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TUG1 weakens the sensitivity of acute myeloid leukemia cells to cytarabine by regulating miR-655-3p/CCND1 axis

B. ZHANG, Y.-F. SUN, X.-M. ZHANG, N. JIANG, Q. CHEN

Department of Pediatrics, Luoyang Central Hospital Affiliated to Zhengzhou University, Luoyang, Henan, China

Abstract. – OBJECTIVE: Long non-coding RNA taurine upregulated gene 1 (lncRNA TUG1) has been demonstrated to promote malignant phenotypes and Adriamycin resistance in acute myeloid leukemia (AML) cells. However, the function and mechanism of TUG1 in cytarabine (Ara-C) sensitivity in AML remain unclear.

PATIENTS AND METHODS: Levels of TUG1, microRNA (miR)-655-3p or cyclin D1 (CCND1) mRNA were examined using quantitative real-time polymerase chain reaction (qRT-PCR). Cell proliferation activity and apoptosis were analyzed using cell counting kit-8 (CCK-8) or flow cytometry, respectively. Western blot was utilized to detect the protein levels of Ki-67, B-cell lymphoma-2-associated X protein (Bax), and CCND1. The interaction between miR-655-3p and TUG1 or CCND1 was confirmed by Dual-Luciferase reporter and pull-down assay.

RESULTS: TUG1 and CCND1 were higher expressed, while miR-655-3p was lower expressed in AML cells compared with that in normal cells. Higher expression levels of TUG1 or CCND1, and lower expression levels of miR-655-3p both notably reversed Ara-C-induced proliferation inhibition and apoptosis promotion in AML cells. TUG1 was a sponge of miR-655-3p, and TUG1 knockdown enhanced the sensitivity of AML cells to Ara-C by regulating miR-655-3p. MiR-655-3p directly targeted CCND1, and CCND1 overexpression attenuated miR-655-3p restoration-mediated reinforcement of Ara-C sensitivity in AML cells. Besides that, TUG1 up-regulated CCND1 expression via miR-655-3p.

CONCLUSIONS: TUG1 weakened the sensitivity of AML cells to Ara-C by up-regulating CCND1 via miR-655-3p, suggesting a new insight into the chemotherapy of AML.

Key Words:
TUG1, Chemosensitivity, Acute myeloid leukemia, MiR-655-3p, CCND1.

Introduction

Acute myeloid leukemia (AML) is a fatal hematopoietic system malignancy with a high relapse rate, which is highlighted by the uncontrolled clonal proliferation of myeloid precursors and differentiation blockage of normal hematopoiesis[1,2]. Currently, hematopoietic stem cell transplantations with chemotherapy are the standard treatments for AML[3]; among them, cytarabine (Ara-C) is the first-line chemotherapy strategy for AML treatment[4]. Unfortunately, the phenomenon of treatment resistance in the majority of AML patients has gradually emerged[5], ultimately causing the incurability of the disease.

Growing evidence has documented the regulatory role of non-coding RNAs (ncRNAs) in the tumorigenesis, maintenance, and progression of AML[6,7]. The ncRNAs are functional RNAs that have no ability to transcribe into a protein, in which longer than –200 nucleotides are regarded as long noncoding RNA (lncRNA)[8]. LncRNAs have been discovered to be closely associated with the regulation of diverse cell biological processes, including cell proliferation, apoptosis, differentiation, chemoresistance, and hematopoiesis[9-11]. LncRNA taurine upregulated gene 1 (TUG1), mapped on chromosome 22q12, is a highly conserved functional RNA molecule[12]. Accumulating evidence has uncovered the oncogenic role of TUG1 in various carcinomas, such as gastric cancer[13], hepatocellular carcinoma[14], and colorectal cancer[15]. In AML, the expression of TUG1 was higher in AML patients and closely related to the poor prognosis[16]. Besides that, TUG1 enhanced malignant phenotypes and Adriamycin resistance of AML cells[17,18]. However, the function and the potential mechanism of TUG1 in the sensitivity of

Corresponding Author: Bei Zhang, MD; e-mail: nvkqrj@163.com

AML cells to Ara-C have not been investigated.

In contrast to lncRNAs, microRNAs (miRNAs) are the best-characterized ncRNAs with approximately 19-25 nucleotides in size, which play significant roles in malignant physiological or pathological cellular processes in cancers[19]. Up to date, emerging studies have identified the implication of miRNAs in nearly all aspects of AML disease development, including cell proliferation, apoptosis, and differentiation[20]. Notably, studies have also identified that the miRNA-target mRNA regulatory network is associated with the development and clinical prognosis of AML[21].

Herein, this investigation evaluated the role of TUG1 in Ara-C sensitivity in AML cells *in vitro*, and the potential miRNA-mRNA pathways in which TUG1 regulated the sensitivity of AML cells to Ara-C were further investigated.

Patients and Methods

Clinical Samples

Human blood samples from 30 untreated AML patients and 30 healthy donors without any diseases were collected at the Luoyang Central Hospital Affiliated to Zhengzhou University. AML patients were clinically diagnosed according to the French American-British (FAB) classification criteria. Serum was obtained from all collected peripheral blood samples and immediately stored at -80°C until further analysis. The research protocols were permitted by the Ethics Committee of Luoyang Central Hospital Affiliated to Zhengzhou University, and manipulated in line with the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all subjects.

Cell Culture

Human AML cell lines (HL-60 and KG-1) and the human normal stromal cells HS-5 were obtained from Shanghai Bank of Tissues (Shanghai, China), and grown in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Invitrogen, Carlsbad, CA, USA) containing with 10% fetal bovine serum (FBS), 1% of penicillin-streptomycin (Gibco, Grand Island, NY, USA) and 2 mM of l-glutamine (Gibco, Grand Island, NY, USA) with 5% CO_2 at 37°C.

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

The isolation of total RNA from clinical samples and cells was carried out using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). 2 μg of total RNA was reversely transcribed into complementary DNAs (cDNAs) using the PrimeScript™ RT reagent Kit (TaKaRa, Dalian, China). Then, the synthesized cDNA template was quantified on ABI7300 with SYBR Green Master Mix II (TaKaRa, Dalian, China). The levels of mRNA expression were analyzed by the $2^{-\Delta\Delta Ct}$ method normalized by gyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 small nuclear B noncoding RNA (U6). The primer sequences were listed as follows: TUG1 F: 5'-CTGAAGAAAGGCAACATC-3' and R: 5'-GTAGGCTACTACAGGATTTG-3'; miR-655-3p F: 5'-CAATCCTTACTCCAGCCAC-3' and R: 5'-GTGTCTTAAGGCTAGGCCTA-3'; cyclin D1 (CCND1) F: 5'-GGCGGAGGAGAACAAACAGA-3'andR:5'-ATGGAGGGCGGATTGGAAA-3'; U6 F: 5'-CTTCGGCAGCACATATAC-3' and R: 5'-GAACGCTTCACGAATTTGC-3'; GADPH F: 5'-CCCACATGGCCTCCAAGGAGTA-3' and R: 5'-GTGTACATGGCAACTGTGAGGAGG-3'.

Cell Proliferation Assay

The untransfected or transfected HL-60 and KG-1 cells (5×10^4/well) were seeded on a 96-well plate overnight, and then were treated with increasing concentrations of cytarabine (Ara-C) (Sigma-Aldrich, St. Louis, MO, USA) (0, 1, 2, or 4 μM) for 48 h. After that, 10 μL of cell counting kit-8 (CCK-8) solution (Beyotime, Shanghai, China) was added per well and incubated for another 2 h. Finally, the absorbance at 450 nm was determined using a microplate reader in the indicated time.

Cell Transfection

The si-TUG1, pcDNA3.1-TUG1 overexpression vector (TUG1) and pcDNA3.1- CCND1 overexpression vector (CCND1), the mimic of miR-655-3p (miR-655-3p) and miR-655-3p inhibitor (anti-miR-655-3p) and their respective negative control (si-NC, Vector, miR-NC, or anti-NC) were synthesized by Genepharma (Shanghai, China). The transfections were performed with Lipofectamine 2000 reagent (Invitrogen) according to the instructions of manufacturer.

Cells Apoptosis Analysis

After transfection and treatment, HL-60 and KG-1 cells were gently dual stained with 10 μL Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) (BD Biosciences, Franklin Lakes, NJ USA). Cell apoptosis rates were detected using a flow cytometer.

Western Blot

Extracted proteins (25 μg) were loaded onto a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) minigel for separating, and then transferred to polyvinylidene difluoride (PVDF) membranes. Afterwards, the membranes were interacted with the antibodies listed below, which were all obtained from Abcam (Cambridge, MA, USA): Ki-67 (1:5000, ab16667), B-cell lymphoma-2-associated X protein (Bax) (1:5000, ab32503), cyclin D1 (CCND1) (1:10000, ab134175) and GAPDH (1:10000, ab181602), followed by incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000, Sangon, Shanghai, China).

Dual-Luciferase Reporter Assay

The TUG1 or CCND1 3'UTR containing the predicted potential wild-type (WT) or mutant (MUT) sites of miR-655-3p were cloned into the pMIR-Report vector (Promega, Madison, WI, USA) to generate Luciferase vectors, named as TUG1-WT, TUG1-MUT, CCND1-WT, or CCND1-MUT. Then, lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used to transfect these constructed Luciferase reporters (100 ng) combined with miR-655-3p or miR-NC (100 nM) into HL-60 and KG-1 cells, respectively. Luciferase activities were determined with the help of the Dual-Luciferase Kit (Promega. Madison, WI, USA) and normalized by *Renilla* Luciferase activity.

Pull-Down Assay

Biotinylated-miR-655-3p (Bio-miR-655-3p) and Biotinylated-miR-NC (Bio-miR-NC) synthesized by Genepharma were transfected into HL-60 and KG-1 cells. After 24 h, cells were lysed, and whole-cell lysates were incubated with Dynabead M-280 streptavidin beads (Invitrogen, Carlsbad, CA, USA) overnight. Then, the bead-bound RNA was eluted, isolated and subjected for qRT-PCR analysis.

Statistical Analysis

Data from thrice-repeated experiments were shown as mean ± standard deviation (SD). Statistical difference was analyzed by Student's *t*-test or one-way analysis of variance (ANOVA) followed by Tukey post-hoc test on GraphPad Prism 7 software (San Diego, CA, USA). *p*-values < 0.05 suggested statistically significant.

Results

TUG1 Is Elevated In AML Patients and Cells

The expression of TUG1 was detected in the serum of AML patients and healthy donors. Results showed that relative to the healthy donors, TUG1 was elevated in the serum of AML patients (Figure 1A). Similarly, the elevation of TUG1 expression level also was observed in AML cell lines (HL-60 and KG-1) compared to the normal HS-5 cells (Figure 1B). These data indicated TUG1 increase might be associated with the progression of AML. Besides, HL-60 and KG-1 cells were treated with increasing doses of Ara-C (0, 1, 2, or 4 μM) for 48 h, and CCK-8 assay displayed that cell proliferation activity was significantly inhibited by Ara-C in a dose-dependent manner (Figure 1C, D), and especially suppressed by 4 μM Ara-C treatment. Thus, 4 μM Ara-C was chosen for following analyses.

TUG1 Attenuates Ara-C-Induced Inhibition of Cell Proliferation and Promotion of Apoptosis In AML

To investigate the detailed function of TUG1 on Ara-C sensitivity in AML, HL-60 and KG-1 cells were transfected with Vector or TUG1, and the level of TUG1 in cells transfected with TUG1 overexpressed plasmid was significantly increased compared with those transfected with empty vector (Figure 2A). Subsequently, after 4 μM Ara-C treatment, we found Ara-C treatment inhibited the proliferation (Figure 2B) and promoted apoptosis in HL-60 and KG-1 cells (Figure 2C), while these effects were reversed by TUG1 overexpression (Figure 2B, C), which proved that TUG1 enhanced Ara-C resistance of AML cells. Besides, TUG1 overexpression also attenuated Ara-C-mediated reduction of Ki-67 expression and elevation of Bax level in HL-60 and KG-1 cells (Figure 2D, E), further suggesting the regulation of TUG1 on Ara-C-induced cell proliferation and apoptosis in AML. Taken together, these findings revealed that Ara-C restrained AML cells proliferation and TUG1 weakened the sensitivity of AML cells to Ara-C.

TUG1 Is a Sponge of MiR-655-3p

The potential pathways in which TUG1 regulated the sensitivity of AML cells to Ara-C, the potential miRNA targets of TUG1, were predicted through searching the bioinformatics tool StarBase3.0 program and miR-655-3p was found that

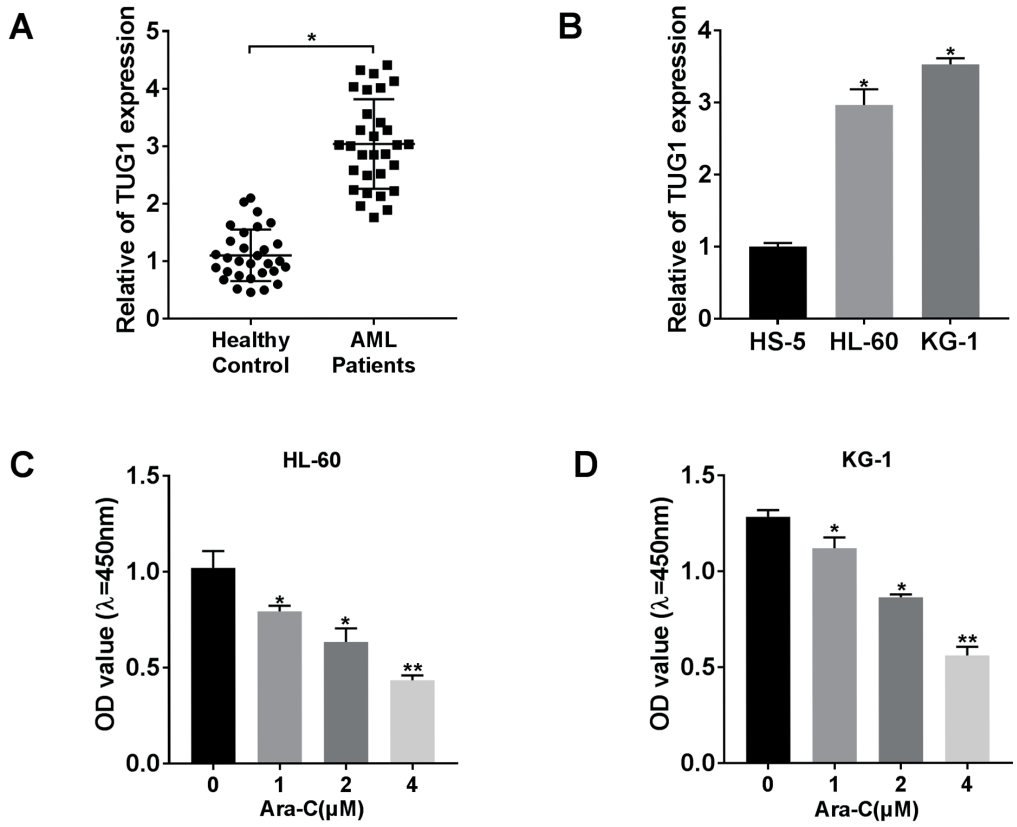

Figure 1. TUG1 is elevated in AML patients and cells. **A-B**, qRT-PCR analysis of TUG1 expression in the serum of AML patients and healthy donors (**A**), as well as in AML cell lines (HL-60 and KG-1) and normal HS-5 cells (**B**). **C-D**, CCK-8 assay analysis of HL-60 and KG-1 cell proliferation combined with increasing doses of Ara-C (0, 1, 2, or 4 μM). *$p<0.05$.

might be a target of TUG1 with putative binding sites (Figure 3A). To confirm the prediction, a Dual-Luciferase reporter assay was conducted, and results showed miR-655-3p overexpression significantly reduced the Luciferase activity of TUG1-WT reporter vector, but not TUG1-MUT reporter vector in HL-60 and KG-1 cells (Figure 3B). Moreover, TUG1 levels in HL-60 and KG-1 cells pulled down using Bio-miR-655-3p were higher than those in cells pulled down by Bio-miR-NC (Figure 3C). These results confirmed the direct interaction between miR-655-3p and TUG1. After that, miR-655-3p expression was detected, qRT-PCR analysis showed miR-655-3p was decreased in HL-60 and KG-1 cells relative to the normal HS-5 cells (Figure 3D), suggesting the potential regulatory role of miR-655-3p in AML progression. Furthermore, we observed TUG1 inhibited miR-655-3p expression in HL-60 and KG-1 cells (Figure 3E). Collectively, TUG1 sponged miR-655-3p and negatively regulated its expression.

MiR-655-3p Knockdown Reverses Ara-C-Induced Inhibition of Cell Proliferation and Promotion of Apoptosis In AML

The function of miR-655-3p on Ara-C sensitivity of AML cells was investigated. HL-60 and KG-1 cells were transfected with anti-NC or anti-miR-655-3p; as expected, anti-miR-655-3p transfection significantly declined the expression level of miR-655-3p in HL-60 and KG-1 cells compared with anti-NC transfection (Figure 4A, B). Following treatment with 4 μM Ara-C, functional experiments showed miR-655-3p down-regulation abated Ara-C-induced inhibition of HL-60 and KG-1 cell proliferation, reflected by the increased proliferation activity (Figure 4C) and Ki-67 levels (Figure 4E, F). Meanwhile, miR-655-3p inhibition also attenuated Ara-C-induced enhancement of HL-60 and KG-1 cell apoptosis, demonstrated by the decreased apoptosis rate (Figure 4D) and Bax levels (Figure 4E, F). These results suggested that same as the action of TUG1, miR-655-3p knockdown aggravated Ara-C

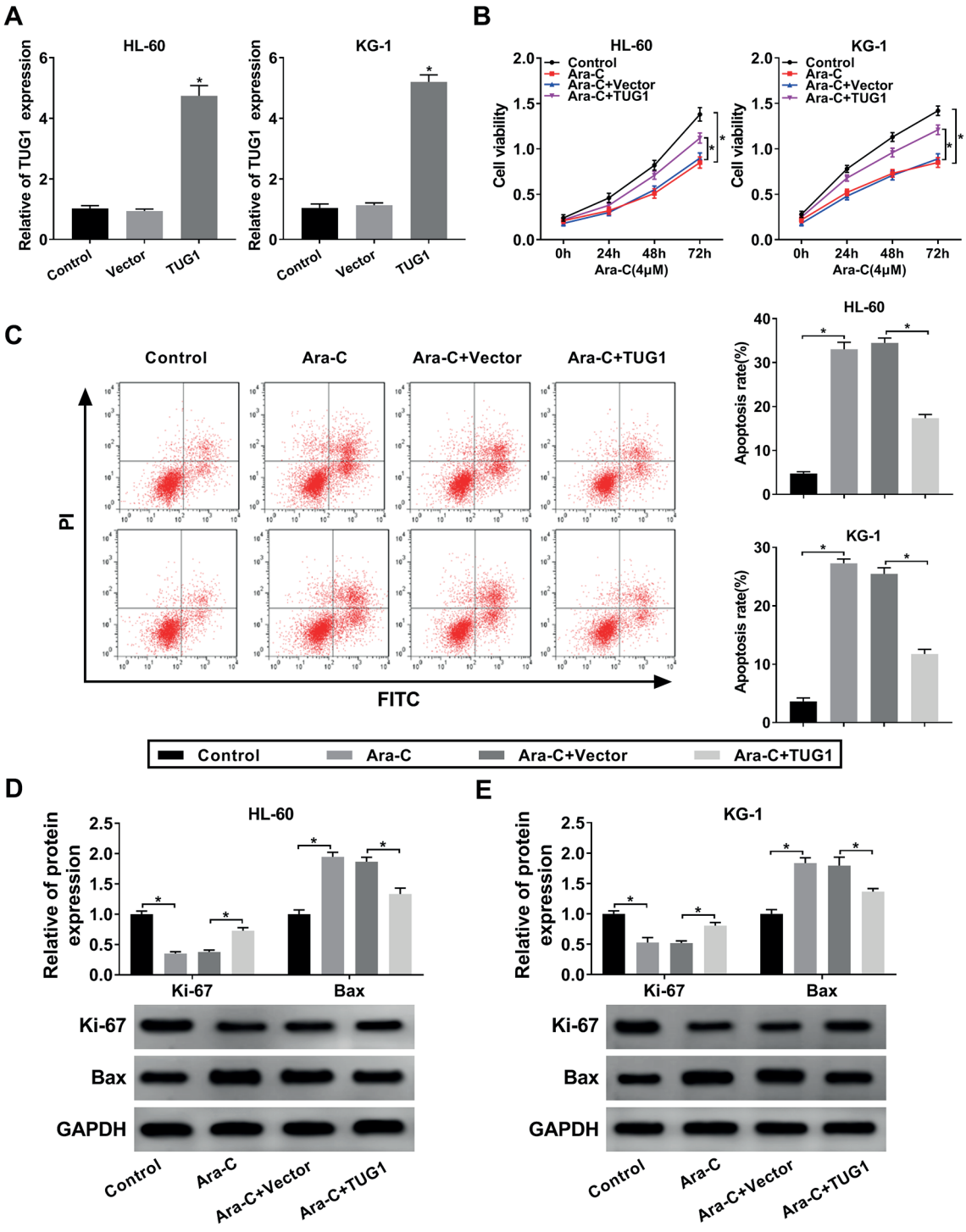

Figure 2. TUG1 attenuates Ara-C-induced inhibition of cell proliferation and promotion of apoptosis in AML. HL-60 and KG-1 cells were transfected with Vector or TUG1. **A**, qRT-PCR analysis of TUG1 expression in HL-60 and KG-1 cells. After treatment with 4 μM Ara-C, (**B**) CCK-8 assay analysis of HL-60 and KG-1 cell proliferation; **C**, detection of apoptosis rate of HL-60 and KG-1 cells with flow cytometry; **D-E**, Western blot analysis of Ki-67 and Bax levels in HL-60 and KG-1 cells. *p<0.05.

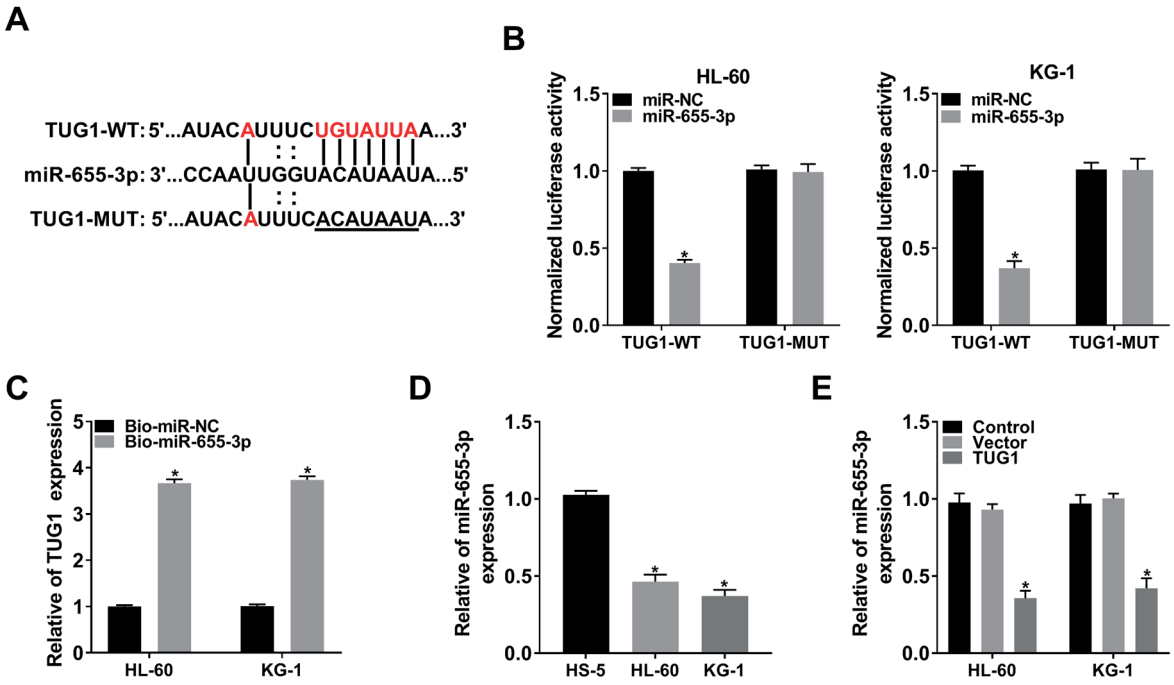

Figure 3. TUG1 is a sponge of miR-655-3p. **A**, The potential binding sites of TUG1 and miR-655-3p. **B**, Dual-Luciferase reporter assay in HL-60 and KG-1 cells co-transfected with the reporter plasmid and the indicated miRNAs. **C**, qRT-PCR analysis of TUG1 expression in HL-60 and KG-1 cells pulled down by Bio-miR-NC or Bio-miR-655-3p. **D**, qRT-PCR analysis of miR-655-3p in AML cell lines (HL-60 and KG-1) and normal HS-5 cells. **E**, qRT-PCR analysis of miR-655-3p in HL-60 and KG-1 cells transfected with Vector or TUG1. *p<0.05.

resistance in AML cells, also meaning that miR-655-3p sensitized AML cells to Ara-C.

TUG1 Knockdown Enhances the Sensitivity of AML Cells to Ara-C by Regulating MiR-655-3p

Based on the relationship of TUG1 and miR-655-3p, we further explored whether miR-655-3p mediated the action of TUG1 on Ara-C sensitivity in AML cells. First, HL-60 and KG-1 cells were transfected with si-NC or si-TUG1, and we verified that si-TUG1 transfection remarkably reduced the level of TUG1 in cells (Figure 5A). Next, HL-60 and KG-1 cells were transfected with si-NC, si-TUG1, si-TUG1 + anti-NC, or si-TUG1 + anti-miR-655-3p to conduct rescue assay. After treatment with 4 μM Ara-C, we found TUG1 knockdown reinforced HL-60 and KG-1 cell proliferation inhibition (Figure 5B, D, E) and apoptosis promotion (Figure 5C, D, E) induced by Ara-C treatment, which testified that TUG1 knockdown reinforced Ara-C sensitivity in AML cells. Of note, these effects induced by TUG1 knockdown on Ara-C sensitivity were all partially overturned by miR-655-3p inhibition (Figure 5B-E). Altogether, it was affirmed that TUG1 knockdown sensitized AML cells to Ara-C by binding to miR-655-3p.

CND1 Is a Target of MiR-655-3p

The targeting genes of miR-655-3p were investigated. According to the prediction of StarBase3.0 program, we found the binding sites of miR-655-3p on CCND1 (Figure 6A). After that, the Dual-Luciferase reporter assay showed miR-655-3p markedly reduced the relative Luciferase activity of CCND1-WT in HL-60 and KG-1 cells, while that in the cells transfected with CCND1-MUT was not declined (Figure 6B). In the meanwhile, pull-down assay exhibited that Bio-miR-655-3p was able to pull down more CCND1 in HL-60 and KG-1 cells than Bio-miR-NC (Figure 6C). In addition, Western blot analysis showed CCDN1 was elevated in AML cell lines (Figure 6D), and miR-655-3p inhibition increased the level of CCDN1 in HL-60 and KG-1 cells (Figure 6E). All these data indicated miR-655-3p targetedly suppressed CCND1 expression.

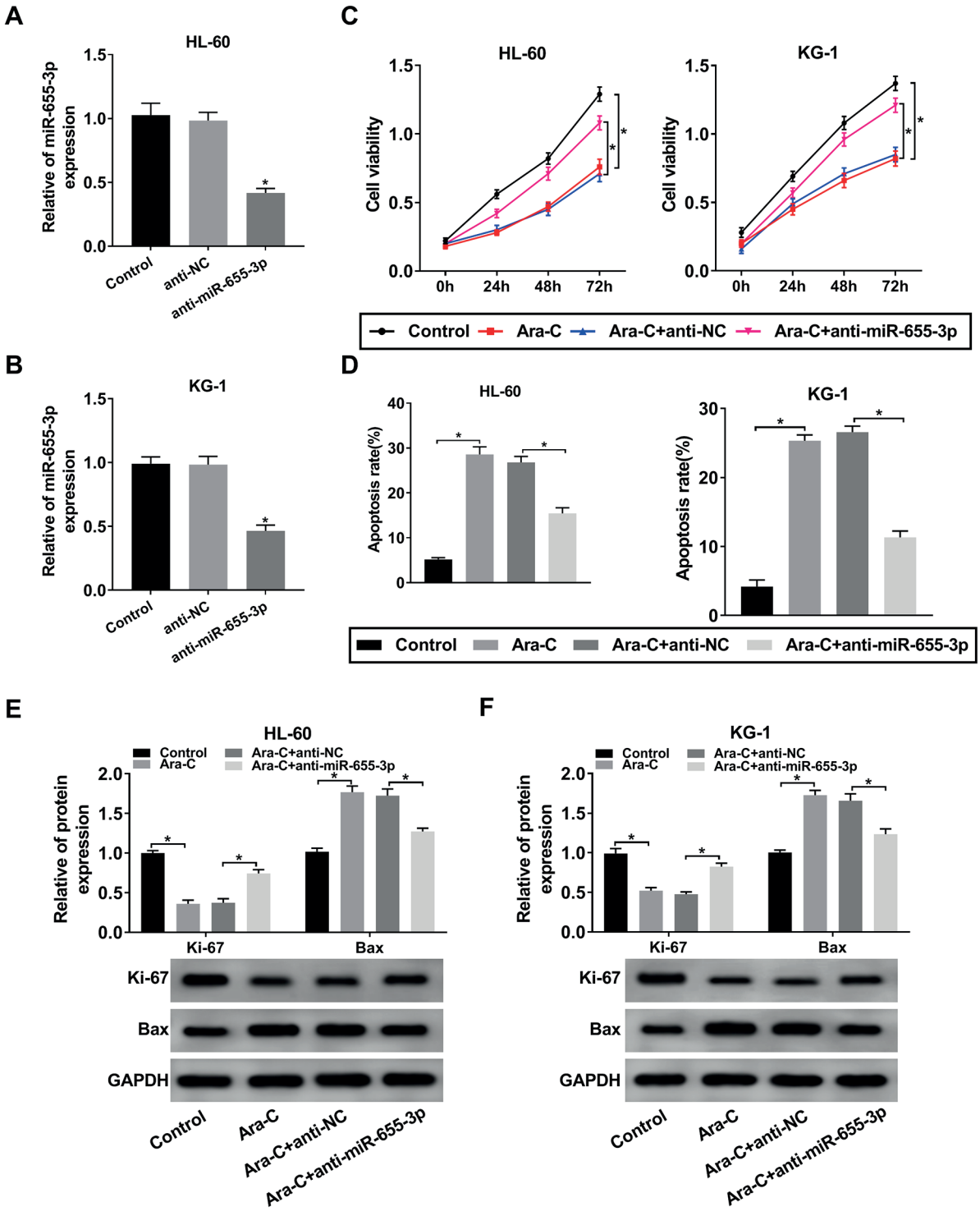

Figure 4. MiR-655-3p knockdown reverses Ara-C-induced inhibition of cell proliferation and promotion of apoptosis in AML. HL-60 and KG-1 cells were transfected with anti-NC or anti-miR-655-3p prior to 4 μM Ara-C treatment. **A-B**, qRT-PCR analysis of miR-655-3p in HL-60 and KG-1 cells. After treatment with 4 μM Ara-C, (**C**) analysis of HL-60 and KG-1 cell proliferation with CCK-8 assay; **D**, detection of HL-60 and KG-1 cell apoptosis using flow cytometry; **E-F**, levels detection of Ki-67 and Bax in HL-60 and KG-1 cells using Western blot. *$p<0.05$.

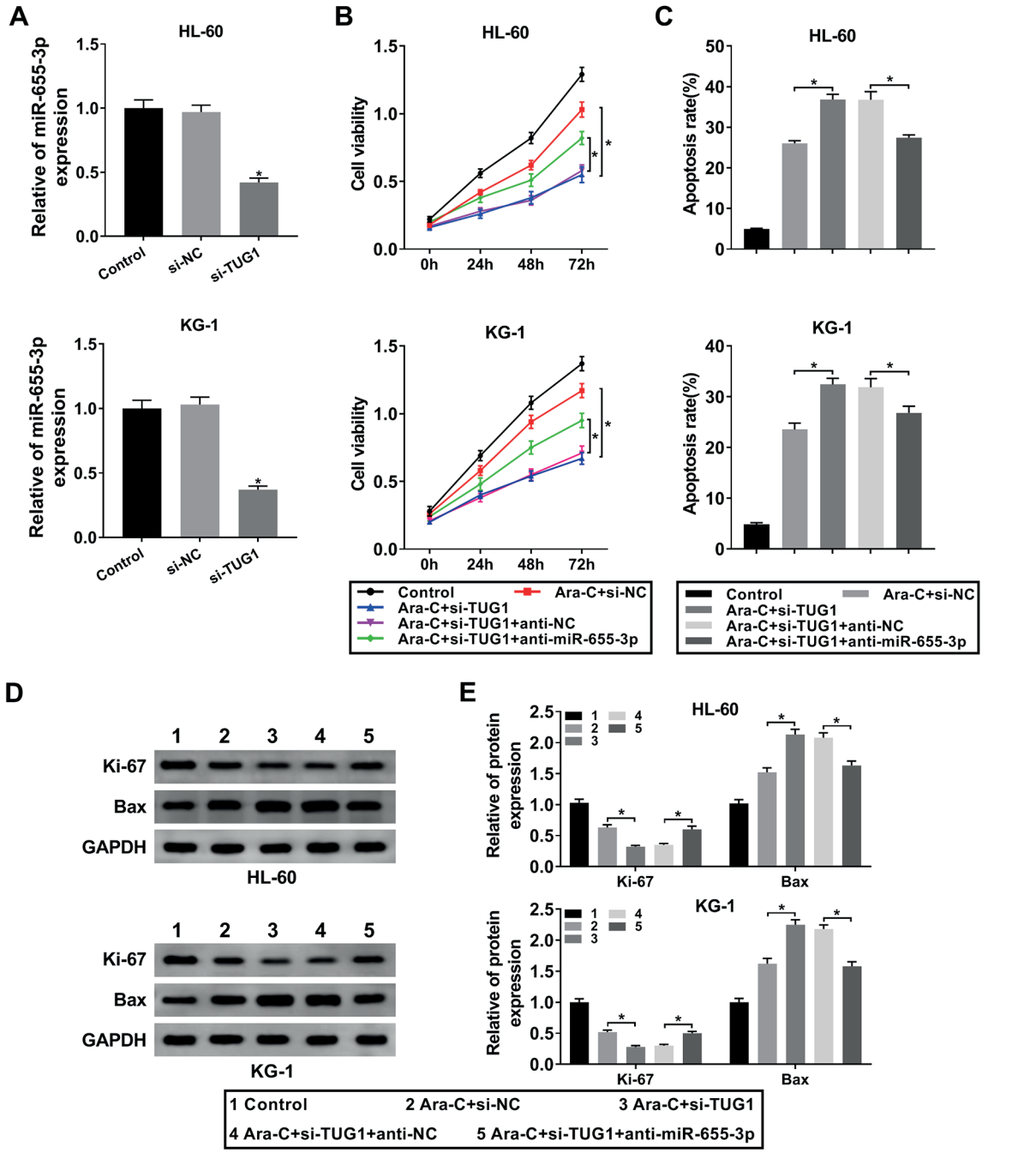

Figure 5. TUG1 knockdown enhances the sensitivity of AML cells to Ara-C by regulating miR-655-3p. **A**, qRT-PCR analysis of miR-655-3p in HL-60 and KG-1 cells transfected with si-NC or si-TUG1. HL-60 and KG-1 cells were transfected with si-NC, si-TUG1, si-TUG1 + anti-NC, or si-TUG1 + anti-miR-655-3p, followed treated with 4 μM Ara-C. **B**, Detection of proliferation of HL-60 and KG-1 cells. **C**, Flow cytometry analysis of HL-60 and KG-1 cell apoptosis. **D-E**, Western blot analysis of Ki-67 and Bax in HL-60 and KG-1 cells. *p<0.05.

CCND1 Overturns AML Cell Proliferation Inhibition and Apoptosis Promotion Mediated by Ara-C

Due to the elevation of CCND1 in AML cells, the function of CCND1 in Ara-C sensitivity in HL-60 and KG-1 cells was evaluated by transfecting with Vector or CCND1 before Ara-C treatment. As presented in Figure 7 A, B, CCND1 protein was significantly elevated in HL-60 and KG-1 cells. Then, we observed CCND1 overex-

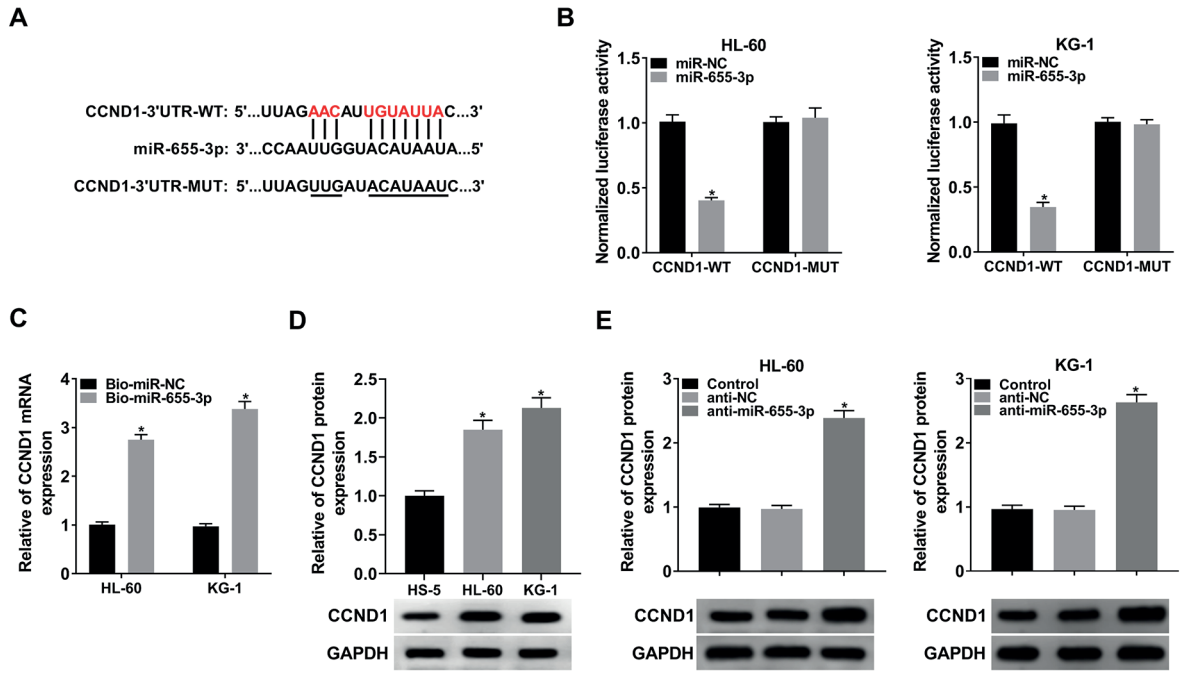

Figure 6. CCND1 is a target of miR-655-3p. **A**, The predicted binding sites of miR-655-3p on CCND1 (CCND1-WT) and CCND1 mutant (CCND1-MUT) sequences. **B**, Luciferase activity detection in HL-60 and KG-1 cells co-transfected with the reporter plasmid and the indicated miRNAs using the dual-Luciferase reporter assay. **C**, qRT-PCR analysis of CCND1 expression in HL-60 and KG-1 cells pulled down by Bio-miR-NC or Bio-miR-655-3p. **D**, Western blot analysis of CCND1 expression in AML cell lines (HL-60 and KG-1) and normal HS-5 cells. **E**, Detection of CCND1 expression in HL-60 and KG-1 cells transfected with anti-NC or anti-miR-655-3p. *$p<0.05$.

pression abolished Ara-C-mediated inhibition of proliferation (Figure 7C, E, F) and enhancement of apoptosis (Figure 7D-F) in HL-60 and KG-1 cells. In all, same as TUG1, CCND1 aggravated the resistance of AML cells to Ara-C.

MiR-655-3p Sensitizes AML Cells to Ara-C by Targeting CCND1

We aimed to assess whether CCND1 involved in the activity of miR-655-3p on Ara-C sensitivity in AML cells. First, HL-60 and KG-1 cells were transfected with miR-NC or miR-655-3p, and a significant increase of miR-655-3p in HL-60 and KG-1 cells transfected with miR-655-3p was observed (Figure 8A). Next, HL-60 and KG-1 cells were transfected with miR-NC, miR-655-3p, miR-655-3p + Vector, or miR-655-3p + CCND1 and treated with Ara-C. After that, CCK-8 assay and flow cytometry assay displayed miR-655-3p restoration enhanced the action of Ara-C on HL-60 and KG-1 cell proliferation inhibition (Figure 8B) and apoptosis promotion (Figure 8C), while CCND1 overexpression remitted these effects (Figure 8B, C). Additionally, CCND1 up-regulation reversed miR-655-3p restoration-mediated Ki-67 decrease and Bax increase in HL-60 and KG-1 cells treated with Ara-C (Figure 8D, E). Thus, miR-655-3p interacted with CCND1 to reinforce the sensitivity of AML cells to Ara-C.

TUG1 Regulates CCND1 Expression by Sponging MiR-655-3p

Given TUG1 sponged miR-655-3p, and CCND1 was a target of miR-655-3p, we then explored whether TUG1 regulated CCND1 via miR-655-3p in AML cells. Notably, as shown in Figure 9A, B, TUG1 up-regulated CCND1 expression level in HL-60 and KG-1 cells both at mRNA and protein levels, while this elevation was attenuated by miR-655-3p restoration. Therefore, TUG1 positively regulated CCND1 expression via miR-655-3p.

Discussion

AML patients usually exhibit a poor long-term survival due to the high relapse resulting from chemoresistance. The current combination treatment, primarily relies on the anchor drug Ara-C,

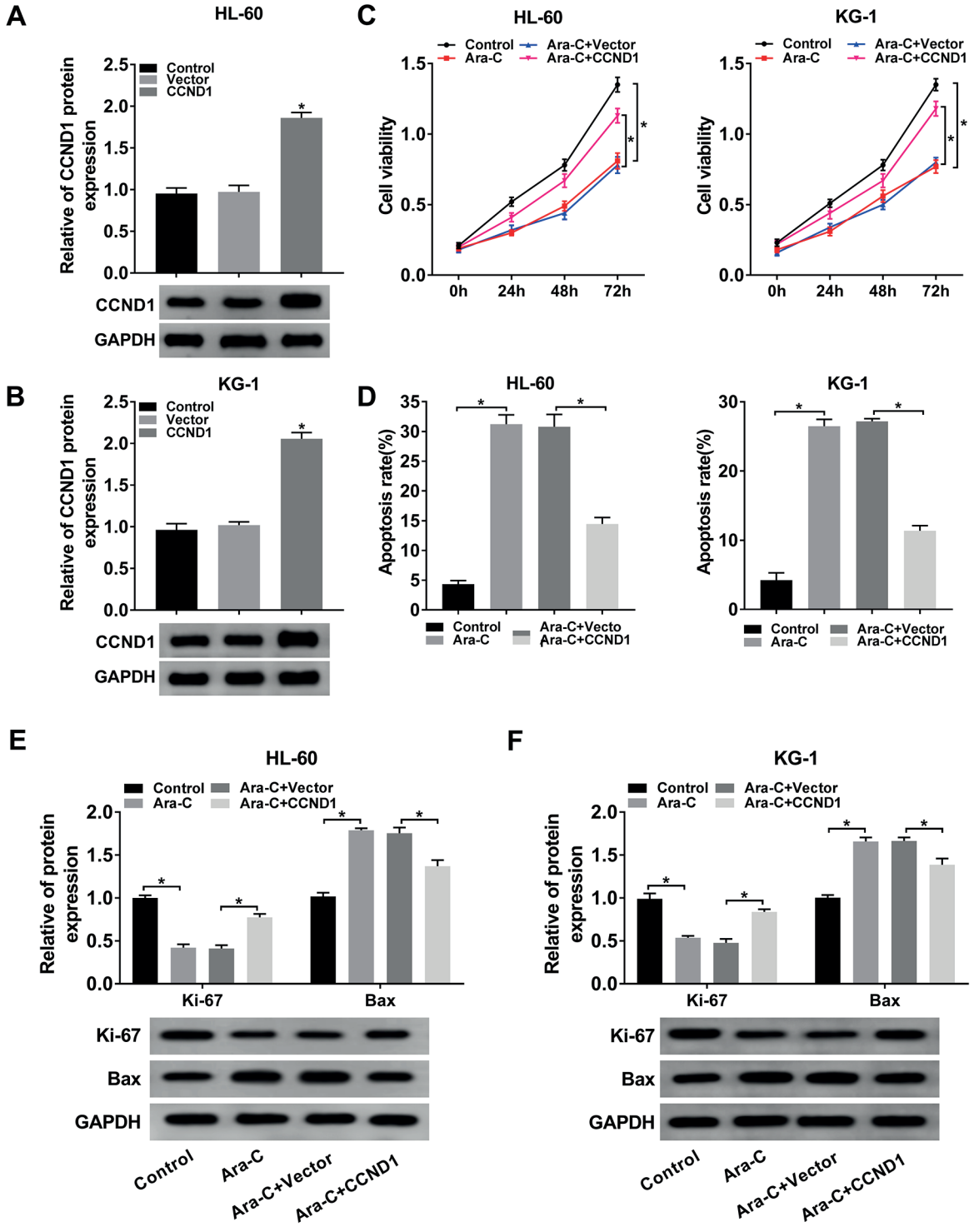

Figure 7. CCND1 overturns proliferation inhibition and apoptosis promotion mediated by Ara-C in AML cells. **A-B**, Western blot analysis of CCND1 expression in HL-60 and KG-1 cells transfected with Vector or CCND1. After treatment with 4 μM Ara-C, (**C**) proliferation analysis of HL-60 and KG-1 cells using CCK-8 assay; **D**, apoptosis rate detection of HL-60 and KG-1 cells with flow cytometry; **E-F**, levels analysis of Ki-67 and Bax in HL-60 and KG-1 cells with Western blot. *p<0.05.

has remained essentially unaltered in the past 50 years and remains the standard induction regimen internationally[22,23]. Although 70-80% of AML patients have remission after induction chemotherapy, 80% of them relapse for which no salvage regimen currently exists[24]. Thus, exploring potential molecular mechanisms involved in the chemoresistance of AML is imperative to treat with AML effectively. The novelty of this study was that the targeted relationship between miR-

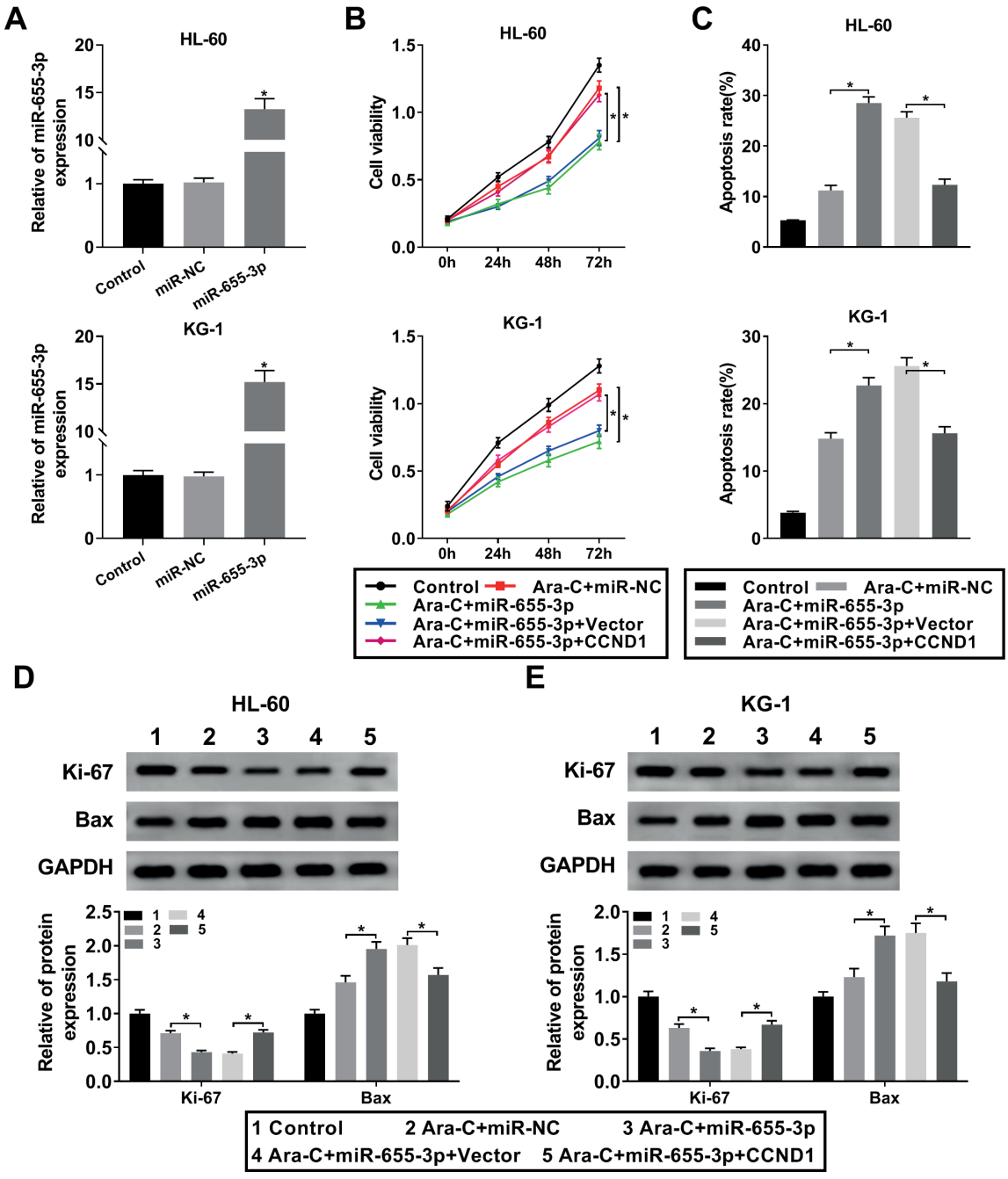

Figure 8. MiR-655-3p sensitizes AML cells to Ara-C by targeting CCND1. **A**, qRT-PCR analysis of miR-655-3p in HL-60 and KG-1 cells transfected with miR-NC or miR-655-3p. HL-60 and KG-1 cells were transfected with miR-NC, miR-655-3p, miR-655-3p + Vector, or miR-655-3p + CCND1 and treated with 4 μM Ara-C, (**B**) CCK-8 assay analysis of HL-60 and KG-1 cell proliferation; **C**, flow cytometry analysis of HL-60 and KG-1 cell apoptosis; **D-E**, Western blot analysis of Ki-67 and Bax levels in HL-60 and KG-1 cells. *p<0.05.

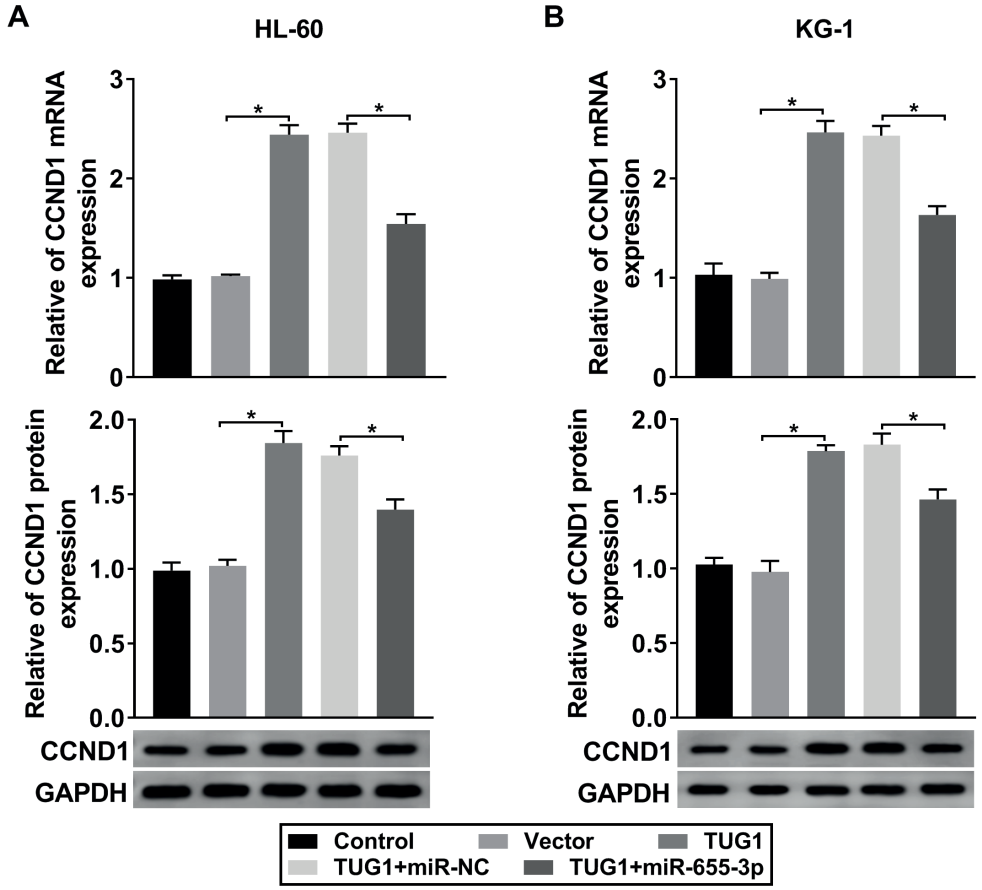

Figure 9. TUG1 regulates CCND1 expression by sponging miR-655-3p. **A-B**, qRT-PCR and Western blot analysis of CCND1 levels in HL-60 and KG-1 cells transfected with Vector, TUG1, TUG1 + miR-NC, or TUG1 + miR-655-3p. *$p<0.05$.

655-3p and TUG1 or CCND1 was first confirmed, and we first constructed the lncRNA-associated competing endogenous RNA (ceRNA) regulatory network of TUG1/miR-655-3p/CCND1 axis in AML cells. LncRNAs are now considered to play roles in various physiological processes and have been involved in multiple malignancies, where lncRNAs can serve as either tumor promoters or tumor suppressors[25]. Hematologic malignancies are no exception, as dysregulated lncRNAs expression benefits blood cancers, particularly AML[26]. For example, lncRNA UCA1 knockdown inhibited glycolysis to enhance the chemosensitivity of pediatric AML via miR-125a/hexokinase 2 pathway[27]. LncRNA HOTTIP promoted AML cell proliferation and cell cycle progression through regulating miR-608/DDA1 axis to accelerate development[28]. TUG1 has been revealed to be related to malignant phenotypes and Adriamycin resistance in AML[17,18], suggesting TUG1 may be a potential biomarker for the interference of AML. In this study, we discovered that TUG1 reversed Ara-C-induced cell proliferation inhibition and cell apoptosis promotion in AML, thus blocking the sensitivity of AML cells to Ara-C.

LncRNAs are usually reported to function as miRNA sponges, which involve in the regulation of gene expression[29]. MiR-655-3p is a functional miRNA which has been demonstrated to act as a tumor suppressor to impede tumor progression in many cancers, such as ovarian cancer[30], hepatocellular carcinoma[31], non-small cell lung cancer[32], and so on. However, there were no studies on the roles of miR-655-3p in AML. In this investigation, we observed that miR-655-3p was decreased in AML cells, miR-655-3p inhibition attenuated Ara-C-induced inhibition of AML cell proliferation and promotion of cell apoptosis, indicating that miR-655-3p sensitized AML cells to Ara-C. Notably, this study confirmed TUG1 was a sponge of miR-655-3p, rescues assay showed TUG1 silence sensitized AML cells to Ara-C by regulating miR-655-3p.

CCDN1 is a gene located on chromosome 11q13 and encodes the cyclin D1 protein[33], which belongs to the highly conserved cyclin family and can regulate CDK kinases[34]. CCDN1 is a pivotal gene, which functions in the regulation of G1/S transition during cell cycle[35]. Mutations, amplification and overexpression of CCDN1 alter cell cycle progression, and occur in a variety of cancers and have a role in carcinogenesis[36]. Additionally, it has also been found alterations in the CCND1 are associated with the aggressive growth of leukemia cell lines in AML[37]. In the current work, CCDN1 was found to be elevated in AML cells, and CCDN1 overexpression abated Ara-C-induced inhibition on AML cell malignant growth. Thus, CCDN1 weakened Ara-C sensitivity in AML cells.

LncRNAs can act as ceRNAs and suppress miRNA function by preventing miRNAs from interacting with target mRNAs and affected translation of protein-coding genes[38]. Recently, it was reported that TUG1 participated in the tumor progression by serving as a ceRNA of miRNAs[39,40]. Nonetheless, whether TUG1 could function as a ceRNA of miR-655-3p in AML progression remains undefined and the lncRNA-miRNA-mRNA ceRNA regulatory network about TUG1 needs to be elucidated. In this study, we verified that CCDN1 was a target of miR-655-3p, and miR-655-3p reinforced the sensitivity of AML cells to Ara-C by down-regulating CCND1. Of note, we also confirmed that TUG1 positively regulated CCND1 via miR-655-3p. Therefore, it was testified that TUG1 could function as a ceRNA by competitively binding to miR-655-3p and thus regulating CCDN1 expression in AML tumorigenesis and progression, thus suppressing the tumorigenic effect of CCDN1. Finally, the lncRNA TUG1-associated TUG1/miR-655-3p/CCND1 ceRNA regulatory network in AML cells was constructed.

Conclusions

We demonstrated that TUG1 or CCND1 overexpression, and miR-655-3p down-regulation enhanced Ara-C resistance of AML cells to Ara-C, and TUG1 could regulate Ara-C sensitivity in AML cells by miR-655-3p/CCND1 axis, suggesting novel therapeutically workable targets to improve disease outcome in AML.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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