimentin in HL-60 and U-937 cells in a dose-dependent manner, while elevated the protein level of Cleaved-casp-3 (Figure 1F,G). Collectively, these results suggested that DNR repressed viability, cell cycle progression, invasion, and induced apoptosis of HL-60 and U-937 cells in a dose-dependent manner.

### DNR treatment decreased UCA1 expression in HL-60 and U-937 cells

To verify the expression of UCA1 in HL-60 and U-937 cells, we executed qRT-PCR analysis to detect UCA1 expression in HL-60 and U-937 cells. We discovered that UCA1 expression was markedly elevated in HL-60 and U-937 cells in comparison with the HS-5 cells (Figure 2A). However, DNR treatment resulted in a decrease in UCA1 expression



**Figure 2.** DNR treatment decreased UCA1 expression in acute leukemia cells

(A) QRT-PCR analysis of UCA1 in HL-60, U-937, and HS-5 cells. (B and C) The expression of UCA1 in HL-60 and U-937 cells treated with different DNR doses (0, 25, 50, and 100 nM) was analyzed by qRT-PCR;  $*P < 0.05$ .

in HL-60 and U-937 cells (Figure 2B,C). Taken together, these data indicated that DNR treatment reduced UCA1 expression in HL-60 and U-937 cells in a dose-dependent manner.

## UCA1 up-regulation decreased the sensitivity of HL-60 and U-937 cells to DNR

Taking into account the above results, we further explored the effects of UCA1 on the viability, apoptosis and invasion of DNR (100 nM)-treated HL-60 and U-937 cells. The overexpression efficiency of pCDNA3.0-UCA1 in HL-60 and U-937 cells was presented in Figure 3A. Moreover, UCA1 overexpression partly reversed the repressive influence of DNR treatment on the viability of HL-60 and U-937 cells (Figure 3B). Moreover, forced UCA1 expression partially abolished the promoting influence of DNR treatment on cell cycle arrest and apoptosis of HL-60 and U-937 cells (Figure 3C,D). Also, transwell assay displayed that UCA1 overexpression reversed the decrease of the invasion ability of HL-60 and U-937 cells caused by DNR treatment (Figure 3E). Additionally, Western blot analysis manifested that UCA1 up-regulation overturned DNR treatment-mediated effects on protein levels of Cyclin D1, Vimentin, and Cleaved-casp-3 in HL-60 and U-937 cells (Figure 3F,G). Therefore, these data indicated that UCA1 up-regulation reduced the sensitivity of HL-60 and U-937 cells to DNR.

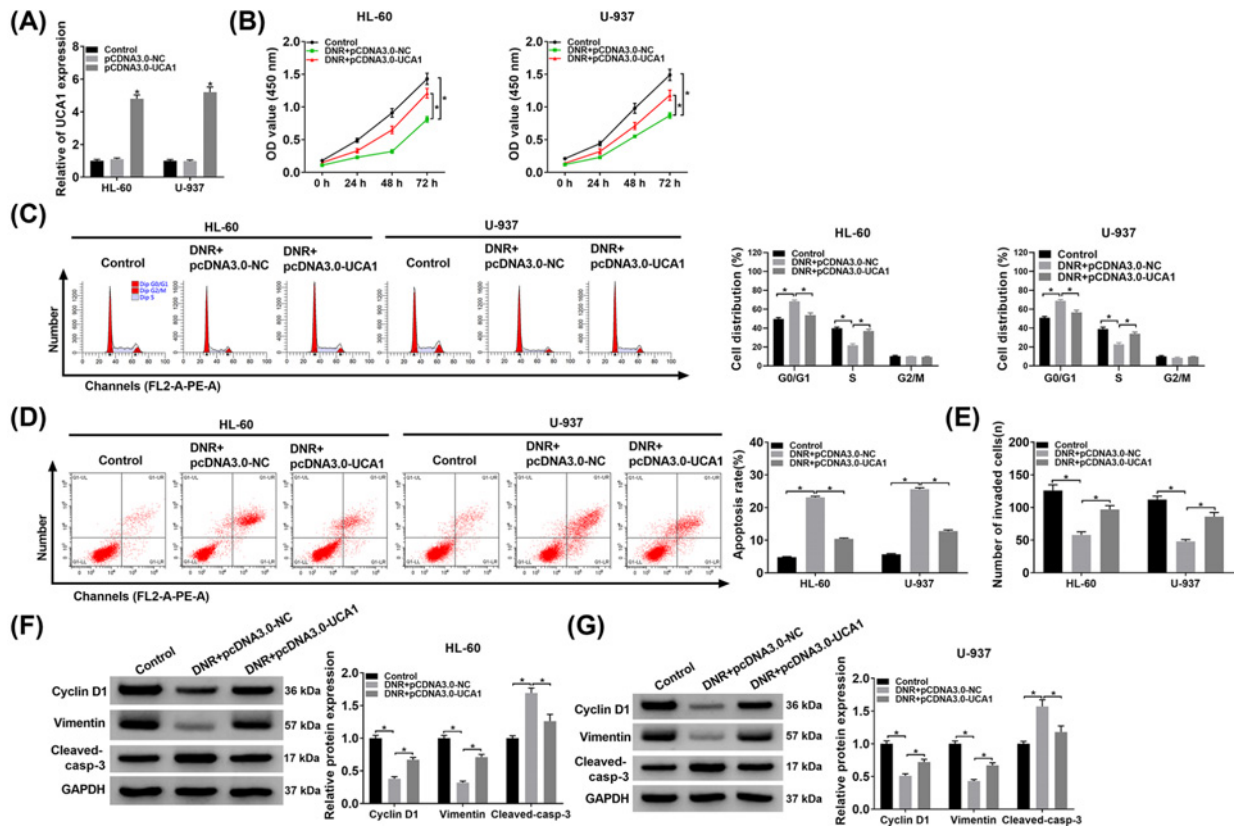
## UCA1 served as a sponge of miR-613 in HL-60 and U-937 cells

Further experiments revealed that UCA1 was mostly distributed in the cytoplasm of HL-60 and U-937 cells, indicating that UCA1 might act as a miRNA sponge (Supplementary Figure S1). To explore the regulatory mechanism of UCA1 in HL-60 and U-937 cells, we predicted miRNAs that might interact with UCA1. We discovered that four miRNAs (miR-206, miR-613, miR-4770, and miR-4735-3p) had complementary sites with UCA1 in the overlapping starbase, LncBase and mircode databases (Supplementary Figure S2A). Moreover, miR-613 mimic reduced the luciferase activity of the UCA1-WT reporter in both HL-60 and U-937 cells (Supplementary Figure S2B). The overexpression efficiency of miR-613 mimic was exhibited in Supplementary Figure S3. The binding sites between miR-613 and UCA1 were presented in Figure 4A. Dual-luciferase reporter assay exhibited that miR-613 mimic curbed the luciferase activity of the UCA1-WT reporter in HL-60 and U-937 cells, whereas the luciferase activity of the UCA1-MUT reporter did not change (Figure 4B). Compared with the HS-5 cells, the expression of miR-613 was markedly down-regulated in HL-60 and U-937 cells (Figure 4C). QRT-PCR displayed that UCA1 expression was observably reduced in HL-60 and U-937 cells after transfection with si-UCA1 (Figure 4D). In addition, silenced UCA1 expression distinctly elevated the expression of miR-613 in HL-60 and U-937 cells (Figure 4E). In sum, these results demonstrated that UCA1 served as a sponge for miR-613 expression in HL-60 and U-937 cells.

## UCA1 decreased the sensitivity of HL-60 and U-937 cells to DNR by interacting with miR-613

Given that UCA1 acted as a sponge for miR-613, we further investigated whether UCA1 affected the sensitivity of HL-60 and U-937 cells to DNR via interacting with miR-613. The knockdown efficiency of miR-613 inhibitor was





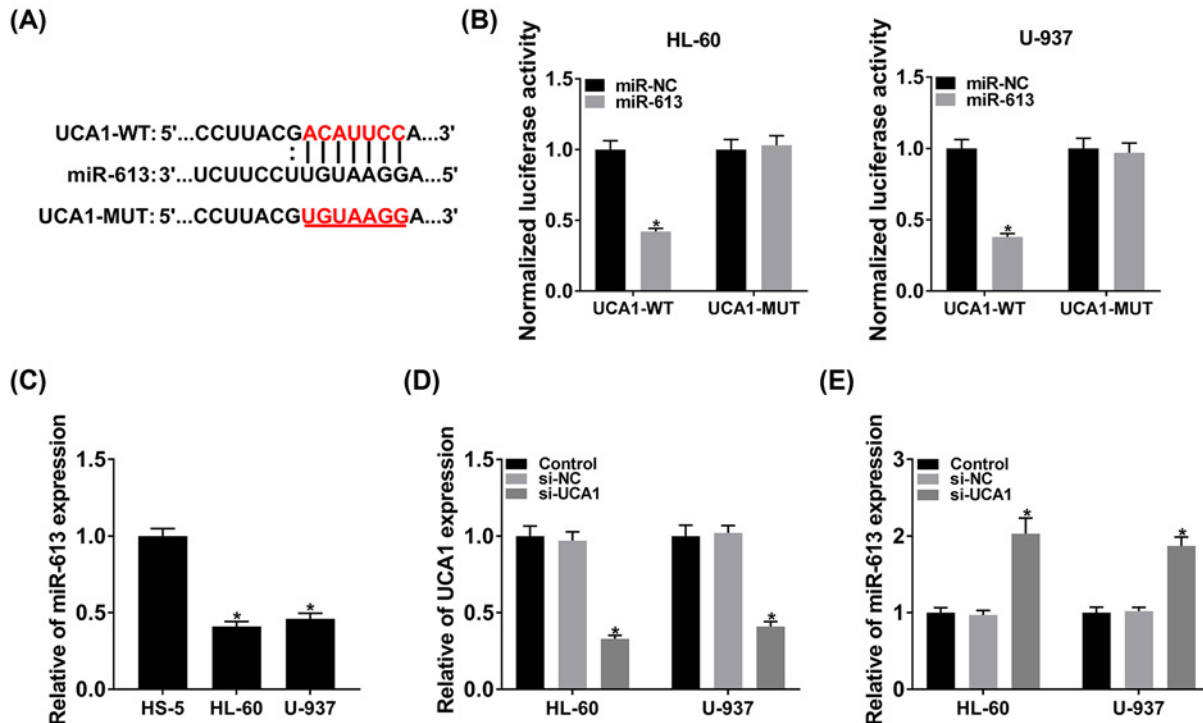
**Figure 3. Effect of UCA1 on the sensitivity of acute leukemia cells to DNR**

(A) The expression of UCA1 in HL-60 and U-937 cells transfected with pCDNA3.0-NC or pCDNA3.0-UCA1 was assessed by qRT-PCR. (B–F) HL-60 and U-937 cells were transfected with pCDNA3.0-NC or pCDNA3.0-UCA1 and then treated with DNR (100 nM). (B) The viability of HL-60 and U-937 cells was determined via CCK-8 assay. (C and D) The cell cycle progression and apoptosis of HL-60 and U-937 cells was detected through flow cytometry assay. (E) The invasion of HL-60 and U-937 cells was analyzed using transwell assay. (F and G) Protein levels of Cyclin D1, Vimentin, and Cleaved-casp-3 protein in HL-60 and U-937 cells were measured by Western blot analysis; \* $P < 0.05$ .

displayed in Supplementary Figure S3. We observed that miR-613 was up-regulated in DNR-treated HL-60 and U-937 cells, while this increase was recovered after Anti-miR-613 introduction (Figure 5A). Moreover, the down-regulation of miR-613 in DNR-treated HL-60 and U-937 cells mediated by miR-613 inhibitor was reversed by UCA1 silencing (Figure 5A). The viability of HL-60 and U-937 cells was elevated by miR-613 inhibition under DNR treatment, while this elevation was abolished by the knockdown of UCA1 (Figure 5B). Furthermore, miR-613 inhibitor promoted cell cycle progression and repressed cell apoptosis in DNR-treated HL-60 and U-937 cells, while these effects caused by miR-613 inhibitor were reversed after UCA1 knockdown (Figure 5C,D). In addition, UCA1 silencing abrogated the promoting impact of miR-613 knockdown on the invasion of DNR-treated HL-60 and U-937 cells (Figure 5E). Also, silenced miR-613 expression increased protein levels of Cyclin D1 and Vimentin and decreased the protein level of Cleaved-casp-3 in DNR-treated HL-60 and U-937 cells, but these trends were reversed by UCA1 inhibition (Figure 5F,G). In short, these results manifested that UCA1 affected the sensitivity of HL-60 and U-937 cells to DNR through miR-613.

### UCA1 regulated the PI3K/AKT pathway via miR-613

To further survey the regulatory mechanism of UCA1 in HL-60 and U-937 cells, we assessed the levels of p-PI3K and p-AKT in DNR-treated HL-60 and U-937 cells transfected Anti-miR-NC, Anti-miR-613, Anti-miR-613+si-NC, or Anti-miR-613+si-UCA1. The results exhibited that DNR reduced the value of p-PI3K/PI3K and p-AKT/AKT in HL-60 and U-937 cells, while this decrease was reversed by miR-613 silencing (Figure 6A,B). However, the effects of miR-613 knockdown on the value of p-PI3K/PI3K and p-AKT/AKT in DNR-treated HL-60 and U-937 cells were



**Figure 4. UCA1 acted as a sponge for miR-613**

(A) The potential binding sites between UCA1 and miR-613. (B) Dual-luciferase reporter assay was carried out to assess the luciferase activities of the UCA1-WT and UCA1-MUT reporters in HL-60 and U-937 cells transfected with miR-613 mimic or miR-NC. (C) The expression of miR-613 in HS-5, HL-60, and U-937 cells was analyzed using qRT-PCR. (D) The knockdown efficiency of si-UCA1 in HL-60 and U-937 cells was validated by qRT-PCR. (E) QRT-PCR was executed to analyze the effect of UCA1 knockdown on miR-613 expression in HL-60 and U-937 cells; \* $P < 0.05$ .

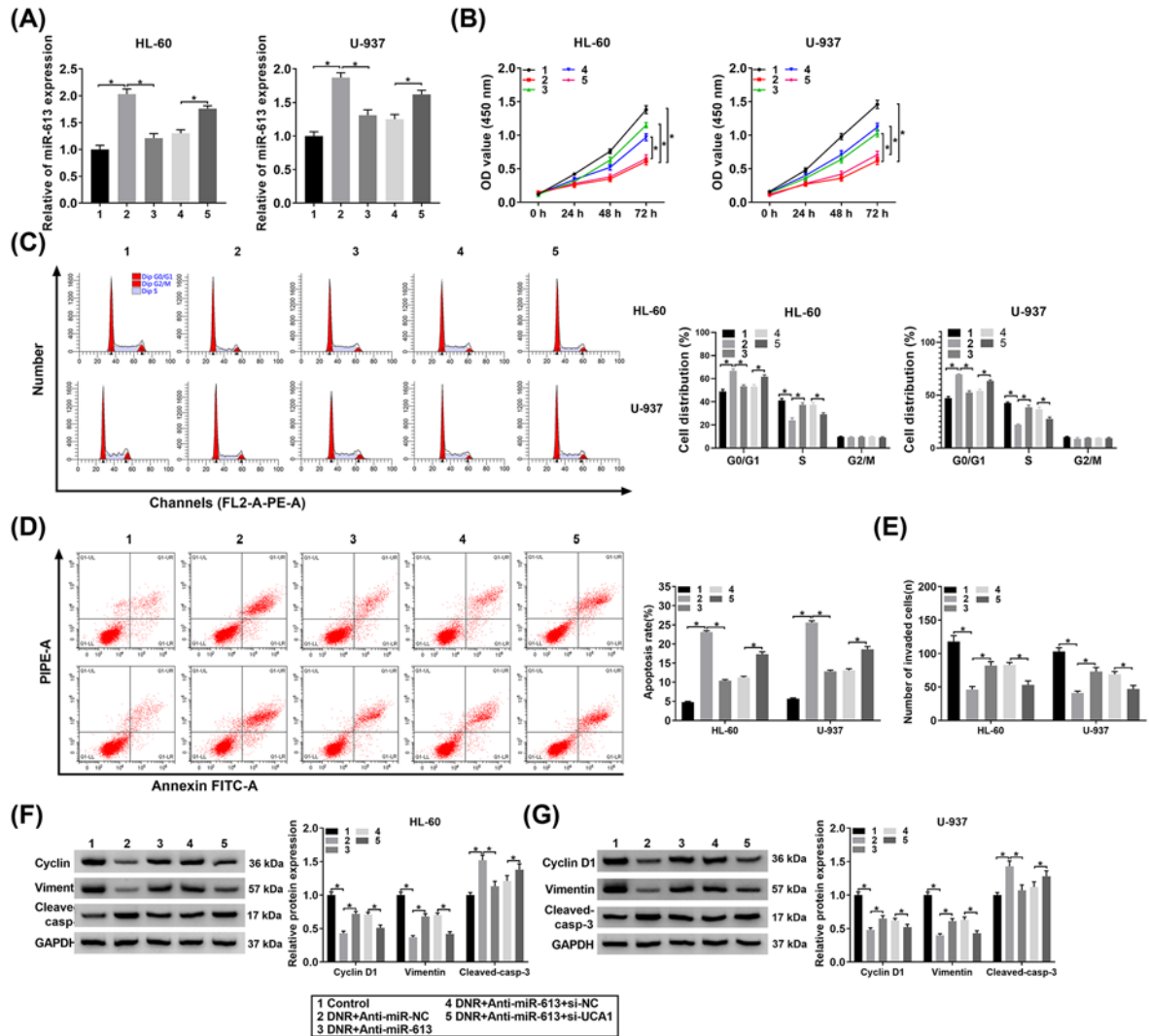
counteracted by UCA1 silencing (Figure 6A,B). Therefore, these data indicated that UCA1 regulated the PI3K/AKT pathway via adsorbing miR-613.

## Discussion

DNR is a chemotherapeutic drug widely utilized in the treatment of various malignant tumors. A number of studies have proved that DNR delayed the growth of cancer cells. Also, the synergy of DNR and celecoxib promotes apoptosis and reduces growth of AML cells [24]. At present, the resistance of acute leukemia cells to DNR is one of the main obstacles to its treatment [25].

Accumulated studies have proved that UCA1 is connected with the chemo-resistance of various cancers. Report of Bian et al. claimed that UCA1 reduced the sensitivity of colorectal cancer cells to 5-fluorouracil and promoted the proliferation of colorectal cancer cells [26]. Another report pointed out that UCA1 elevated doxorubicin resistance in gastric cancer [27]. Xiao et al. stated that UCA1 accelerated the resistance of chronic myeloid leukemia cells to imatinib [15]. Additionally, UCA1 silencing could elevate the cytotoxic effect of adriamycin to adriamycin-treated pediatric AML cells [28]. In the present study, DNR induced apoptosis and impeded viability, cell cycle progression, and invasion of HL-60 and U-937 cells in a dose-dependent manner. Also, DNR restrained the expression of UCA1 in HL-60 and U-937 cells, and forced UCA1 reversed DNR treatment-mediated impacts on viability, cell cycle progression, invasion, and apoptosis of HL-60 and U-937 cells. Thus, we concluded that UCA1 upregulation could reduce the sensitivity of HL-60 and U-937 cells to DNR.

Recent researches have pointed out that UCA1 act as a miRNA sponge and participate in the regulation of chemo-resistance in diversified cancers [14,28,29]. MiR-613 had been reported as an anti-tumor gene in bladder cancer [30], non-small cell lung cancer [31], and laryngeal squamous cell cancer [32]. Moreover, miR-613 mimic elevated cell sensitivity to cisplatin by down-regulating SOX9 in gastric cancer [23]. Herein, miR-613 was down-regulated in HL-60 and U-937 cells. Also, UCA1 was identified as a miR-613 sponge. Furthermore, DNR treatment restored the



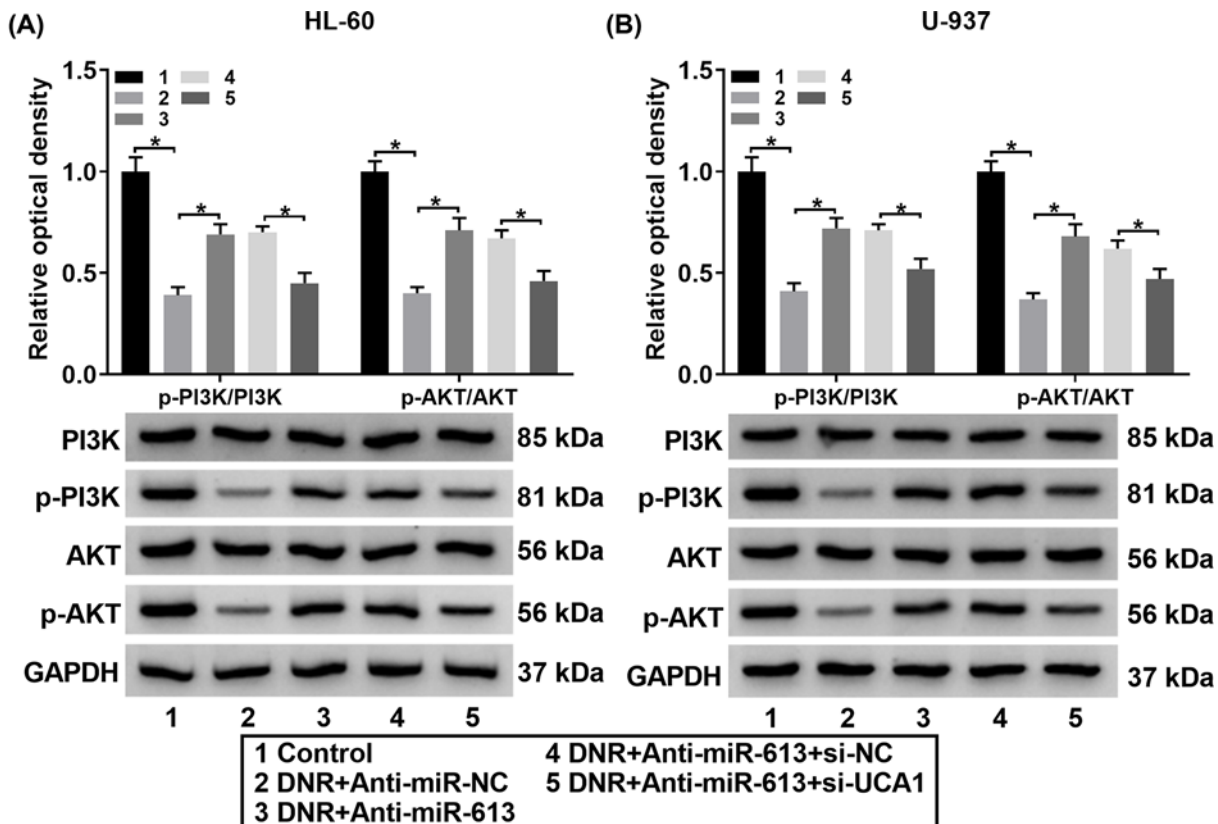
**Figure 5.** UCA1 interacted with miR-613 to affect acute leukemia cell sensitivity to DNR

(A–F) HL-60 and U-937 cells were transfected with Anti-miR-NC, Anti-miR-613, Anti-miR-613+si-NC, or Anti-miR-613+si-UCA1 and then treated with DNR (100 nM). (A) The expression of miR-613 in HL-60 and U-937 cells was evaluated by qRT-PCR. (B) CCK-8 assay was carried out to determine the viability of HL-60 and U-937 cells. (C and D) The cell cycle progression and apoptosis of HL-60 and U-937 cells was determined with flow cytometry assay. (E) The invasion of HL-60 and U-937 cells was assessed via transwell assay. (F and G) Western blot analysis was performed to detect protein levels of Cyclin D1, Vimentin, and Cleaved-casp-3 in HL-60 and U-937 cells; \* $P < 0.05$ .

down-regulation of miR-613 in HL-60 and U-937 cells. In addition, the effects of DNR treatment on viability, cell cycle progression, apoptosis, and invasion of HL-60 and U-937 cells were reversed by miR-613 inhibition, but these effects caused by miR-613 silencing were restored after UCA1 silencing. However, miR-613 was revealed as an oncogene in colon cancer [33] and cervical cancer [34], which might be related to tissues specificity. Thus, we inferred that UCA1 mediated the sensitivity of HL-60 and U-937 cells to DNR via miR-613.

It was reported that the PI3K/AKT pathway was involved in the chemo-resistance of diverse cancers [35,36]. Yang et al. reported that lncRNA linc00239 contributed to malignant behaviors of AML cells and elevated doxorubicin resistance through activating the PI3K/AKT/mTOR pathway in AML [37]. In the present study, DNR treatment blocked the PI3K/AKT pathway, but miR-613 silencing reversed the inactivation of the PI3K/AKT pathway in HL-60 and U-937 cells caused by DNR treatment. Nevertheless, UCA1 suppression offset the influence of miR-613 inhibitor





**Figure 6. UCA1 mediated the PI3K/AKT pathway via miR-613**

(A and B) Protein levels of PI3K, p-PI3K, AKT, and p-AKT in HL-60 and U-937 cells transfected with Anti-miR-NC, Anti-miR-613, Anti-miR-613+si-NC, or Anti-miR-613+si-UCA1 under DNR treatment were detected through Western blot analysis; \* $P < 0.05$ .

on the PI3K/AKT pathway in DNR-treated HL-60 and U-937 cells. Therefore, we inferred that UCA1 regulated the sensitivity of HL-60 and U-937 cells to DNR through mediating PI3K/AKT pathway via sponging miR-613.

In sum, UCA1 up-regulation decreased the sensitivity of acute leukemia cells to DNR. Moreover, UCA1 overexpression activated the PI3K/AKT pathway via sponging miR-613. The present study provided an evidence to support UCA1 as a promising target for acute leukemia treatment.

### Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

### Author Contribution

Qiyang Yao and Li Zhang designed the research study. Yuchuan Wang performed the research. Junli Liu and Liu Yang analyzed the data. Qiyang Yao, Li Zhang, and Yingjie Wang wrote the paper. All authors revised the paper and approved the final version.

### Abbreviations

AKT, protein kinase B; AML, acute myeloid leukemia; CCK-8, cell counting kit-8; cleaved-casp-3, cleaved-caspase-3; DNR, daunorubicin; miR-613, microRNA-613; p-AKT, phosphorylated-AKT; p-PI3K, phosphorylated PI3K; PI3K, phosphatidylinositol 3-kinase; qRT-PCR, quantitative real-time polymerase chain reaction; UCA1, urothelial cancer-associated 1.

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