

Kinesin family member 2A acts as a potential prognostic marker and treatment target via interaction with PI3K/AKT and RhoA/ROCK pathways in acute myeloid leukemia

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Abstract. KIF2A has been shown to be involved in the regulation of AML pathology, however, the mechanistic role of KIF2A in AML has not been fully identified. The present study aimed to identify the underlying mechanism of KIF2A regulation of AML cell function and chemosensitivity. A total of 58 patients with AML and 30 healthy subjects were enrolled for clinical analysis. AML cells (KG-1 and Kasumi-1) were transfected with KIF2A or control small interfering (si)RNA. PI3K/AKT pathway activator (740 Y-P) and RhoA overexpression plasmid were added to rescue the effect of KIF2A siRNA. Cell proliferation, apoptosis, chemosensitivity to ADR and AraC, expression levels of mRNA/proteins associated with PI3K/AKT and RhoA/ROCK pathways were measured by Cell Counting Kit-8, flow cytometry, reverse transcription-quantitative PCR and western blotting. KIF2A was overexpressed, and correlated with higher levels of bone marrow blast, poor risk classification, lower treatment response and unfavorable survival profile in patients with AML. KIF2A siRNA inhibited proliferation but enhanced apoptosis and chemosensitivity to ADR and AraC in KG-1 and Kasumi-1 cells, which also inactivated PI3K/AKT and RhoA/ROCK pathways. Subsequent rescue experiments showed that 740 Y-P and RhoA overexpression plasmid promoted cell survival and decreased chemosensitivity, which reversed

the effect of KIF2A siRNA in KG-1 and Kasumi-1 cells. KIF2A was correlated with worse clinical features and survival in patients with AML; its knockdown promoted apoptosis and chemosensitivity by inactivating PI3K/AKT and RhoA/ROCK signaling pathways in AML cells. These data suggested KIF2A may be a potential prognostic marker and treatment target for AML management.

Introduction

Acute myeloid leukemia (AML), a heterogeneous disease of, but not restricted to, the bone marrow is characterized by abnormal clonal augmentation of immature myoblasts and presents with a poor prognosis due to rapid progress (1,2). AML accounts for more than a half of leukemia-associated mortality, with a 5-year survival rate of ~27% (3). Especially, the elderly are more sensitive to the development of AML; most cases present in individuals aged >65 years, which adds the difficulty of management due to relatively poor physical condition (4). Although novel therapies have been introduced for AML treatment over past decades, limited progress has been made in enhancing patient survival profile (5,6). Hence, to develop novel treatment options for AML, its associated molecule mechanisms, such as driver genes and pathways, have been extensively studied (7,8).

Kinesins are a class of motor proteins that are key for intracellular transport of multiple materials, such as adenovirus, and participate in almost all important cellular functions (9-11). Kinesin family member 2A (KIF2A), a member of the Kinesin-13 family, regulates mitosis via supporting the formation of the bipolar spindle (12). KIF2A is promising target in the exploration of cancer pathology, as indicated by reports of its function and potential clinical utility in multiple types of cancer, including nasopharyngeal carcinoma, non-small cell lung cancer and AML (13-15). Previously, KIF2A has been shown to be correlated with clinical features of patients with AML and regulate AML cell proliferation and apoptosis, indicating that KIF2A is involved in the regulation of AML pathology (15). However, the mechanistic role of KIF2A in AML has not been fully identified.

The present study aimed to detect the effect of KIF2A on AML cell survival and chemosensitivity, as well as the pathways by which it exerts these effects.

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Abbreviations: KIF2A, kinesin family member 2A; AML, acute myeloid leukemia; ADR, Adriamycin; RT-q, reverse transcription-quantitative; EFS, Event-free survival; OS, overall survival; NC, negative control; CCK-8, Cell Counting Kit-8; AV/PI, Annexin V/propidium iodide; siRNA, small interfering RNA

Key words: acute myeloid leukemia, kinesin family member 2A, PI3K/AKT pathway, ras homolog family member A/rho-associated coiled-coil containing protein kinase pathway, cell function, chemosensitivity

Materials and methods

Subjects. The present study was approved by the Institutional Review Board of First Affiliated Hospital of Anhui Medical University. A total of 58 newly diagnosed patients with AML and 30 healthy subjects (56.7% males and 43.3% females) with a mean age of 53.2 ± 6.6 years (range, 33 to 79 years) from First Affiliated Hospital of Anhui Medical University (Anhui, China) were enrolled from January 2015 to December 2018. All patients were newly diagnosed with AML rather than acute promyelocytic leukemia and were aged >18 years, without a history of other hematological malignancy or cancer or radiotherapy or chemotherapy before enrollment. The healthy subjects were all aged >18 years, recruited during scheduled bone marrow donation and blood routine indexes, tumor markers and blood biochemistry tests were normal. All subjects provided written informed consent.

Data and sample collection. After recording the clinical characteristics of patients with AML (Table I), bone marrow samples were collected before starting induction therapy. Bone marrow samples were also collected from healthy donors. Immediately following collection, bone marrow mononuclear cells (BMMCs) were isolated from bone marrow by gradient centrifugation (at $600 \times g$ for 15 min at 25°C) using Percoll (Sigma-Aldrich; Merck KGaA), followed by reverse transcription quantitative (RT-q)PCR assay for determination of KIF2A expression levels. For patients with AML, remission status was documented following completion of the response evaluation for induction therapy. Clinical follow-up was conducted until December 31, 2019. Event-free survival (EFS) and overall survival (OS) were estimated for analysis of survival prognosis; in addition, patients with AML were divided into high- and low KIF2A expression groups according to the median value of KIF2A expression (2.638).

Cell line culture and KIF2A expression detection. Human AML cell lines, including KG-1 [American Type Culture Collection (ATCC)], Kasumi-1 (ATCC), MOLM-13 [Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)] and OC-AML2 (DSMZ), were cultured with 90% RPMI-1640 supplemented with 10% fetal bovine serum (FBS; both Hyclone; Cytiva) according to the manufacturer's instructions. The cells were cultured in 5% CO_2 at 37°C . The expression of KIF2A in AML cell lines was determined by RT-qPCR and western blotting using BMMCs from healthy subjects as the control.

KIF2A small interfering RNA (siRNA) transfection. The transfection of 50 nM negative control (NC) or KIF2A siRNA (both Shanghai GenePharma Co., Ltd.) into AML cells (KG-1 and Kasumi-1) was performed using HillyMax reagent (Dojindo Molecular Technologies, Inc.) at 37°C for 6 h. Untreated cells were labeled as Untreated cells. At 48 h after transfection, RT-qPCR, western blotting and Annexin V/propidium iodide (AV/PI) and chemosensitivity assay were performed. At 0, 24, 48 and 72 h after transfection, Cell Counting Kit-8 (CCK-8) assay was performed. The target sequence of KIF2A siRNA was 5'-GGCAAAGAG AUUGACCUUG-3' and the scrambled sequence used for NC siRNA was 5'-AAGAACAACACAAAAGAACAG-3'.

740 Y-P incubation. The concentration of 740 Y-P (Selleck Chemicals) incubated with AML cells (KG-1 and Kasumi-1) was $25 \mu\text{g/ml}$, and the cells were incubated for 48 h at 37°C . The transfection of NC or KIF2A siRNA was performed as aforementioned at the same time as 740 Y-P incubation. Cells were divided into five groups as follows: i) Untreated cells; ii) NC siRNA, cells transfected with NC siRNA; iii) KIF2A siRNA, cells transfected with KIF2A siRNA; iv) NC siRNA + 740 Y-P, cells were transfected with NC siRNA and incubated with 740 Y-P and v) KIF2A siRNA + 740 Y-P, cells were transfected with KIF2A siRNA and incubated with 740 Y-P. Following incubation, RT-qPCR, western blotting, CCK-8 and AV/PI and chemosensitivity assay were performed.

Ras homolog family member A (RhoA) plasmid transfection. The sequence of RhoA was obtained from the National Center of Biotechnology Information (reference no. NM_001664.4). The sequence used for NC was 5'-AACACCGAACGAGAC AGGATT-3'. NC or RhoA overexpression plasmids were constructed using pcDNA3.1 vector (Shanghai GenePharma Co., Ltd.). Then, 50 nM NC or RhoA overexpression plasmid was co-transfected with 50 nM NC or KIF2A siRNA into AML cells (KG-1 and Kasumi-1) using HillyMax (Dojindo Molecular Technologies, Inc.) at 37°C for 6 h. Cells were divided into groups as follows: i) Untreated cells; ii) NC siRNA + NC plasmid, cells transfected with NC siRNA and NC overexpression plasmid; iii) KIF2A siRNA + NC plasmid, cells transfected with KIF2A siRNA and NC overexpression plasmid; iv) NC siRNA + RhoA plasmid, cells transfected with NC siRNA and RhoA overexpression plasmid and v) KIF2A siRNA + RhoA plasmid, cells transfected with KIF2A siRNA and RhoA overexpression plasmid. RT-qPCR, western blotting and CCK-8, AV/PI and chemosensitivity assay were performed 48 h after transfection.

CCK-8 assay. CCK-8 (Sigma-Aldrich; Merck KGaA) assay was performed. The KG-1 and Kasumi-1 cells (3×10^3) were plated, and $10 \mu\text{l}$ reagent was added for 3 h. Then, the optical density value was read using a microplate reader (BioTek Instruments, Inc.) at 450 nm.

AV/PI assay. AV/PI assay was performed using an Annexin V Apoptosis Detection kit I (BD Biosciences). The KG-1 and Kasumi-1 cells (1×10^5 ; $100 \mu\text{l}$) were stained with AV ($5 \mu\text{l}$) and PI ($5 \mu\text{l}$) for 15 min at room temperature after being digested. A FACSCalibur flow cytometer (BD Biosciences) was used to analyze the cells. The data was analyzed by FlowJo 7.6 (BD Biosciences).

RT-qPCR. The extraction of total RNA from BMMCs from healthy subjects was performed using RNeasy Mini kit (Qiagen GmbH) in accordance with the manufacturer's protocol. The complementary DNA was generated using qPCR RT Master Mix (Toyobo Life Science) and $1 \mu\text{g}$ RNA. Realtime PCR Master Mix (Toyobo Life Science) was used to perform qPCR. The thermocycling conditions were as follows: 95°C for 60 sec, followed by 40 cycles of 95°C for 15 sec and 61°C for 30 sec. Relative expression was calculated via the $2^{-\Delta\Delta\text{C}_q}$ method (16). The primers for KIF2A and β -actin (internal reference) were as follows: KIF2A forward, 5'-GCCGAATACATCAAGCAAT-3'

Table I. Clinical features of patients with AML.

Characteristic	Patients (n=58)
Age, years	
Mean \pm SD	57.9 \pm 11.6
Median (IQR)	60.0 (48.8-68.0)
Sex (%)	
Male	34.0 (58.6)
Female	24.0 (41.4)
WBC ($\times 10^9/l$)	
Mean \pm SD	17.2 \pm 16.1
Median (IQR)	11.6 (4.6-25.1)
BM blasts, %	
Mean \pm SD	73.8 \pm 13.5
Median (IQR)	75.0 (61.8-85.0)
FAB classification (%)	
M1	3.0 (5.2)
M2	16.0 (27.6)
M4	17.0 (29.3)
M5	22.0 (37.9)
Cytogenetic abnormality (%)	
NK	29.0 (50.0)
CK	7.0 (12.1)
inv(16) or t(16;16)	4.0 (6.9)
MK	2.0 (3.4)
+8	2.0 (3.4)
t(9;11)	2.0 (3.4)
-7 or 7q-	1.0 (1.7)
-5 or 5q-	1.0 (1.7)
t(8;21)	1.0 (1.7)
Other non-defined	11.0 (19.0)
Molecular abnormality (%)	
Mutated NPM1	17.0 (29.3)
Mutated FLT3-ITD	15.0 (25.9)
Mutated WT1	7.0 (12.1)
Mutated CEBPA	6.0 (10.3)
Risk classification (%)	
Favorable	11.0 (19.0)
Intermediate	31.0 (53.4)
Poor	16.0 (27.6)

AML, acute myeloid leukemia; IQR, interquartile range; WBC, white blood cell; BM, bone marrow; FAB, French-American-British; NK, normal karyotype; CK, complex karyotype; MK, monosomal karyotype; NPM1, nucleophosmin 1; FLT3-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; WT1, Wilms' tumor 1; CEBPA, CCAAT/enhancer binding protein α .

and reverse, 5'-CTCTCCAGGTCAATCTCTT-3' and β -actin forward, 5'-TCGTGCGTGACATTAAGGAGAAG-3' and reverse, 5'-AGGAAGGAAGGCTGGAAGAGTG-3'.

Western blot analysis. RIPA lysis buffer (Beyotime Institute of Biotechnology) was used to isolate total protein from AML cells

and BMBCs from healthy subjects. The quantification of total protein was performed via BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Then, the total protein (20 μ g) was separated by 4-20% SDS-PAGE (Beyotime Institute of Biotechnology) and transferred to nitrocellulose membranes (Pall Life Sciences). The membranes were then blocked with 5% BSA (Beyotime, China) at 37°C for 1 h. Afterwards, the diluted primary antibodies (4°C, overnight) and secondary antibody (37°C, 1 h) were then incubated with the membrane. Protein bands were visualized using ECL western blotting substrate (Thermo Fisher Scientific, Inc.) and quantified with ImageJ V 1.52v (National Institutes of Health). The antibodies are listed in Table SI.

Chemosensitivity assay. Following incubation as aforementioned, the KG-1 and Kasumi-1 cells (5×10^3) were treated with different concentrations of Adriamycin (ADR; 0.0, 0.1, 0.2, 0.4, 0.8 and 1.6 μ M) (Selleck Chemicals) or Arabinofuranosyl Cytidine (AraC; 0, 10, 20, 40, 80 and 160 nM) in a 96-well plate for 48 h at 37°C. Then, CCK-8 assay was performed as aforementioned.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 7.02 (GraphPad Software Inc.). Data are presented as the mean \pm standard deviation or median and interquartile range. Differences in expression levels between groups were determined by Kruskal-Wallis (with post hoc Dunn's multiple comparisons test) or Mann-Whitney U test. Correlation analysis was performed using Spearman's test. Receiver operating characteristic (ROC) curve analysis was used to estimate the value of KIF2A in distinguishing patients with AML from healthy donors as well as CR from non-CR AML. EFS and OS are shown as Kaplan-Meier curves and were analyzed by Log-rank test. Comparison among groups was assessed by one-way ANOVA, subsequently, the multiple comparisons were assessed by Dunnett or Tukey's test. A two-side $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Correlation of KIF2A with risk, feature and prognosis of AML. A total of patients with 58 AML (58.6% males and 41.4% females) were enrolled, with a mean age of 57.9 \pm 11.6 years (Table I). A total of 30 healthy donors were also recruited. KIF2A was upregulated in patients with AML compared with healthy donors ($P < 0.001$; Fig. 1A). ROC analysis revealed the ability of KIF2A to differentiate between patients with AML and healthy donors, with the area under curve (AUC) of 0.922 (95% CI, 0.865-0.979; Fig. 1B). Furthermore, KIF2A was correlated with increased bone marrow blasts ($P = 0.018$; Fig. 2A) and poor National Comprehensive Cancer Network risk classification ($P = 0.016$; Fig. 2C) but was not correlated with FAB classification ($P = 0.528$; Fig. 2B) in patients with AML. In addition, KIF2A was downregulated in patients with complete response (CR) compared with non-CR patients ($P = 0.029$; Fig. 2D). ROC analysis demonstrated that KIF2A differentiated between CR and non-CR patients, with an AUC of 0.695 (95% CI, 0.529-0.861; Fig. 2E). EFS ($P = 0.026$; Fig. 2F) exhibited a less favorable association whereas OS ($P = 0.092$; Fig. 2G) was not significantly different between patients with high and low KIF2A expression.

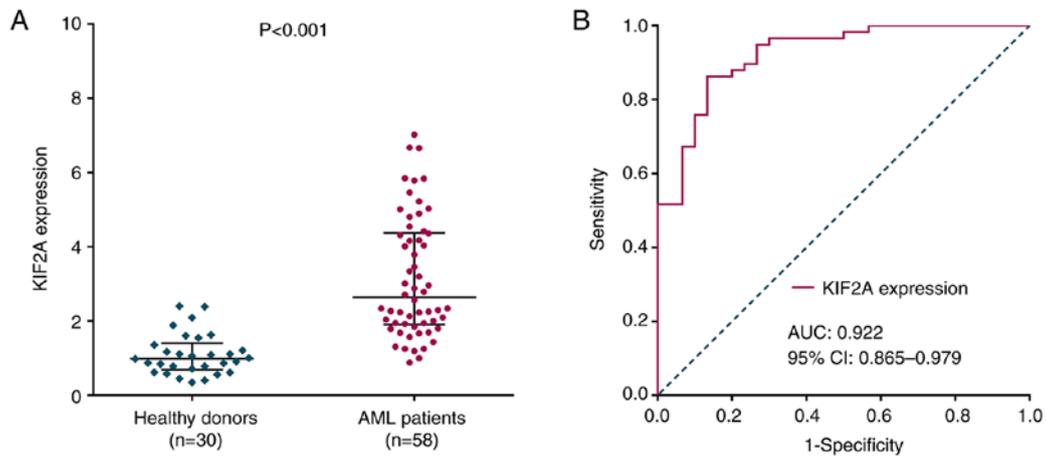


Figure 1. Comparison of KIF2A expression levels between patients with AML and healthy subjects. (A) KIF2A expression in patients with AML and healthy subjects. (B) receiver operating curve analysis demonstrated KIF2A expression is able to differentiate between patients with AML and healthy donors. KIF2A, kinesin family member 2A; AML, acute myeloid leukemia; AUC, area under curve.

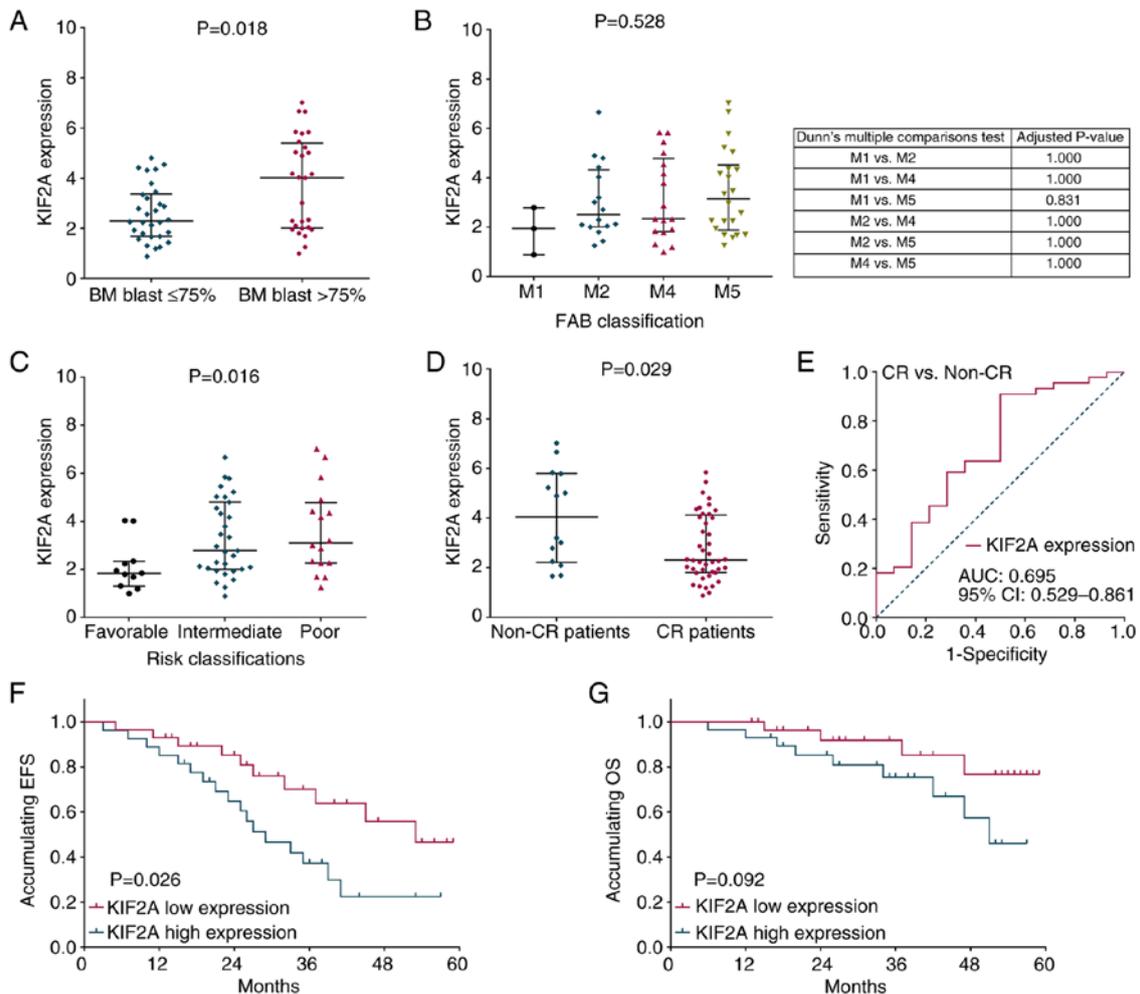


Figure 2. Correlation of KIF2A expression with clinical features and efficacy in patients with AML. The correlation of KIF2A expression with (A) BM blasts, (B) FAB classification, (C) risk classification and (D) CR. (E) KIF2A expression can differentiate between patients with and without CR. Correlation of KIF2A expression with (F) EFS and (G) OS. KIF2A, kinesin family member 2A; AML, acute myeloid leukemia; BM, bone marrow; FAB, French-American-British; CR, complete response; EFS, event-free survival; OS, overall survival; AUC, area under curve.

KIF2A expression in AML cell lines. KIF2A mRNA expression levels were increased in AML cell lines KG-1 ($P < 0.01$), Kasumi-1 ($P < 0.01$), MOLM-13 ($P < 0.05$) and OCI-AML2

($P < 0.05$) compared with NC cells (Fig. 3A). Protein expression of KIF2A was also elevated in KG-1 ($P < 0.01$), Kasumi-1 ($P < 0.01$), MOLM-13 ($P < 0.05$) and OCI-AML2 ($P < 0.05$) cell

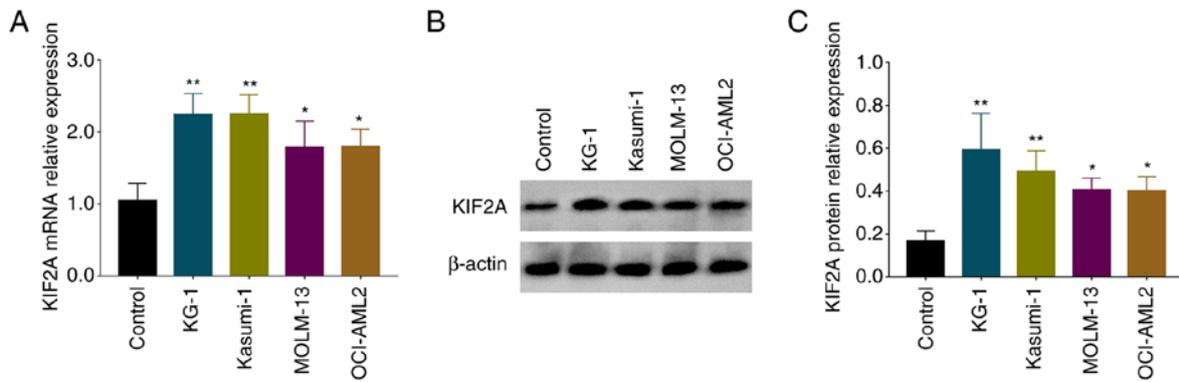


Figure 3. KIF2A expression is upregulated in AML cells. KIF2A (A) mRNA and (B and C) protein expressions in AML cell lines and NC cells. * $P < 0.05$, ** $P < 0.01$ vs. Control. KIF2A, kinesin family member 2A; AML, acute myeloid leukemia.

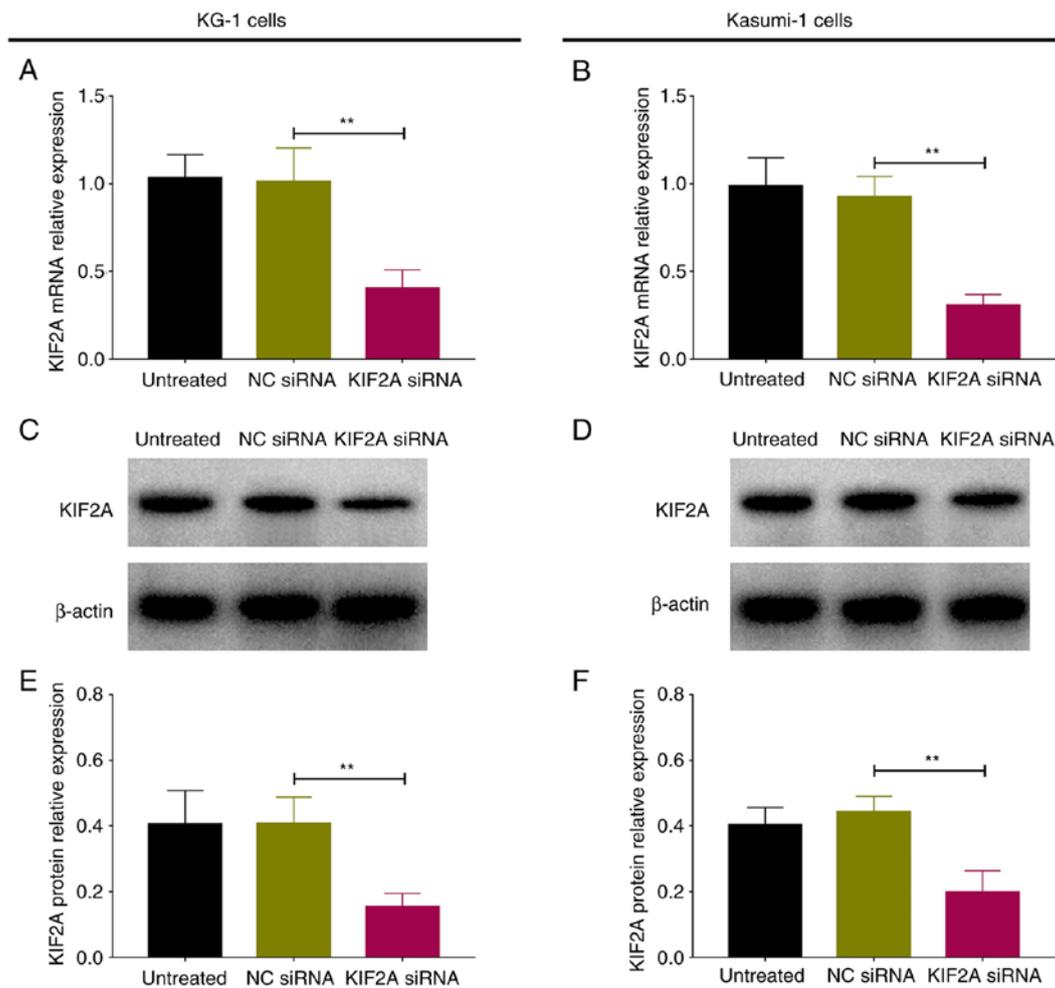


Figure 4. KIF2A siRNA was successfully transfected in AML cells. KIF2A mRNA expression levels in (A) KG-1 and (B) Kasumi-1 cells. Western blot analysis of (C) KG-1 and (D) Kasumi-1 cells. Relative KIF2A protein expression levels in (E) KG-1 and (F) Kasumi-1 cells. ** $P < 0.01$. KIF2A, kinesin family member 2A; AML, acute myeloid leukemia; NC, negative control; si, small interfering.

lines compared with NC cells (Fig. 3B and C). Expression of KIF2A mRNA and protein levels was higher in KG-1 and Kasumi-1 cell lines, thus, KG-1 and Kasumi-1 cell lines were selected for subsequent molecular experiments.

Effect of KIF2A inhibition on AML cell proliferation, apoptosis and chemosensitivity. Following transfection, KIF2A mRNA

and protein relative expression levels were downregulated in KIF2A siRNA compared with NC siRNA group in KG-1 and Kasumi-1 cells (all $P < 0.01$; Fig. 4). These data indicated that transfection of KIF2A siRNA was successful in both cell lines.

Following transfection, in KG-1 cells, KIF2A siRNA inhibited proliferation but promoted cell apoptosis (both $P < 0.01$; Fig. 5A-C). Chemosensitivity to ADR was enhanced

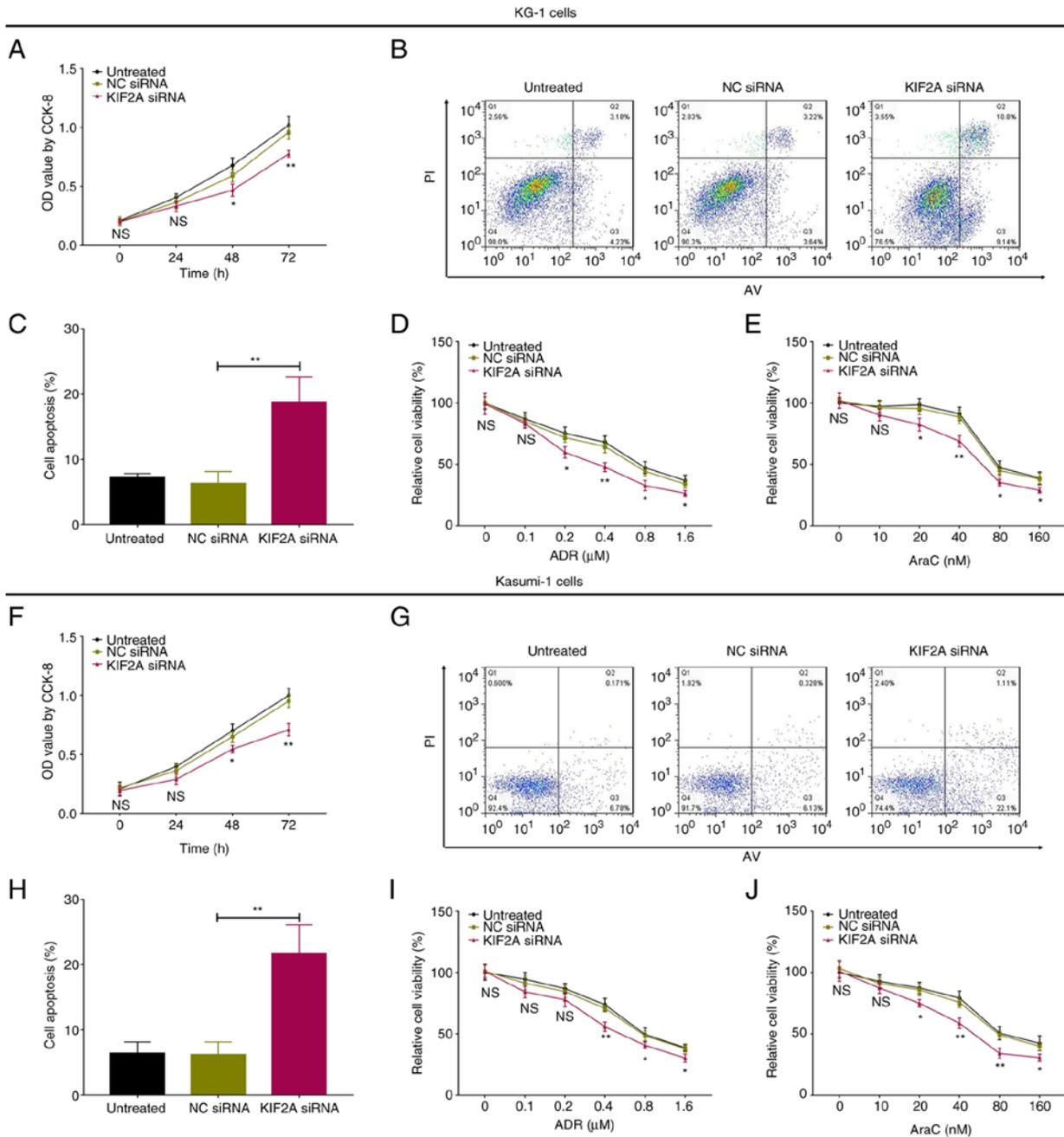


Figure 5. KIF2A siRNA regulates proliferation, apoptosis and chemosensitivity of AML cells. Cell (A) proliferation, (B and C) cell apoptosis and chemosensitivity to (D) ADR and (E) AraC in KG-1 cells transfected with KIF2A siRNA. Cell (F) proliferation, (G and H) apoptosis and chemosensitivity to (I) ADR and (J) AraC in Kasumi-1 cells transfected with KIF2A siRNA. * $P < 0.05$, ** $P < 0.01$ KIF2A vs. NC siRNA. KIF2A, kinesin family member 2A; AML, acute myeloid leukemia; NC, negative control; ADR, Adriamycin; AraC, Arabinofuranosyl Cytidine; si, small interfering; OD, optical density; CCK-8, Cell Counting Kit-8.

by KIF2A siRNA at 0.2 ($P < 0.05$), 0.4 ($P < 0.01$), 0.8 ($P < 0.05$) and 1.6 μ M ($P < 0.05$; Fig. 5D). Chemosensitivity to AraC was enhanced by KIF2A siRNA at 20 ($P < 0.05$), 40 ($P < 0.01$), 80 ($P < 0.05$) and 160 μ M ($P < 0.05$; Fig. 5E). In Kasumi-1 cells, KIF2A also decreased cell proliferation ($P < 0.05$; Fig. 5F), but increased cell apoptosis ($P < 0.01$; Fig. 5G and H). In addition, chemosensitivity to ADR was promoted by KIF2A siRNA at 0.4 ($P < 0.01$), 0.8 ($P < 0.05$) and 1.6 μ M ($P < 0.05$; Fig. 5I); chemosensitivity to AraC was enhanced by KIF2A siRNA at 20 ($P < 0.05$), 40 ($P < 0.01$), 80 ($P < 0.01$) and 160 μ M ($P < 0.05$; Fig. 5J).

PI3K/AKT and RhoA/rho-associated coiled-coil containing protein kinase (ROCK) signaling pathways are inhibited by KIF2A inhibition in AML cells. In KG-1 cells, phosphorylated (p-)PI3K ($P < 0.01$), p-AKT ($P < 0.05$), RhoA ($P < 0.05$) and ROCK1 ($P < 0.05$) protein expression levels were inhibited by KIF2A siRNA (Fig. 6A and C). Similarly, in Kasumi-1 cells, KIF2A siRNA suppressed p-PI3K ($P < 0.01$), p-AKT ($P < 0.01$), RhoA ($P < 0.01$) and ROCK1 ($P < 0.05$) protein expression levels.

PI3K/AKT pathway activation regulates proliferation, apoptosis and chemosensitivity in KIF2A-knockdown AML

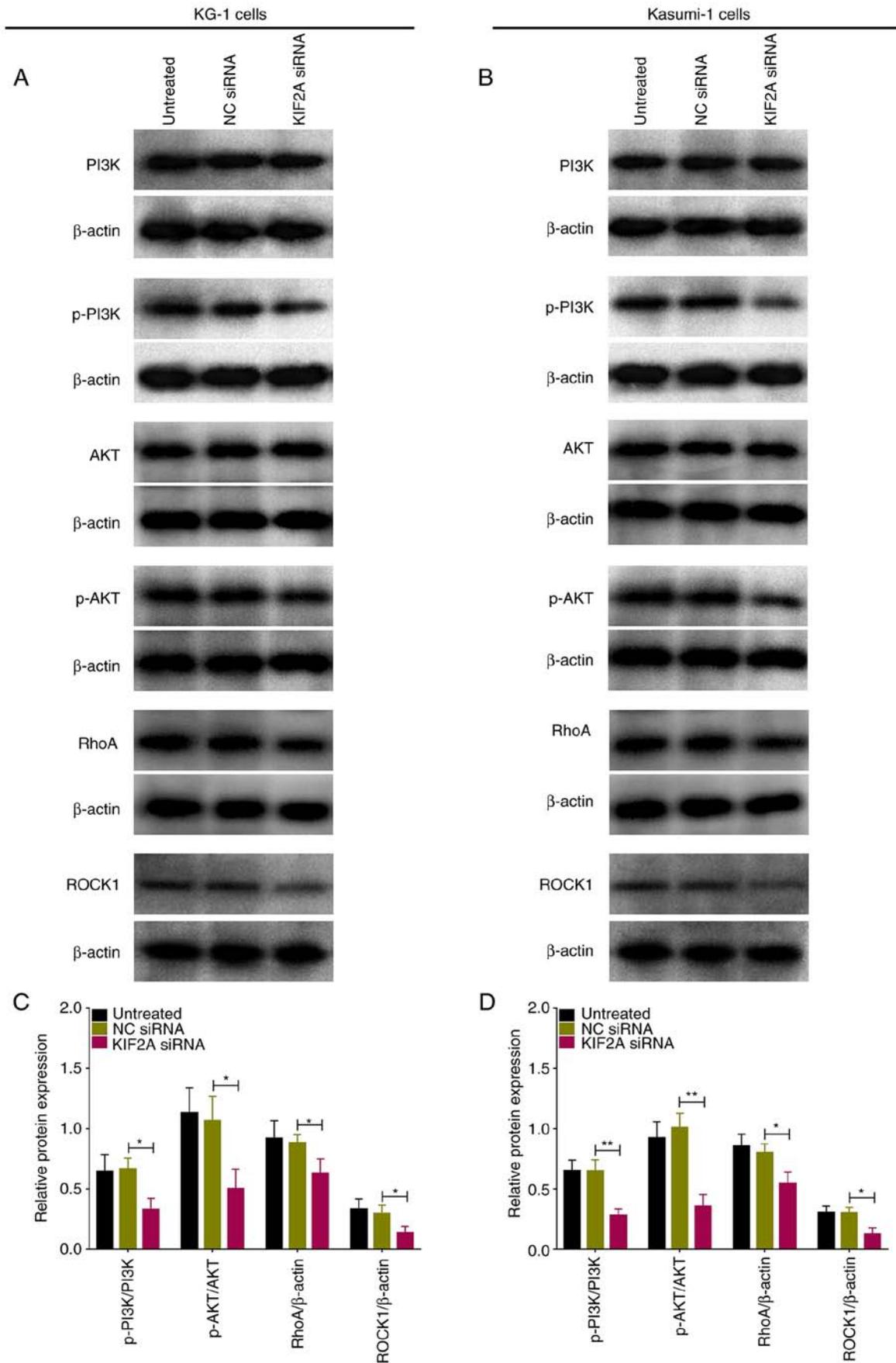


Figure 6. KIF2A siRNA regulates PI3K/AKT and RhoA/ROCK pathways in AML cells. Western blot analysis of (A) KG-1 and (B) Kasumi-1 cells following transfection with KIF2A siRNA. Relative PI3K, p-PI3K, AKT, p-AKT, RhoA and ROCK1 protein expression levels in (C) KG-1 and (D) Kasumi-1 cells transfected with KIF2A siRNA. * $P < 0.05$, ** $P < 0.01$. KIF2A, kinesin family member 2A; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; RhoA, ras homolog family member A; ROCK1, Rho associated coiled-coil containing protein kinase 1; AML, acute myeloid leukemia; NC, negative control; si, small interfering; p-, phosphorylated.

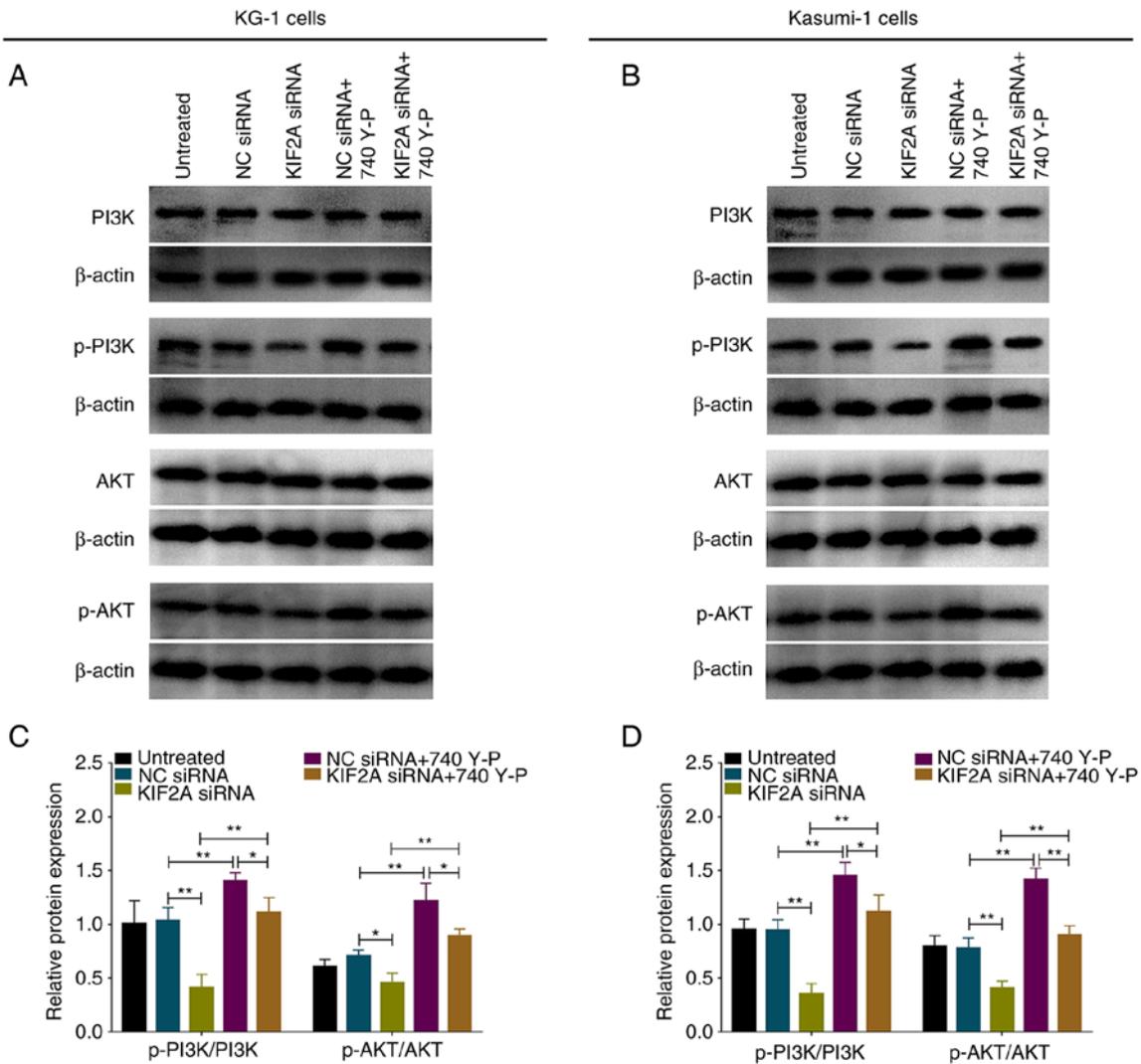


Figure 7. PI3K/AKT activator treatment in AML cells. Western blot analysis of (A) KG-1 and (B) Kasumi-1 cells. Relative PI3K, p-PI3K, AKT and p-AKT protein expression levels in (C) KG-1 and (D) Kasumi-1 cells (B and D). * $P < 0.05$, ** $P < 0.01$. PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; AML, acute myeloid leukemia; p-, phosphorylated; NC, negative control; KIF2A, kinesin family member 2A; si, small interfering.

cells. In rescue experiments, the PI3K/AKT pathway activator (740 Y-P) elevated p-PI3K and p-AKT expression levels in both wild-type ($P < 0.05$ and $P < 0.01$) and KIF2A-knockdown KG-1 cells (both $P < 0.01$; Fig. 7A and C). Additionally, PI3K/AKT pathway activator also elevated p-PI3K and p-AKT expression in both wild-type and KIF2A-knockdown Kasumi-1 cells (all $P < 0.01$; Fig. 7B and D).

In KG-1 cells, the PI3K/AKT pathway activator increased cell proliferation but inhibited cell apoptosis and chemosensitivity to ADR and AraC (all $P < 0.05$; Fig. 8A-E); furthermore, PI3K/AKT activation also reversed the effect of KIF2A siRNA on cell proliferation, apoptosis and chemosensitivity (all $P < 0.05$). In Kasumi-1 cells, the PI3K/AKT pathway activator exhibited similar effects as in KG-1 cells (Fig. 8F-J).

RhoA/ROCK pathway activation regulates proliferation, apoptosis and chemosensitivity in KIF2A-knockdown AML cells. In rescue experiments, RhoA overexpression plasmid elevated RhoA and ROCK1 expression levels in both wild-type and KIF2A-knockdown KG-1 and Kasumi-1 cells (all $P < 0.05$; Fig. 9A-D).

In both KG-1 (Fig. S1A) and Kasumi-1 cells (Fig. S1B), RhoA expression was elevated in RhoA plasmid compared with NC plasmid group, indicating successful transfection (both $P < 0.001$). Moreover, in KG-1 cells, RhoA overexpression plasmid enhanced cell proliferation, but inhibited cell apoptosis and chemosensitivity to ADR and AraC (all $P < 0.05$); it also eliminated the effect of KIF2A siRNA on KG-1 cell proliferation, apoptosis and chemosensitivity ($P < 0.05$; Fig. 10A-E). In Kasumi-1 cells, RhoA overexpression plasmid showed similar effects as in KG-1 cells (Fig. 10F-J).

Discussion

Similarly to other kinesins, KIF2A is essential for cell mitosis. Moreover, KIF2A may serve as a biomarker or therapeutic target in patients with cancer, including AML (17,18). To the best of our knowledge, there is limited evidence to support the potential clinical use of KIF2A in AML. However, KIF2A has been found to be correlated with clinical features or prognosis in other types of cancer. For example, a previous study based on The Cancer Genome Atlas database demonstrated

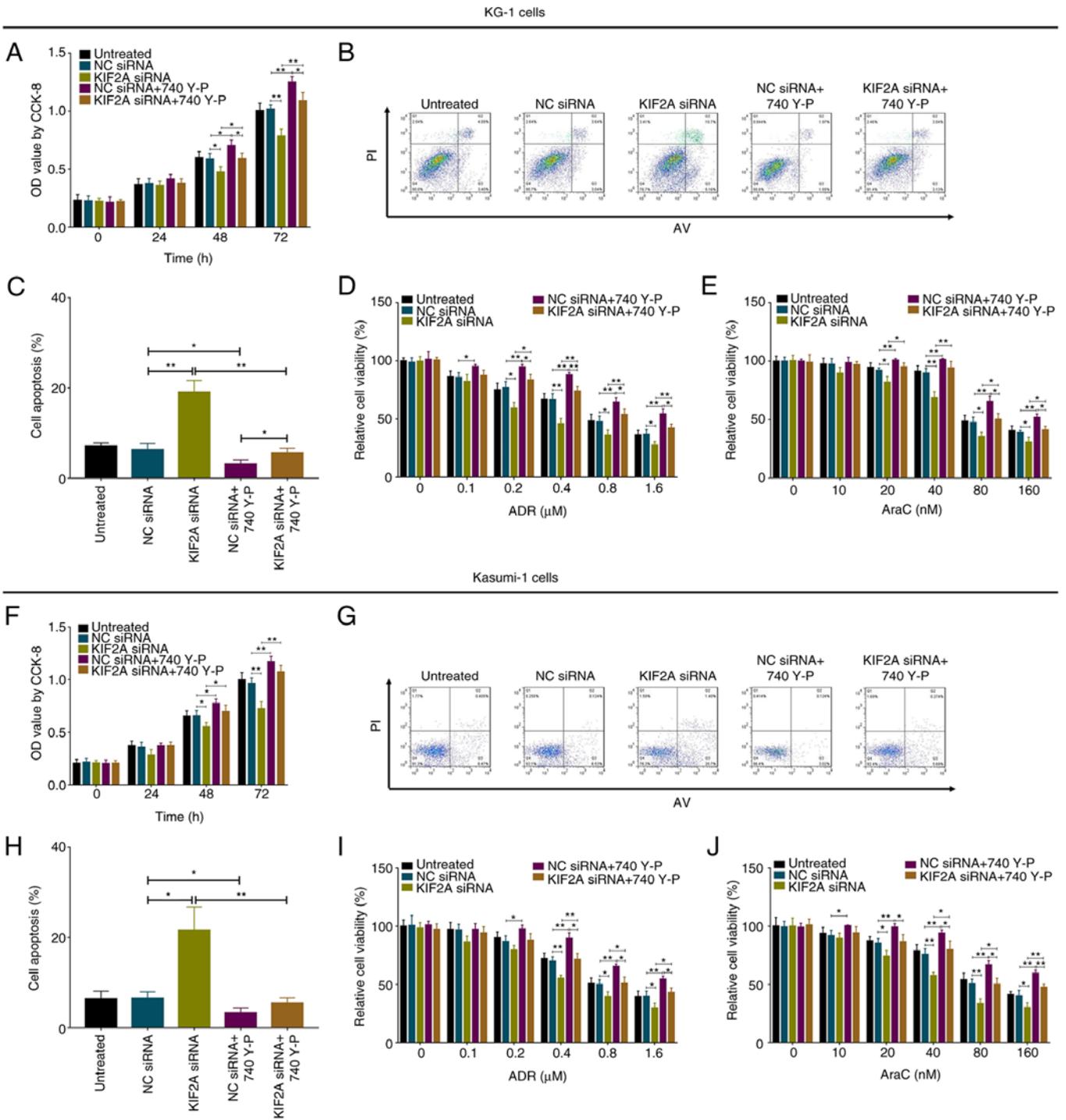


Figure 8. PI3K/AKT activator rescues the effect of KIF2A siRNA on AML cells. (A) Proliferation, (B and C) apoptosis and chemosensitivity to (D) ADR and (E) AraC in KG-1 cells. (F) Proliferation, (G and H) apoptosis and chemosensitivity to (I) ADR and (J) AraC in Kasumi-1 cells. *P<0.05, **P<0.01. PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; KIF2A, kinesin family member 2A; AML, acute myeloid leukemia; NC, negative control; si, small interfering; OD, optical density; CCK-8, Cell Counting Kit-8.

that KIF2A is upregulated in esophageal squamous cell carcinoma tissue and its expression is correlated with worse disease-free survival in patients (19). Another study observed increased expression of KIF2A in patients with gastric cancer, and KIF2A expression is associated with worse histological type, higher TNM stage, lymph node metastasis and decreased 5-year survival rate; in addition, KIF2A is also an independent predicting factor for worse prognosis in patients

with gastric cancer (20). These studies indicated that KIF2A may be a prognostic biomarker for patients with cancer. However, further studies of the clinical value of KIF2A in cancer are required.

In view of the mechanistic role of KIF2A in cancer, a study revealed that KIF2A short hairpin RNA notably suppresses tumor cell proliferation, migration and invasion, and also inhibits tumor growth and metastasis in osteosarcoma

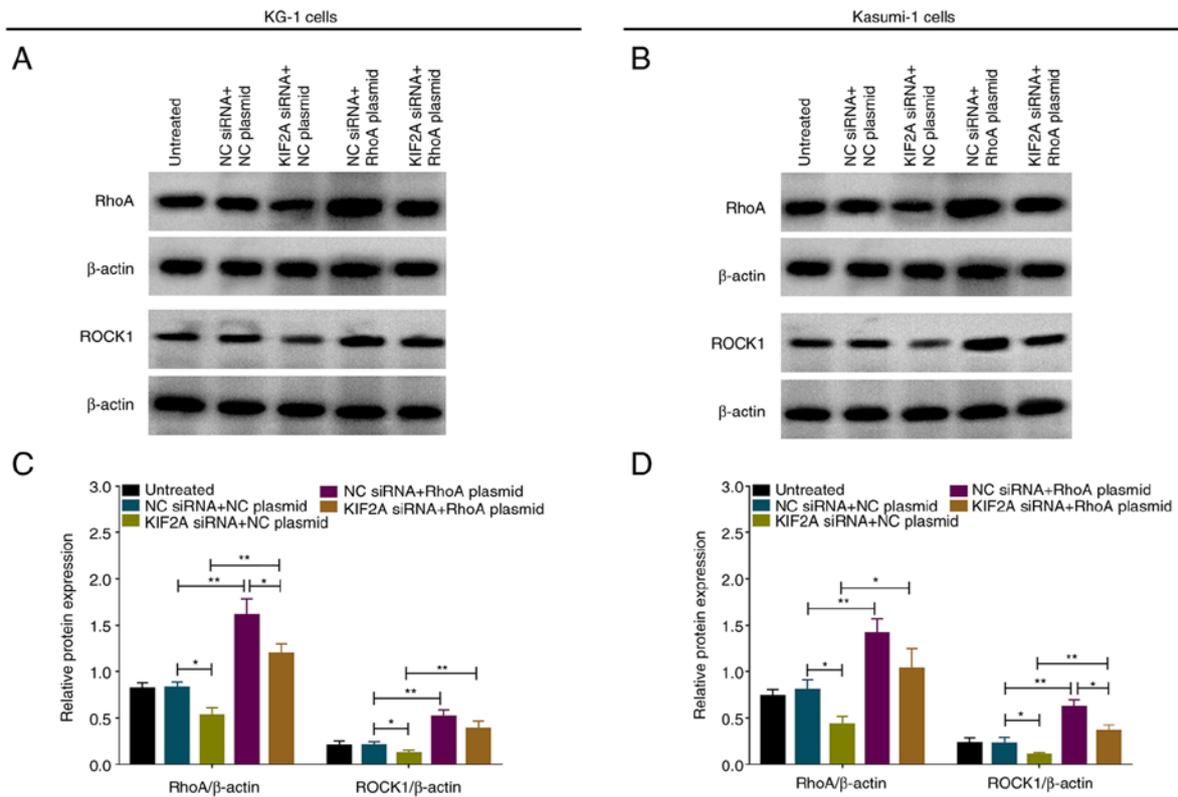


Figure 9. Successful transfection of RhoA overexpression plasmid in AML cells. Western blot analysis of (A) KG-1 and (B) Kasumi-1 cells following RhoA overexpression. RhoA and ROCK1 protein expression levels in (C) KG-1 and (D) Kasumi-1 cells following RhoA overexpression. * $P < 0.05$, ** $P < 0.01$. RhoA, ras homolog family member A; AML, acute myeloid leukemia; ROCK1, Rho associated coiled-coil containing protein kinase 1; NC, negative control; KIF2A, kinesin family member 2A; si, small interfering.

mice (21). In addition, KIF2A expression is elevated in gastric cancer cells; inhibiting KIF2A expression in gastric cancer cells suppresses cell invasion via downregulating membrane type 1-matrix metalloproteinase (22). A previous study demonstrated that KIF2A presents with higher expression in grade III-IV glioma tissue compared with grade I-II glioma tissue, and KIF2A inhibition results in suppression of glioma cell proliferation, migration, invasion and promotion of apoptosis (23). A previous study revealed that in lung adenocarcinoma, KIF2A suppression decreases migration and proliferation but promotes apoptosis in A549 cells (24). These previous studies suggest that KIF2A participates in cancer pathology primarily by regulating cell function. In the present study, KIF2A was upregulated in patients with AML and positively correlated with BM blast percentage and risk classification but negatively correlated with treatment response and survival profile. It was hypothesized that KIF2A exerted its clinical effect in the pathology of AML by modulating cancer cell functions via interaction with multiple factors, as indicated by previous studies (21-24). Here, KIF2A siRNA inhibited proliferation but enhanced apoptosis, chemosensitivity to ADR and AraC and expression levels of mRNA/proteins associated with PI3K/AKT and RhoA/ROCK pathway in AML cells. These data suggested a regulatory role of KIF2A in AML.

The PI3K/AKT and RhoA/ROCK signaling pathways are involved in the development of AML, especially the PI3K/AKT signaling pathway, which has been widely studied (25,26). To the best of our knowledge, however, the role of RhoA/ROCK is less reported in AML. A previous study demonstrated that

an inhibitor of both PI3K and histone deacetylase elevates the antitumor activity of venetoclax in preclinical AML mice (27). Additionally, in AML cells, Metrine[®] notably suppresses cell viability and enhances cell apoptosis in a time- and dose-dependent manner by downregulating the PI3K/AKT/mTOR signaling pathway (28). Another study reported that ISC-4, a PI3K/AKT signaling pathway inhibitor, decreases cell survival and clonogenicity but promotes apoptosis and sensitivity to cytarabine in AML cells; it also enhances disease progression in a mouse model of preclinical AML (29). With regard to the RhoA/ROCK signaling pathway, a study demonstrated that ROCK1 is targeted by microRNA (miR)-592 to suppress cell proliferation, migration and invasion and enhance apoptosis in AML cells (30). Moreover, a long intergenic non-coding RNA LINC00662 increases proliferation but decreases apoptosis in AML cells by activating ROCK1 via sponging of miR-340-5p (31). These previous findings identified the role of the PI3K/AKT and RhoA/ROCK signaling pathways in AML by enhancing disease progression disease via modulation of cell function, most of which are accomplished by interacting with other factors.

To the best of our knowledge, there are few reports on the regulation of PI3K/AKT and RhoA/ROCK signaling pathways by KIF2A. A previous study revealed that KIF2A inhibition enhance squamous cell carcinoma of the oral tongue (SCCOT) cell apoptosis by downregulating the PI3K/AKT signaling pathway, which indicates that KIF2A acts as a tumor promotor by activating this pathway in SCCOT (32). Another study demonstrated that silencing of KIF2A notably elevates

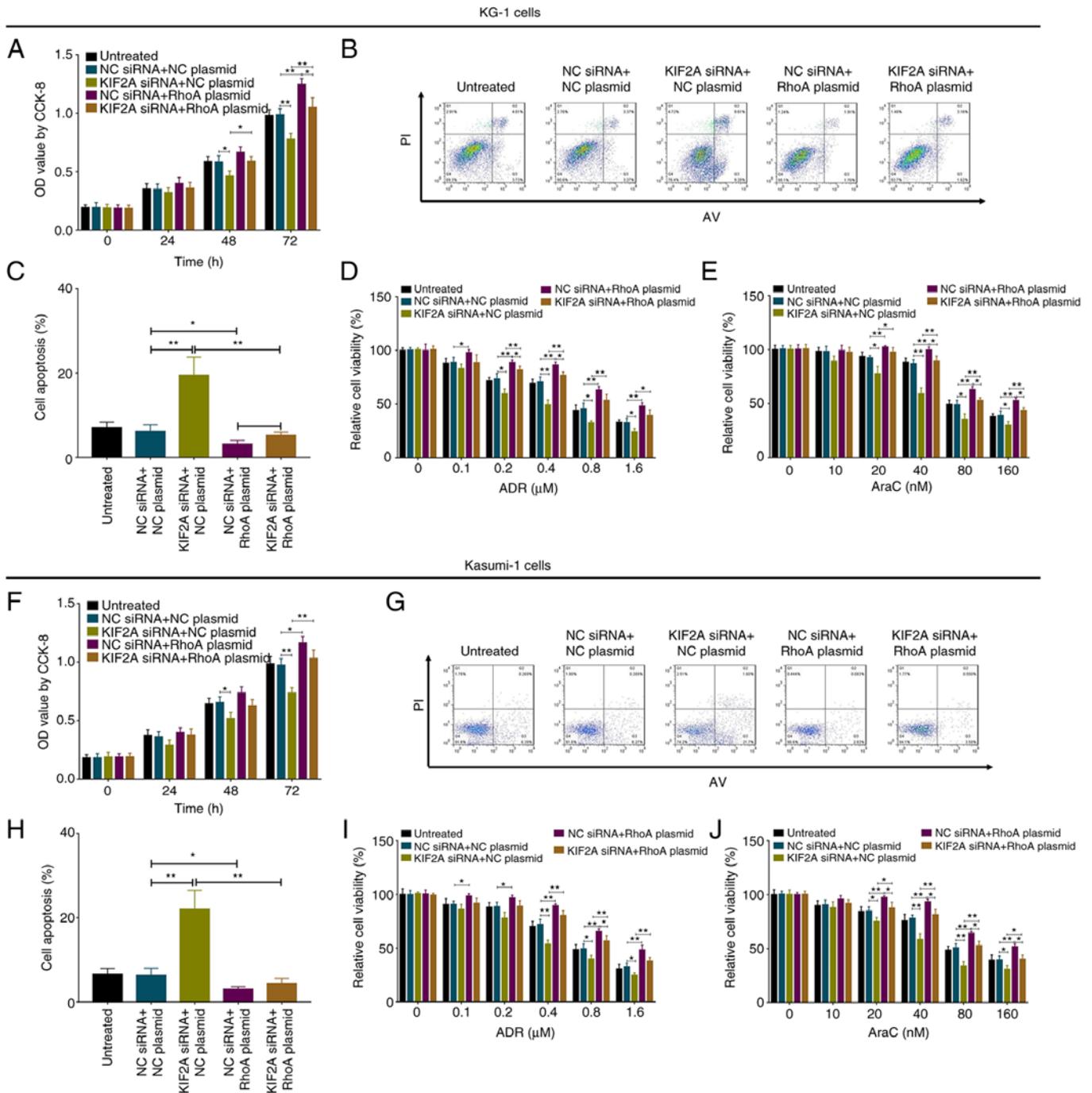


Figure 10. RhoA overexpression plasmid decreases the effect of KIF2A siRNA on AML cells. (A) Proliferation, (B and C) apoptosis and chemosensitivity to (D) ADR and (E) AraC in KG-1 cells following RhoA overexpression. (F) Proliferation, (G and H) apoptosis and chemosensitivity to (I) ADR and (J) AraC in Kasumi-1 cells following RhoA overexpression. *P<0.05, **P<0.01. RhoA, ras homolog family member A; KIF2A, kinesin family member 2A; AML, acute myeloid leukemia; ADR, Adriamycin; AraC, Arabinofuranosyl Cytidine; NC, negative control; si, small interfering; OD, optical density; CCK-8, Cell Counting Kit-8.

apoptosis but decreases proliferation, migration and invasion of gastric cancer cells by mediating AKT signaling (17). To the best of our knowledge, however, no studies have reported regulation of RhoA/ROCK signaling pathway by KIF2A in AML or other types of cancer. The present study identified a role of KIF2A in the regulation of AML pathology and demonstrated that KIF2A silencing inhibited proliferation, enhanced apoptosis and chemosensitivity; it also found that KIF2A regulated the PI3K/AKT and RhoA/ROCK signaling

pathways to affect the malignant behavior of AML cells. These data enriched understanding of the mechanism underlying the progression of AML and provided a basis for investigation of novel targeted therapy.

There was a limitation of the present study: Few people were willing to give bone marrow, thus, it the sample size of controls was smaller than that of patients with AML.

In conclusion, KIF2A was correlated with worse clinical features and survival profile in patients with AML; its

knockdown suppressed proliferation but promoted apoptosis and chemosensitivity via inactivating PI3K/AKT and RhoA/ROCK signaling pathways in AML cells. These data suggested the potential of KIF2A as a prognostic marker and treatment target for AML management.

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Availability of data and materials

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

Authors' contributions

RX designed the experiments. XL analyzed the data and drafted the manuscript. XL and RX confirm the authenticity of all the raw data. XL and RX revised the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of First Affiliated Hospital of Anhui Medical University (approval no. PJ2020-12-39). All subjects provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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