

Original Article

Kinesin family member 2A promotes cancer cell viability, mobility, stemness, and chemoresistance to cisplatin by activating the PI3K/AKT/VEGF signaling pathway in non-small cell lung cancer

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Abstract: Kinesin family member 2A (KIF2A), a member of the kinesin-13 protein family that functions as a regulator in mitosis, neuron branch extension, etc., is reported to be involved in the pathogenesis of multiple cancers. This study assessed KIF2A effects on cancer cell functions and sensitivity to chemotherapy and its interaction with PI3K/AKT/VEGF signaling when mediating cancer cell functions, and chemosensitivity in non-small cell lung cancer (NSCLC). Human bronchial epithelial cell line BEAS-2B and human NSCLC cell lines NCI-H1299, NCI-H385, NCI-H1650, and A549 were used. The KIF2A and negative control (NC) overexpression plasmids were transfected into A549 cells; KIF2A and NC knock-down plasmids were transfected into NCI-H1299 cells. Rescue experiments were conducted by transfecting PI3K and NC knock-down plasmids into KIF2A overexpression A549 cells and transfecting PI3K and NC overexpression plasmids into KIF2A knock-down NCI-H1299 cells. Proliferation, apoptosis, migration, invasion, CD133⁺ proportion, sensitivity to chemotherapeutics, and PI3K/AKT/VEGF pathway were assessed. KIF2A mRNA and protein expression levels were elevated in NCI-H1299, NCI-H385, NCI-H1650, and A549 cells compared to BEAS-2B cells. KIF2A overexpression elevated proliferation, migration, invasion, stemness, and resistance to cisplatin but did not affect apoptosis or resistance to pemetrexed in A549 cells. Furthermore, KIF2A knock-down repressed proliferation, migration, invasion, stemness, and resistance to cisplatin, but not to pemetrexed, and it enhanced apoptosis in NCI-H1299 cells. Rescue experiments showed that the PI3K/AKT/VEGF pathway compensated for KIF2A effects on cell functions and sensitivity to cisplatin in A549 and NCI-H1299 cells. In conclusion, KIF2A advocates NSCLC cell viability, mobility, stemness, and chemoresistance to cisplatin by activating the PI3K/AKT/VEGF signaling pathway.

Keywords: Non-small cell lung cancer, kinesin family member 2A, cell viability and motility, stemness, chemosensitivity, PI3K/AKT/VEGF signaling pathway

Introduction

Lung cancer remains the most prevalent and lethal cancer, despite advancements in screening and reduction in tobacco use. Notably, lung cancer prevalence is increasing constantly due to aging and growing risk factors in the worldwide population [1, 2]. Non-small cell lung cancer (NSCLC) accounts for the majority of the lung cancer cases, and the treatment for NSCLC has progressed considerably over the past two decades. The current treatment options for NSCLC patients include surgery, radiotherapy, chemotherapy, molecular targeted therapy, and

immunotherapy [3-5]. Although there have been reports revealing hopeful outcomes in NSCLC patients treated with novel targeted drugs, such as the tyrosine kinase inhibitors and programmed death receptor 1/programmed death ligand 1 inhibitors, challenges still exist in improving the survival rate of patients with NSCLC [6]. The 5-year survival rate of NSCLC ranges approximately from 4% to 70%, depending on the clinical stages [7, 8].

Kinesin family member 2A (KIF2A), constructed by kinesin heavy chain and kinesin light chain, is a member of the kinesin-13 protein family

that functions as a regulator in mitosis, neuron branch extension, cilium disassembly, etc. [9-11]. Since its first discovery in mammalian cells, KIF2A has been extensively researched in various areas, including the field of oncology. The growing interest in KIF2A in oncology has greatly promoted research and findings in multiple carcinomas. For instance, studies have shown that KIF2A overexpression is correlated with more advanced tumor features and worse survival profiles in cancer patients [12, 13]. In addition to the clinical value of KIF2A in cancers, its involvement in molecular oncological mechanisms has been reported; for example, KIF2A is involved in the regulation of various cancer cell functions, including migration, invasion, apoptosis, etc. [14, 15]. These findings indicate a very promising role of KIF2A in oncology.

Recent studies also found that KIF2A might be engaged in regulating the pathogenesis of lung cancer, shedding light on its potential value in lung cancer management. However, to the best of our knowledge, these studies are very limited. One study reveals that KIF2A inhibition reduces cancer cell migration, epithelial-mesenchymal transition (EMT), and proliferation while it increases apoptosis in lung adenocarcinoma [16]. Another study reveals that KIF2A could independently predict unfavorable survival in lung squamous cell carcinoma [17]. Nonetheless, these previous studies were conducted on only a certain number of subtypes of NSCLC. The current knowledge about the intricate molecular mechanisms through which KIF2A exerts its capacities in regulating NSCLC pathogenesis is still very limited. Therefore, in the present study, we assessed the regulatory role of KIF2A in cancer cell functions and sensitivity to chemotherapy as well as its interaction with phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT)/vascular endothelial growth factor (VEGF) signaling when mediating cancer cell functions and chemosensitivity in NSCLC cell lines.

Materials and methods

Cell culture

Human bronchial epithelial cell line BEAS-2B and human NSCLC cell lines NCI-H1299, NCI-H385, NCI-H1650, and A549 were all purchased from American Type Culture Collection [ATCC], Manassas, VA, USA]. All cells were cul-

tured according to the ATCC protocols. The NCI-H1299, NCI-H385 and NCI-H1650 cells were maintained in 90% RPMI1640 medium (Sigma, Whitehouse Station, NJ, USA) supplemented with 10% fetal bovine serum [(FBS), Sigma, Whitehouse Station, NJ, USA]. BEAS-2B and A549 cells were maintained in 90% DMEM medium (Sigma, Whitehouse Station, NJ, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma, Whitehouse station, NJ, USA). All cells were cultured in a humid incubator at 37°C with 5% CO₂. After being cultured, the KIF2A mRNA and protein expressions in each cell line were determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot assay, respectively. BEAS-2B cells served as the control.

Transfection

The pcDNA 3.1(+) vector was used to construct KIF2A overexpression [KIF2A(+)] plasmid and control overexpression [NC(+)] plasmid. The pRNAT-U6.1/Neo (YouBio, Xi'an, ShaanXi Province, China) vector was used to construct KIF2A knock-down [KIF2A(-)] plasmid and negative control knock-down [NC(-)] plasmid. After construction, the KIF2A(+) and NC(+) plasmids were transfected into A549 cells and the KIF2A(-) and NC(-) plasmids were transfected into NCI-H1299 cells using Lipofectamine 3000 Transfection Reagent (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. After incubation for 24 h, the mRNA expression of KIF2A was determined by RT-qPCR assay, and the protein expressions of KIF2A, VEGF, PI3K, phospho-PI3K (p-PI3K), AKT and phospho-AKT (p-AKT) were detected by western blot assay. During the incubation, cell proliferation was assessed at 0, 24, 48, and 72 h using a Cell Counting Kit-8 (CCK-8) assay. After incubation for 48 h, cell apoptosis, cell migration, and invasion ability were respectively determined by the Annexin V-FITC, propidium iodide (AV/PI) assay, scratch assay, and transwell assay. CD133 positive (CD133⁺) cells were detected by flow cytometry. In addition, a drug-sensitivity assay regarding cisplatin and pemetrexed was also carried out 48 h after transfection.

RT-qPCR

The total RNA was extracted from the cells using TRIzol (Invitrogen, Waltham, MA, USA). Then the RNA was reversely transcribed by

ReverTra Ace[®] qPCR RT Kit (Toyobo, Osaka, Japan). Next, qPCR was performed using SYBR[®] Green Realtime PCR Master Mix (Toyobo, Osaka, Japan). Reaction conditions were 95°C for 60 s, then 95°C for 15 s, followed by 61°C for 30 s, for a total of 40 cycles. The expression of KIF2A mRNA was calculated using glyceraldehyde-phosphate dehydrogenase (GAPDH) as an internal reference. The sequence of the primers used in RT-qPCR were as follows: KIF2A, forward: 5'-GCCGAATACATCAAGCAAT-3', reverse: 5'-CTCTCCAGGTCAATCTCTT-3'; GAPDH, forward: 5'-GACCACAGTCCATGCCATCAC-3', reverse: 5'-ACGCCTGCTTACCACCTT-3'.

Western blot assay

Cells were lysed using RIPA buffer (Sigma, Whitehouse Station, NJ, USA), and then quantification of protein concentration was performed using a Pierce[™] BCA Protein Assay Kit (Thermo, Wilmington, MA, USA). Subsequently, equal amounts of total protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at 140 V constant voltage, and then transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules CA, USA) at 250 mA electricity for 2.5 h. The membranes were blocked with 5% bovine serum albumin (BSA, Sigma, Whitehouse Station, NJ, USA) at 37°C with gentle shaking in TBS-T (10 mM Tris HCl (pH 7.6), 150 mM NaCl, 0.05% Tween 20). Then the membranes were incubated overnight at 4°C with the following primary antibodies: Rabbit Anti-KIF2A antibody, 1:1000 dilution (Abcam, Cambridge, UK); Rabbit Anti-VEGF antibody, 1 µg/mL (Invitrogen, Waltham, MA, USA), Rabbit Anti-PI3K antibody, 1:3000 dilution (Invitrogen, Waltham, MA, USA), Rabbit anti-Phospho-PI3K antibody, 1:2000 dilution (Invitrogen, Waltham, MA, USA), Rabbit Anti-AKT antibody, 1:1000 dilution (Invitrogen, Waltham, MA, USA), Rabbit anti-Phospho-AKT antibody, 1:1000 dilution (Invitrogen, Waltham, MA, USA), Rabbit anti-GAPDH antibody, 1:5000 dilution (Invitrogen, Waltham, MA, USA). The membranes were then incubated with Goat Anti-Rabbit IgG H&L (HRP) secondary antibody in 1:10000 dilution (Abcam, Cambridge, UK) for 1.5 h at 37°C. Specific bands were detected using Pierce[™]ECL Plus Western Blotting Substrate (Invitrogen, Waltham, MA, USA) on autoradiographic film.

CCK-8 assay

The CCK-8 assay was performed to detect cell proliferation. In brief, cells were seeded in 96-well plates at a density of 1×10^4 cells in each well. Following the transfection, 10 µL of CCK-8 reagents (Dojindo, Kumamoto Prefecture, Japan) were added to each well at 0, 24, 48, and 72 h and incubated for an additional 2 h at 37°C. Finally, to assess the cell proliferation, the absorbance at 450 nm was measured using a microplate reader (BioTek Instruments, Winooski, VT, USA), and the optical density (OD) value was calculated.

AV/PI assay

An AV/PI assay was used to determine cell apoptosis 48 h after transfection. In brief, the transfected cells were collected and seeded into 6-well plates (2.5×10^5 /well) and then digested in EDTA-free trypsin (Beyotime, Shanghai, China). After centrifugation at 1,500 rpm for 3 min, cell density was adjusted to 1×10^5 /mL using binding buffer. Subsequently, each 100-µL cell suspension was stained with 5 µL of Annexin V-FITC Apoptosis Detection Kit (R&D, Minneapolis, MN, USA) and 5 µL of PI (Thermo Fisher Scientific, Waltham, MA, USA) in the dark for 15 min at room temperature. The percentage of apoptotic cells, including early apoptosis and late apoptosis, were measured by flow cytometry (BD Biosciences, San Jose, CA, USA).

Scratch assay

A scratch assay was carried out to assess the cell migration ability 48 h after transfection. Briefly, cells were inoculated at a density of 4×10^4 /mL and washed with base culture solution, as mentioned in the cell culture section, next, the cell monolayer was scratched using a sterile 200-µL tip and photographed immediately. A second photograph was taken 24 h later at the same site. The cell growth in the denuded area was assessed, and the cell migration was quantified as the percentage of the wound-healed area. In addition, the migration rate was calculated as follows: migration rate = (scratch area-residual area) ÷ scratch area.

Transwell assay

A transwell assay was performed to assess cell invasion ability 48 h after transfection. In brief, cells were seeded into a Matrigel basement membrane matrix (BD Biosciences, San Jose, CA, USA) coated upper chamber (Corning, Lowell, MA, USA) at a density of 4×10^4 /mL, and the lower chambers were filled with complete medium. After incubated for another 24 h at 37°C in 5% CO₂, the cells on the upper surface of the chamber were removed, and the cells on the lower surface of chamber were fixed with 4% methanol (Sigma, Whitehouse Station, New Jersey, USA) for 10 min at room temperature. After stained with 0.5% crystal violet at room temperature for 15 min, cells were counted and photographed with an inverted microscope (Olympus, Tokyo, Japan).

CD133⁺ cell determination

CD133⁺ cells were determined by flow cytometry 48 h after transfection. Cells were collected and washed, and the cell suspension concentration was adjusted to 1×10^6 cells/mL with cold phosphate buffer saline (PBS) (Sigma-Aldrich, Whitehouse Station, NJ, USA), 10% fetal calf serum (FCS) (Sigma-Aldrich, Whitehouse Station, NJ, USA) and 1% sodium azide (Sigma-Aldrich, Whitehouse Station, NJ, USA). Then, rabbit anti-CD133 antibody (Abcam, Cambridge, UK) was added to the cells at 4°C for 30 min in the dark. Following that, the cells were washed three times and centrifuged at 400 g for 5 min. After resuspension in 1 mL of cold PBS, 10% FCS and 1% sodium azide, CD133⁺ cells were detected using flow cytometry (BD Biosciences, San Jose, CA, USA).

Drug-sensitivity assay

After transfection for 48 h, the transfected cells were seeded into a 96-well plate at a density of 0.5×10^4 cells/well. The transfected cells were then treated with cisplatin or pemetrexed. Cisplatin (Sigma-Aldrich, Whitehouse Station, NJ, USA) was given at concentrations of 0, 1, 2, 4, 8, and 16 μM for both transfected A459 cells and NCI-H1299 cells. Pemetrexed (Sigma-Aldrich, Whitehouse Station, NJ, USA) was given at concentrations of 0, 0.5, 1, 2, 4, and 8 μM for transfected A459 cells, and at 0, 0.25, 0.5, 1, 2, and 4 μM for transfected NCI-H1299 cells.

The preparation of cisplatin and pemetrexed was performed according to the manufacturer's manuals. After incubation with cisplatin or pemetrexed for another 48 h, cell viability of the treated cells was assessed using CCK-8 (Dojindo, Kumamoto Prefecture, Japan). The relative cell viability was calculated using the cell viability of 0 μM cisplatin-treated or 0 μM pemetrexed-treated cells as references.

Rescue experiments

PI3K inhibitor LY294002 (MedChem Express, Monmouth Junction, NJ, USA) was dissolved in DMSO (Sigma-Aldrich, Whitehouse Station, NJ, USA) to obtain a final concentration of 20 μM. Also, PI3K activator 740 Y-P (MedChem Express, Monmouth Junction, NJ, USA) was dissolved in H₂O to obtain a final concentration of 3 μM. The preparations of LY294002 and 740 Y-P were performed in accordance with the manufacturer's manuals. Using Lipofectamine™ 3000 Transfection Reagent (Invitrogen, Waltham, MA, USA), the KIF2A(+) plasmids were transfected into A549 cells, which were termed as KIF2A(+) cells. The KIF2A(-) plasmids were transfected into NCI-H1299 cells, which were termed as KIF2A(-) cells. Then the KIF2A(+) cells were incubated in the RPMI1640 medium (Sigma, Whitehouse station, NJ, USA) and treated with 20 μM LY294002 or DMSO for 48 h, and these cells were respectively marked as KIF2A(+) & PI3K(-) or KIF2A(+) & NC(-). The KIF2A(-) cells were incubated in the DMEM medium (Sigma, Whitehouse station, NJ, USA) and treated with 3 μM 740 Y-P or H₂O for 48 h. These cells were marked as KIF2A(-) & PI3K(+) or KIF2A(-) & NC(+), respectively. After incubation for 48h, the mRNA expression of KIF2A was detected by RT-qPCR, and the protein expressions of KIF2A, VEGF, PI3K, p-PI3K, AKT, and p-AKT were determined by western blot assay. During the incubation, cell proliferation was assessed at 0, 24, 48, and 72 h post transfection by CCK-8 assay. After incubation for 48 h, cell apoptosis, cell migration, and invasion ability were respectively determined by AV/PI assay, scratch assay, and transwell assay, respectively. CD133⁺ cells were detected by flow cytometry. In addition, a drug-sensitivity assay for cisplatin and pemetrexed was also carried out 48 h after transfection as described above.

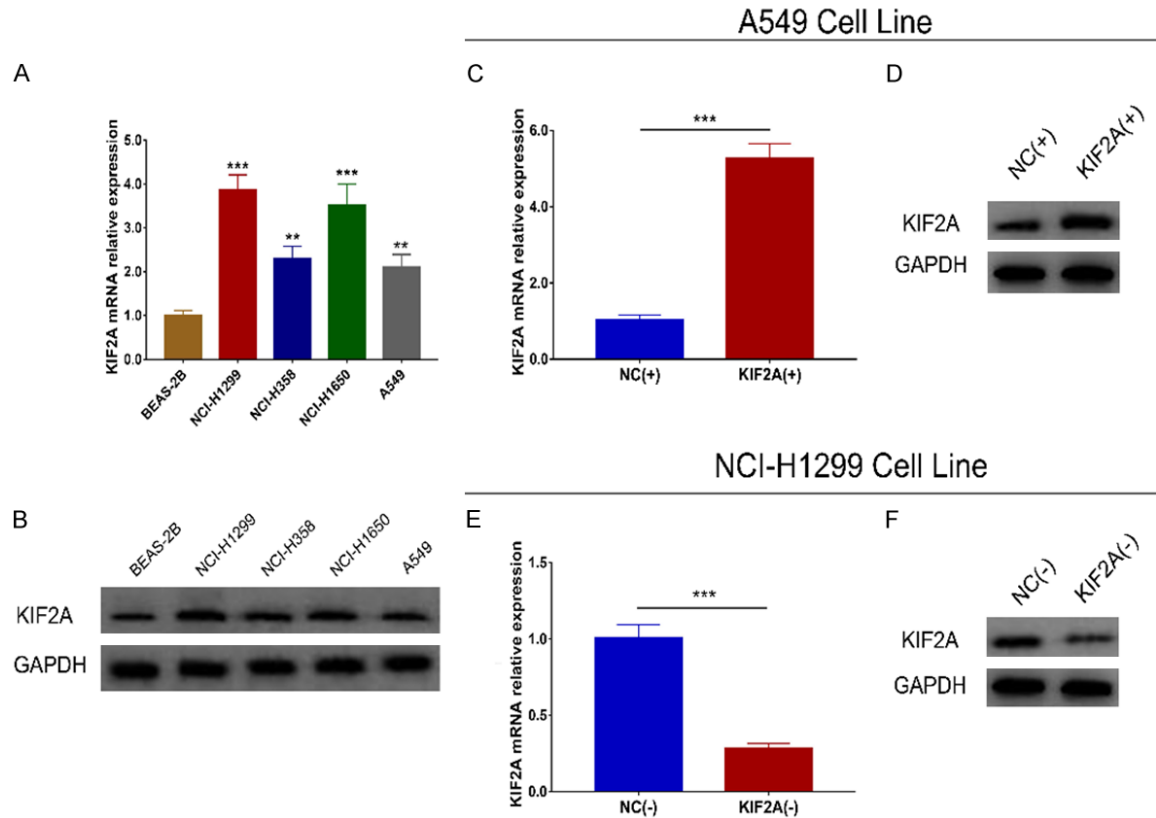


Figure 1. KIF2A was upregulated in NSCLC cell lines compared to a human bronchial epithelial cell line. Altered KIF2A expression was detected after transfection in NSCLC cells. The mRNA (A) and protein (B) expression levels of KIF2A in NCI-H1299, NCI-H385, NCI-H1650, A549, and BEAS-2B cell lines. KIF2A mRNA (C) and protein (D) expression levels in the KIF2A(+) group and NC(+) group in A549 cells. KIF2A mRNA (E) and protein (F) expression levels in the KIF2A(-) group as well as NC(-) group in NCI-H1299 cells after transfection. KIF2A, kinesin family member 2A; NSCLC, non-small cell lung cancer; NC, negative control.

Statistical analysis

Data were displayed as mean and standard deviation (SD). Comparison between two independent samples was determined by the unpaired t-test. Comparison among groups was determined by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Statistical analysis and graph plotting were performed on the GraphPad Prism 7.01 software (GraphPad Software, NY, New York, USA). A P -value of <0.05 was considered to be statistically significant. Levels of significance are shown as $*P<0.05$, $**P<0.01$, and $***P<0.001$ in all figures. The abbreviation "NS" indicated no significance in all figures.

Results

KIF2A expression in NSCLC and normal lung cell lines

Compared to the BEAS-2B cell line, KIF2A mRNA was increased in NCI-H1299 ($P<0.001$),

NCI-H358 ($P<0.01$), NCI-H1650 ($P<0.001$), and A549 ($P<0.01$) cell lines (**Figure 1A**). In addition, the protein expression of KIF2A was also elevated in NCI-H1299, NCI-H358, NCI-H1650, and A549 cell lines compared to BEAS-2B cell line (**Figure 1B**).

Effect of KIF2A on NSCLC cell proliferation, apoptosis, migration, and invasion

The A549 and NCI-H1299 cell lines were selected for additional experiments. In the A549 cell line, the KIF2A mRNA ($P<0.001$) (**Figure 1C**) and protein (**Figure 1D**) expressions were elevated in the KIF2A(+) group compared to the NC(+) group. In the NCI-H1299 cell line, KIF2A mRNA ($P<0.001$) (**Figure 1E**) and protein (**Figure 1F**) expressions were reduced in the KIF2A(-) group compared to the NC(-) group. Together these results show that the transfections were successful. In regard to cell proliferation and apoptosis, KIF2A overexpression promoted cell proliferation at 48h ($P<0.05$) and 72

h ($P < 0.05$) (**Figure 2A**) but did not regulate cell apoptosis ($P > 0.05$) (**Figure 2B, 2C**) in the A549 cell line. In addition, KIF2A knock-down repressed cell proliferation at 48 h ($P < 0.05$) and 72 h ($P < 0.05$) (**Figure 2D**), whereas it enhanced cell apoptosis ($P < 0.05$) (**Figure 2E, 2F**) in the NCI-H1299 cell line. In terms of cell invasion and migration, KIF2A overexpression enhanced cell migration ($P < 0.01$) (**Figure 3A, 3B**) and invasion ($P < 0.05$) (**Figure 3C, 3D**) in the A549 cell line, whereas KIF2A knock-down suppressed cell migration ($P < 0.05$) (**Figure 3E, 3F**) and invasion ($P < 0.05$) (**Figure 3G, 3H**) in the NCI-H1299 cell line.

Effect of KIF2A on NSCLC cell stemness

In regard to cell stemness, KIF2A overexpression increased the CD133⁺ cell proportion in A549 cell line ($P < 0.05$) (**Figure 4A, 4B**); In contrast, KIF2A knock-down decreased CD133⁺ cell proportion in the NCI-H1299 cell line ($P < 0.01$) (**Figure 4C, 4D**).

Effect of KIF2A on NSCLC cell chemosensitivity

In terms of drug sensitivity to cisplatin and pemetrexed, KIF2A overexpression increased relative cell viability of A549 cells treated with cisplatin at 4 μM ($P < 0.01$), 8 μM ($P < 0.01$), and 16 μM ($P < 0.05$) (**Figure 4E**) and the relative cell viability of A549 cells treated with pemetrexed at 4 μM ($P < 0.05$) (**Figure 4F**). KIF2A knock-down reduced relative cell viability NCI-H1299 cells treated with cisplatin at 1 μM ($P < 0.05$), 2 μM ($P < 0.05$), 4 μM ($P < 0.05$), 8 μM ($P < 0.01$), and 16 μM ($P < 0.01$) (**Figure 4G**). In contrast, KIF2A knock-down did not affect the relative cell viability of pemetrexed-treated NCI-H1299 cells (all $P > 0.05$) (**Figure 4H**).

Regulatory role of KIF2A on PI3K/AKT/VEGF pathway in NSCLC cells

With respect to the regulation of KIF2A on PI3K/AKT/VEGF pathway, KIF2A overexpression increased the protein expressions of p-PI3K, p-AKT, and VEGF in A549 cell line (**Figure 5A**). In contrast, KIF2A knock-down decreased the protein expressions of p-PI3K, p-AKT, and VEGF in NCI-H1299 cell line (**Figure 5B**).

Interaction between KIF2A and PI3K in regulation of NSCLC cell proliferation, apoptosis, migration and invasion

The rescue experiments show that PI3K knock-down decreased PI3K, p-PI3K, p-AKT, and

VEGF protein expressions in the KIF2A overexpressed A549 cell line (**Figure 5C**), and PI3K overexpression elevated PI3K, p-PI3K, p-AKT, and VEGF protein expressions in the KIF2A knock-down NCI-H1299 cell line (**Figure 5D**). Furthermore, PI3K knock-down reduced cell proliferation at 48 h ($P < 0.05$) and 72 h ($P < 0.01$) (**Figure 6A**), but enhanced cell apoptosis ($P < 0.01$) (**Figure 6B, 6C**) in the KIF2A overexpressed A549 cell line. Furthermore, PI3K overexpression promoted cell proliferation at 48 h ($P < 0.05$) and 72 h ($P < 0.01$) (**Figure 6D**) but repressed cell apoptosis ($P < 0.05$) (**Figure 6E, 6F**) in the KIF2A knock-down NCI-H1299 cell line. PI3K knock-down also repressed cell migration ($P < 0.01$) (**Figure 7A, 7B**) and cell invasion ($P < 0.05$) (**Figure 7C, 7D**) in the KIF2A overexpressed A549 cell line. In contrast, PI3K overexpression enhanced cell migration ($P < 0.01$) (**Figure 7E, 7F**) and invasion ($P < 0.05$) (**Figure 7G, 7H**) in the KIF2A knock-down NCI-H1299 cell line.

Interaction between KIF2A and PI3K in regulation of NSCLC cell stemness

With regard to cell stemness, PI3K knock-down reduced the CD133⁺ cells proportion in the KIF2A overexpressed A549 cell line ($P < 0.05$) (**Figure 8A, 8B**); however, PI3K overexpression upregulated the CD133⁺ cells proportion in the KIF2A knock-down NCI-H1299 cell line ($P < 0.05$) (**Figure 8C, 8D**).

Interaction between KIF2A and PI3K in the regulation of NSCLC cell chemosensitivity

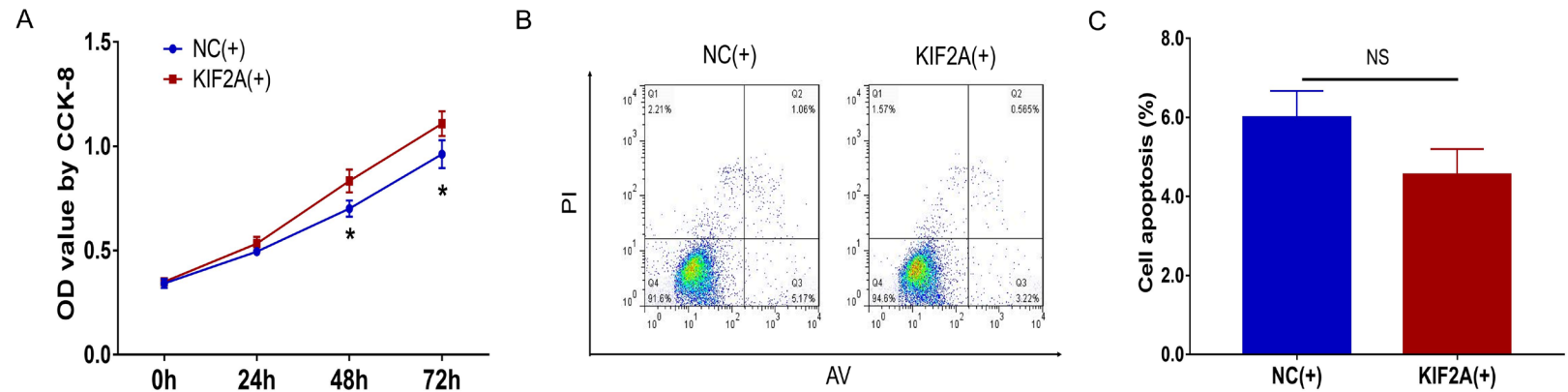
With regard to drug sensitivity to cisplatin and pemetrexed, PI3K knock-down reduced relative cell viability of 4 μM ($P < 0.05$) and 8 μM ($P < 0.01$) cisplatin-treated KIF2A overexpressed A549 cell line (**Figure 8E**), and 1 μM ($P < 0.05$), 2 μM ($P < 0.01$), and 4 μM ($P < 0.05$) pemetrexed-treated KIF2A overexpressed A549 cell line (**Figure 8F**). In addition, PI3K overexpression enhanced relative cell viability in 4 μM ($P < 0.05$) and 8 μM ($P < 0.05$) cisplatin-treated KIF2A knock-down NCI-H1299 cell line (**Figure 8G**), and 1 μM ($P < 0.05$) pemetrexed-treated KIF2A knock-down NCI-H1299 cell line (**Figure 8H**).

Discussion

It is undeniable that investigations of KIF2A in carcinomas, as a factor intimately related to the regulation of microtubules that are crucial

KIF2A in NSCLC

A549 Cell Line



NCI-H1299 Cell Line

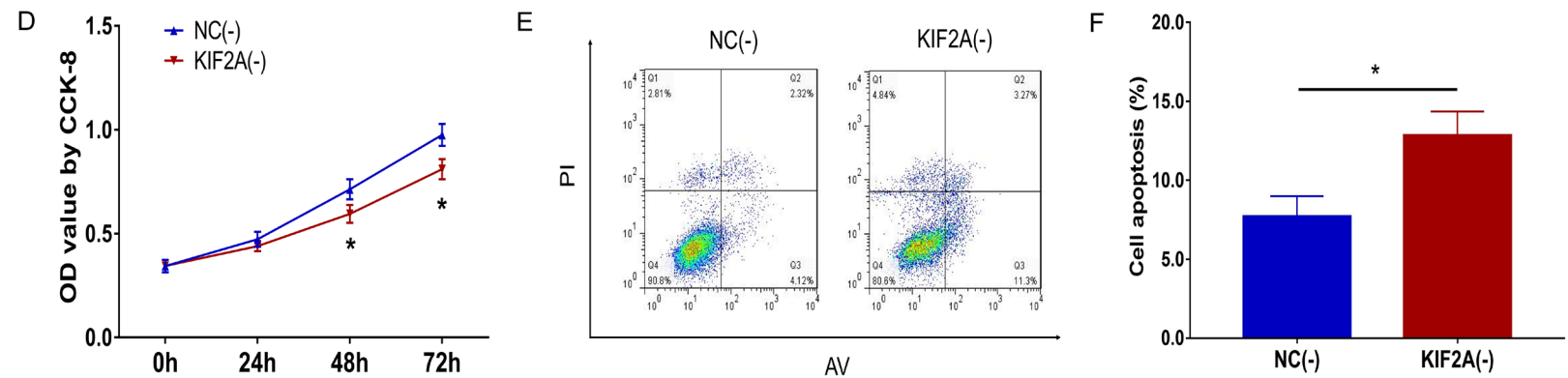
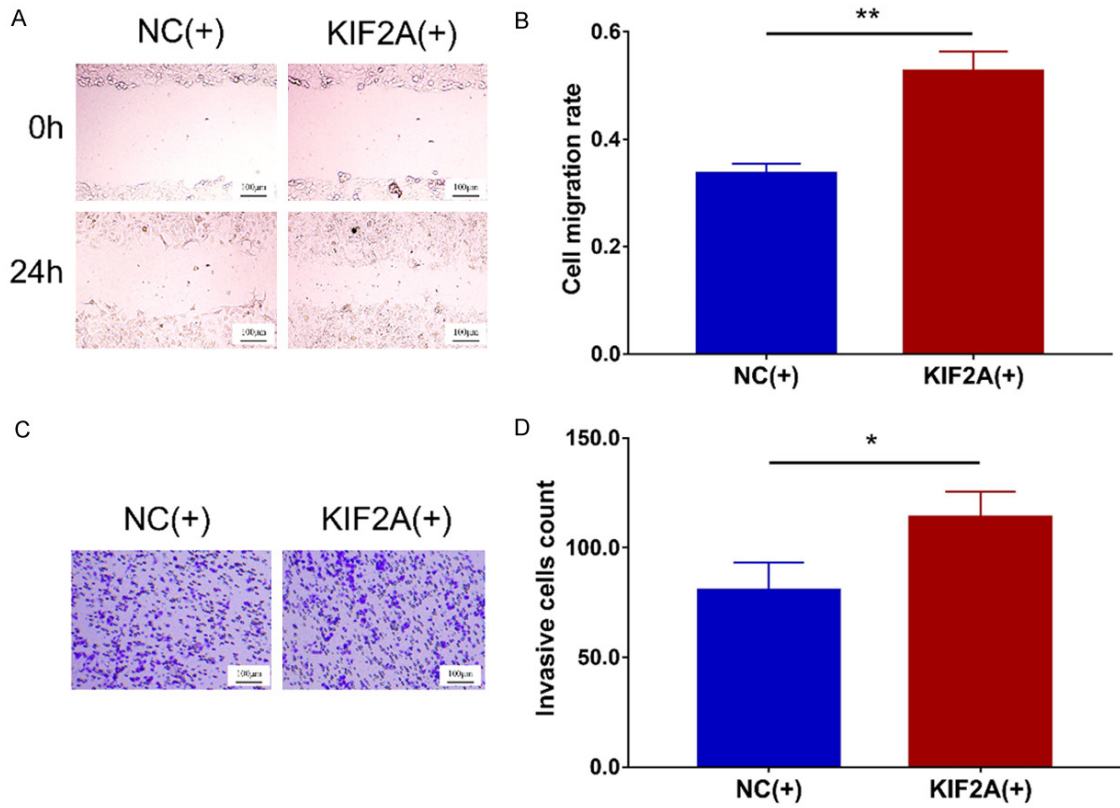
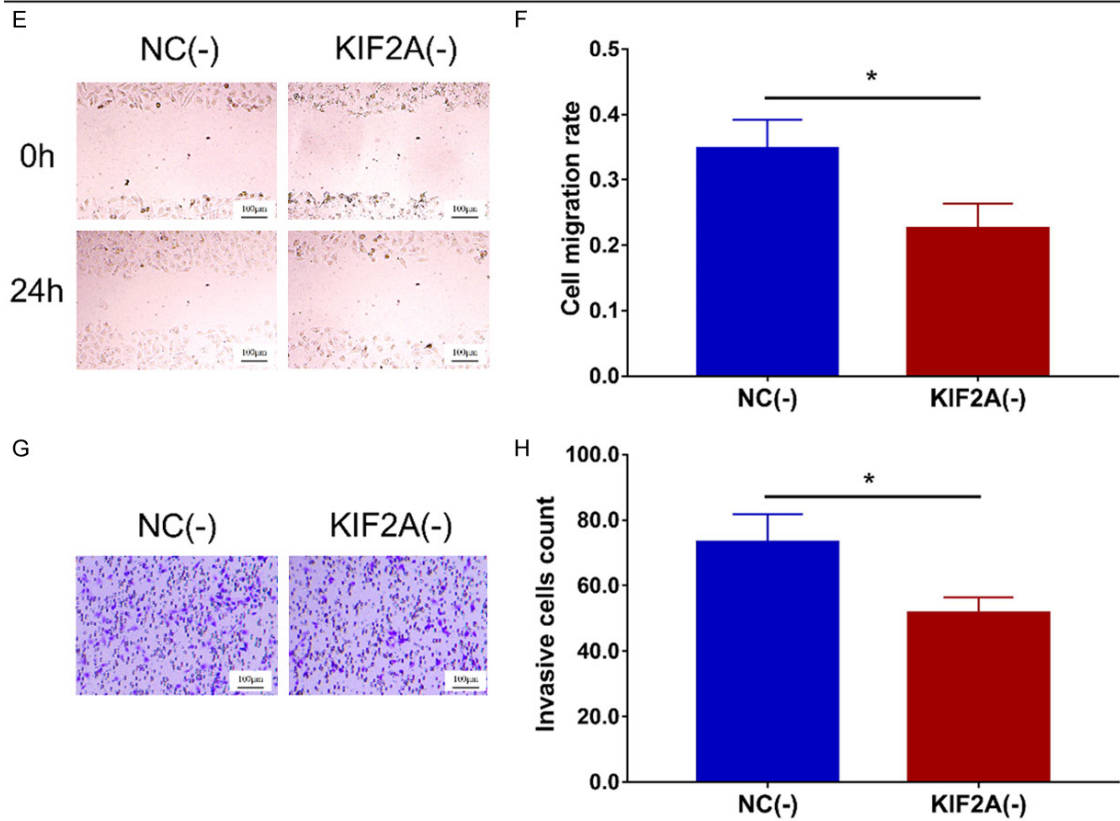


Figure 2. KIF2A promoted proliferation and inhibited apoptosis in NSCLC cells. Cell proliferation (A) and cell apoptosis (B, C) in the KIF2A(+) group and NC(+) group in A549 cells. Cell proliferation (D) and cell apoptosis (E, F) in the KIF2A(-) group as well as NC(-) group in NCI-H1299 cells. KIF2A, kinesin family member 2A; NSCLC, non-small cell lung cancer; NC, negative control; OD, optical density; CCK-8, Cell Counting Kit-8; PI, propidium iodide; AV, Annexin V-FITC.

A549 Cell Line



NCI-H1299 Cell Line



KIF2A in NSCLC

Figure 3. KIF2A promoted migration and invasion in NSCLC cells. Cell migration (A, B) and cell invasion (C, D) in KIF2A(+) group and NC(+) group in A549 cells. Cell migration (E, F) and invasion (G, H) in KIF2A(-) group and NC(-) group in NCI-H1299 cells. KIF2A, kinesin family member 2A; NSCLC, non-small cell lung cancer; NC, negative control.

to multiple cellular functions, are growing rapidly. A previous study reveals that silencing KIF2A mRNA represses cell proliferation, migration, and invasion, while enhancing cell apoptosis in human glioma cells [18]. Another study elucidates that KIF2A is notably upregulated in gastric cancer cells compared with normal gastric mucosa epithelial cells, and KIF2A mRNA inhibition decreases cancer cell invasion by repressing the signaling of membrane type 1-matrix metalloproteinase (MT1-MMP) in gastric cancer [19]. In addition, another study finds that KIF2A mRNA is targeted by miR-206, which subsequently suppresses ovarian cancer cell proliferation, migration, and invasion, but promotes cancer cell apoptosis [20]. Besides, another study illuminates that KIF2A could be upregulated by the transcription factor ETV4, and downregulation of the KIF2A gene represses cancer cell proliferation and enhances cancer cell apoptosis by mediating AKT signaling in gastric cancer [15]. As for the clinical value of KIF2A in cancers found by the previous researchers, another study reports that KIF2A is upregulated in tumor tissue compared with paired adjacent non-tumor tissue, and it is positively correlated with increased tumor stage and lymph node metastasis in patients with laryngeal squamous cell carcinoma [21]. In addition, KIF2A mRNA and protein expression levels are both increased in colorectal cancer tumor tissue compared with paired adjacent non-tumor tissue, and its high tumor expression is associated with elevated TNM stage as well as a worse 5-year survival rate. Additionally, increased tumor KIF2A expression is an independent predictor for a worse 5-year survival rate in colorectal cancer patients [12]. These studies all indicate that KIF2A can promote the malignant behaviors of cancer cells, and KIF2A overexpression is correlated with more severe disease conditions and a worse prognosis in multiple cancers.

More importantly, KIF2A has also been reported in lung cancer, but the findings are quite insufficient. To the best of our knowledge, only three studies have been reported. Two of them reveal that KIF2A high expression correlates

with worse prognosis in lung cancer patients; the remaining study shows that KIF2A promotes migration and EMT in lung cancer cells [16, 17, 22]. Nonetheless, a comprehensive regulatory role of KIF2A on multiple cancer cell functions in NSCLC has not been adequately evaluated. Hence, in this study, we assessed the effect of KIF2A on cancer cell functions of NSCLC and found that KIF2A promoted NSCLC cell proliferation, migration, and invasion but inhibited apoptosis. The reason for these results could be that KIF2A modulated NSCLC cell functions via interaction with some other factors, as reported in the previous studies conducted on other cancers, such as MT1-MMP and miRNAs [12, 15, 18-21]. In addition, KIF2A may promote the progression of NSCLC by enhancing the malignant behaviors of cancer cells via modulating microtubules, which have been elucidated as a crucial factor in cancer progression [23, 24]. Also, our subsequent rescue experiments displayed that KIF2A modulated NSCLC cell functions via mediating PI3K/AKT/VEGF signaling pathway, which also provided a possible explanation to this result.

The stemness of cancer cell has been recognized as a critical feature that drives the progression of the disease. Moreover, this theory of cancer stemness is also relevant in lung cancers, as evidenced by its critical role in the regulation of tumor growth, resistance to therapeutics, and metastasis [25]. Many potential regulators of NSCLC cell stemness have been identified, such as mitochondrial citrate carrier SLC25A1, Six2, interleukin-10, and ubiquitin-protein ligase E3C [26-29]. Although not in NSCLC, KIF2A has been demonstrated as a regulator of cell stemness as well. A previous study reveals that inhibiting the KIF2A gene suppresses proliferation while it increases neuronal differentiation in neural stem cells [30]. These previous findings suggest that KIF2A participates in the regulation of cancer stem cells in several cancers. Nevertheless, the findings regarding the mediating role of KIF2A in the stemness of cancer cells has not yet been reported yet. In the present study, we elucidated that KIF2A promotes stemness of the NSCLC

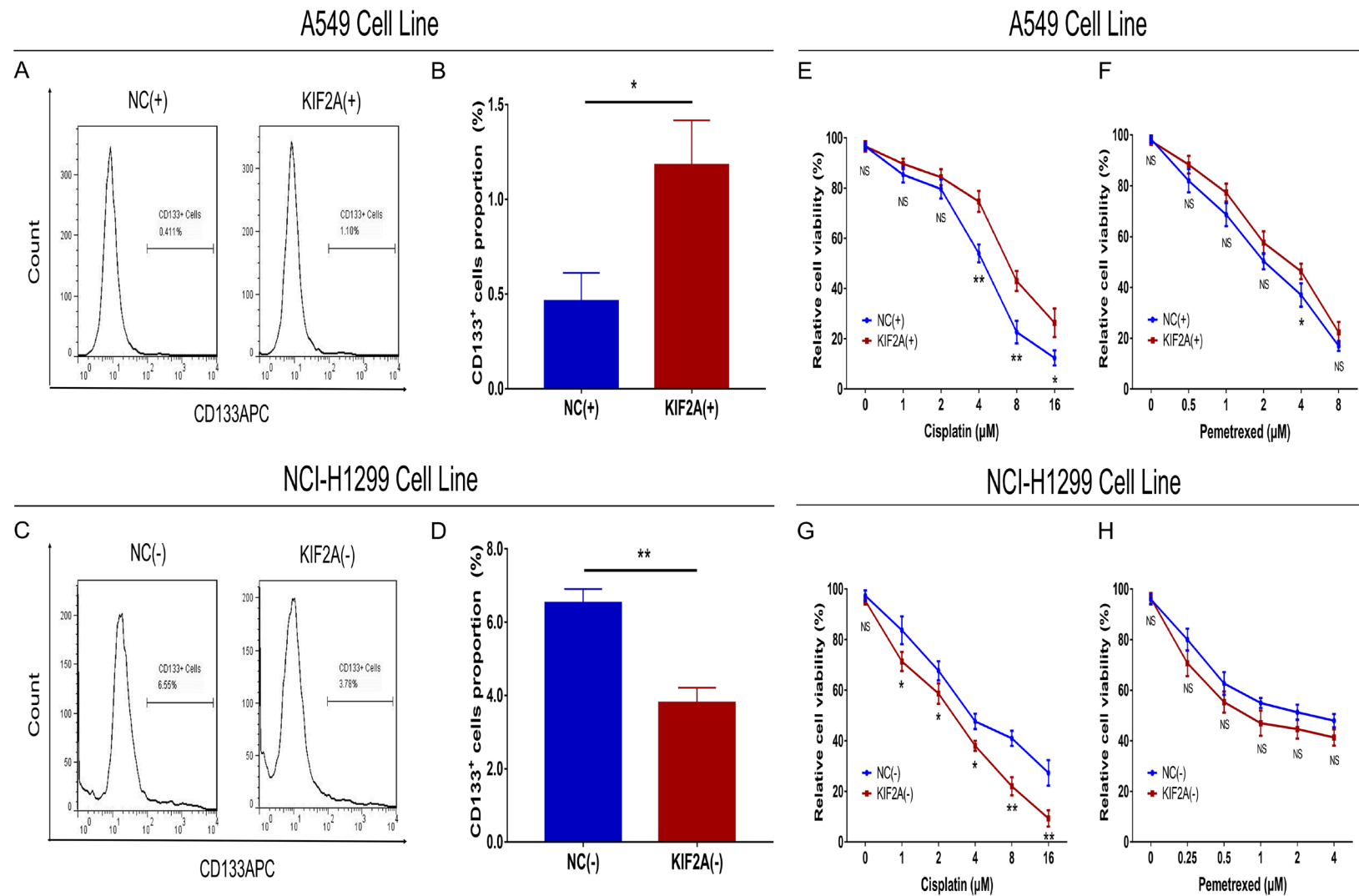


Figure 4. KIF2A elevated CD133⁺ cell proportion but reduced sensitivity to cisplatin in NSCLC cells. CD133⁺ cell proportions in the KIF2A(+) group and NC(+) group in A549 cells (A, B); CD133⁺ cell proportions in the KIF2A(-) group and NC(-) group in NCI-H1299 cells (C, D). Relative cell viability between the KIF2A(+) group and NC(+) group in cisplatin-treated A549 cells (E) and pemetrexed-treated A549 cells (F). Relative cell viability between KIF2A(-) group and NC(-) group in cisplatin-treated NCI-H1299 cells (G) and pemetrexed-treated NCI-H1299 cells (H). KIF2A, kinesin family member 2A; CD133⁺, CD133 positive; NSCLC, non-small cell lung cancer; NC, negative control; NS, not significant.

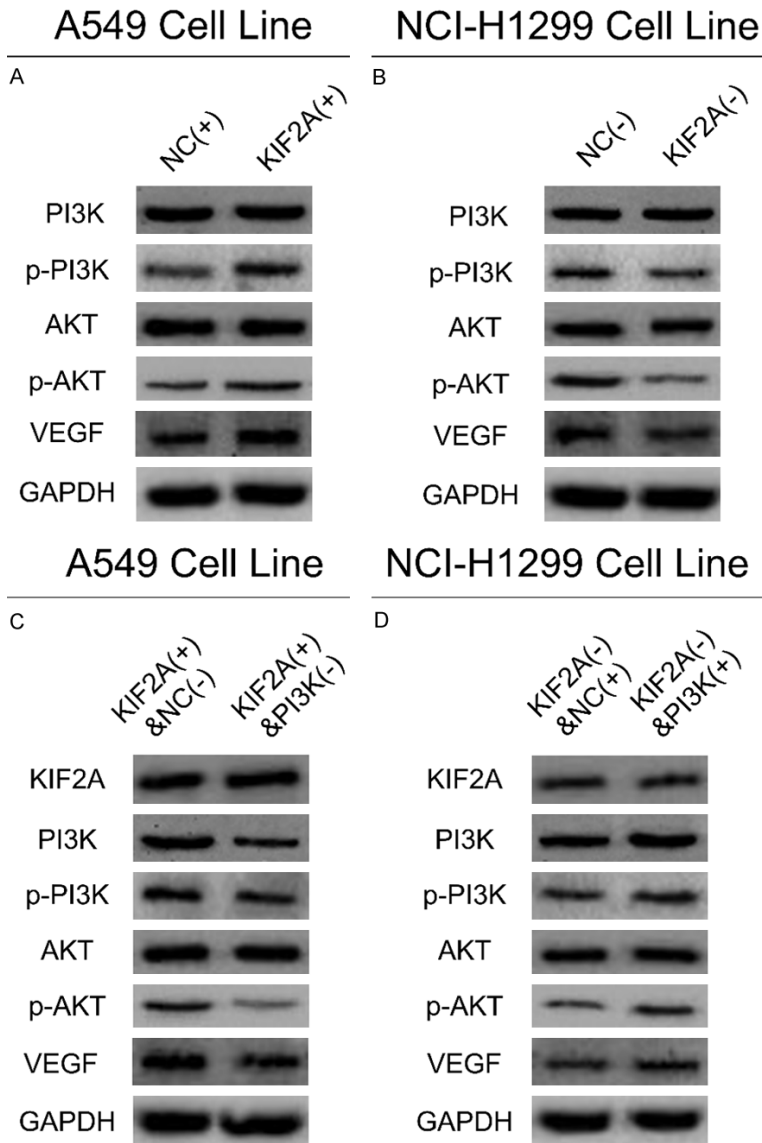


Figure 5. KIF2A activated the PI3K/AKT/VEGF signaling pathway, and PI3K decreased the effect of KIF2A on p-AKT and VEGF expression levels in NSCLC cells. The protein expression levels of PI3K, p-PI3K, AKT, p-AKT, and VEGF in the KIF2A(+) group and NC(+) group in A549 cells (A) and in the KIF2A(-) group as well as NC(-) group in NCI-H1299 cells (B). The protein expression levels of KIF2A, PI3K, p-PI3K, AKT, p-AKT, and VEGF in the KIF2A(+) & NC(-) group and KIF2A(+) & PI3K(-) group in A549 cells (C) and in the KIF2A(-) & NC(+) as well as in the KIF2A(-) & PI3K(+) group in NCI-H1299 cells (D). KIF2A, kinesin family member 2A; PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; VEGF, vascular endothelial growth factor; NSCLC, non-small cell lung cancer; p-PI3K, phospho-PI3K; p-AKT, phospho-AKT; NC, negative control.

cells. This result indicated that KIF2A might be a regulator of cancer cell stemness in NSCLC, which shed new light on the clinical utility of KIF2A in NSCLC patients' management.

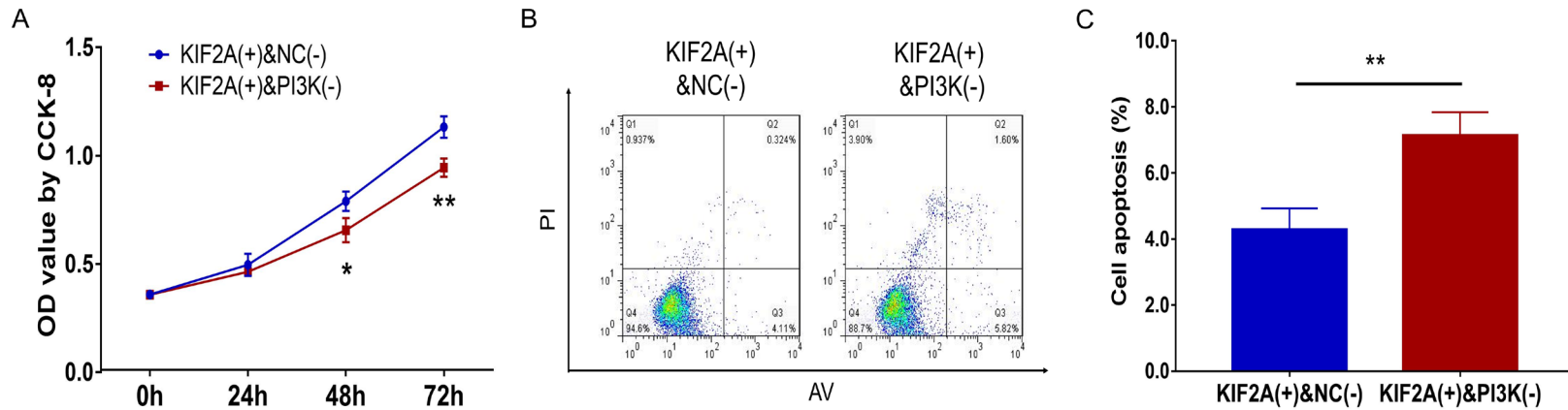
Aside from the advancement of other treatment modalities for NSCLC, chemotherapy con-

tinues to be the mainstay of treatment. Unfortunately, the development of chemoresistance has blocked the use of chemotherapy in the clinical practice [31, 32]. It is thus of considerable interest to investigate the mechanism of chemotherapy drug resistance in NSCLC patients [33-35]. A previous study reported that KIF2A is possibly involved in the drug therapy for cancer, despite the reality that no study about its role in chemoresistance has been published. The study elucidates that the expression of KIF2A may serve as a biomarker for predicting the efficiency of an anti-microtubule agent in *Drosophila* S2 cells treated with colchicine [36]. Our study, for the first time, found that KIF2A reduced the sensitivity to cisplatin in NSCLC cell lines, indicating that KIF2A could be considered as a promising target in enhancing the efficacy of cisplatin treatment in NSCLC. However, this effect should be evaluated by more experiments.

Previously, several studies report that PI3K and AKT are downstream regulatory genes of KIF2A, therefore, we investigated the regulatory role of KIF2A on PI3K/AKT signaling pathway in this study. As an example, KIF2A inhibition elevates the cell apoptosis rate via downregulating the PI3K/AKT pathway in oral squamous cell carcinoma of the tongue [37]. In gastric cancer, KIF2A enhances cancer cell proliferation, migration, and invasion, while it reduces cancer cell apoptosis through modulating AKT signaling [15]. To the best of our knowledge, however, the regulatory role of KIF2A for VEGF has not yet been reported. Although VEGF may be regulated by KIF2A in NSCLC, VEGF is also a downstream gene of the PI3K/AKT signaling pathway [38, 39]. Therefore,

KIF2A in NSCLC

A549 Cell Line



NCI-H1299 Cell Line

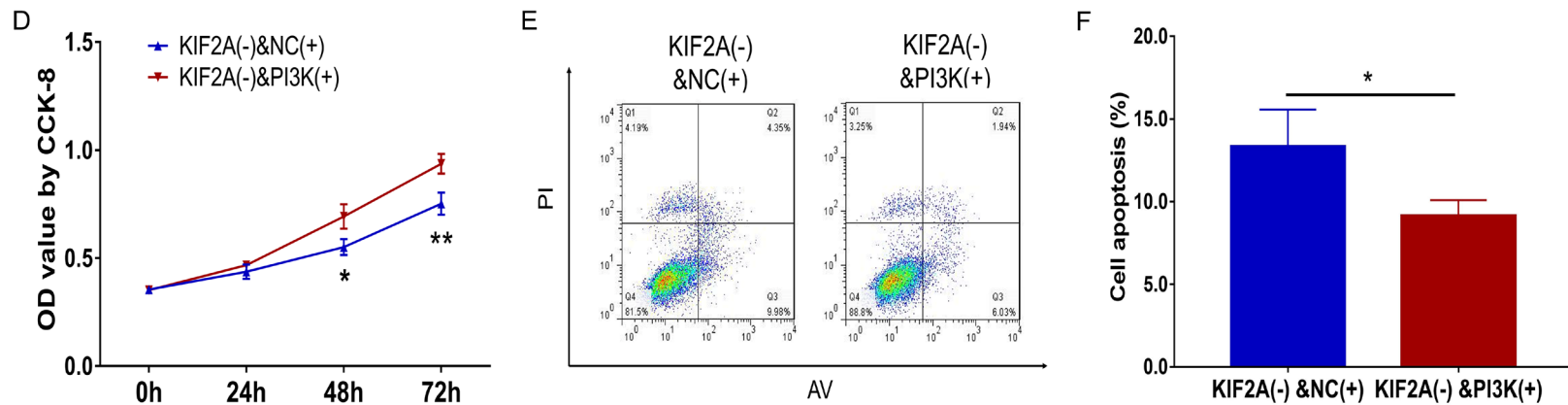
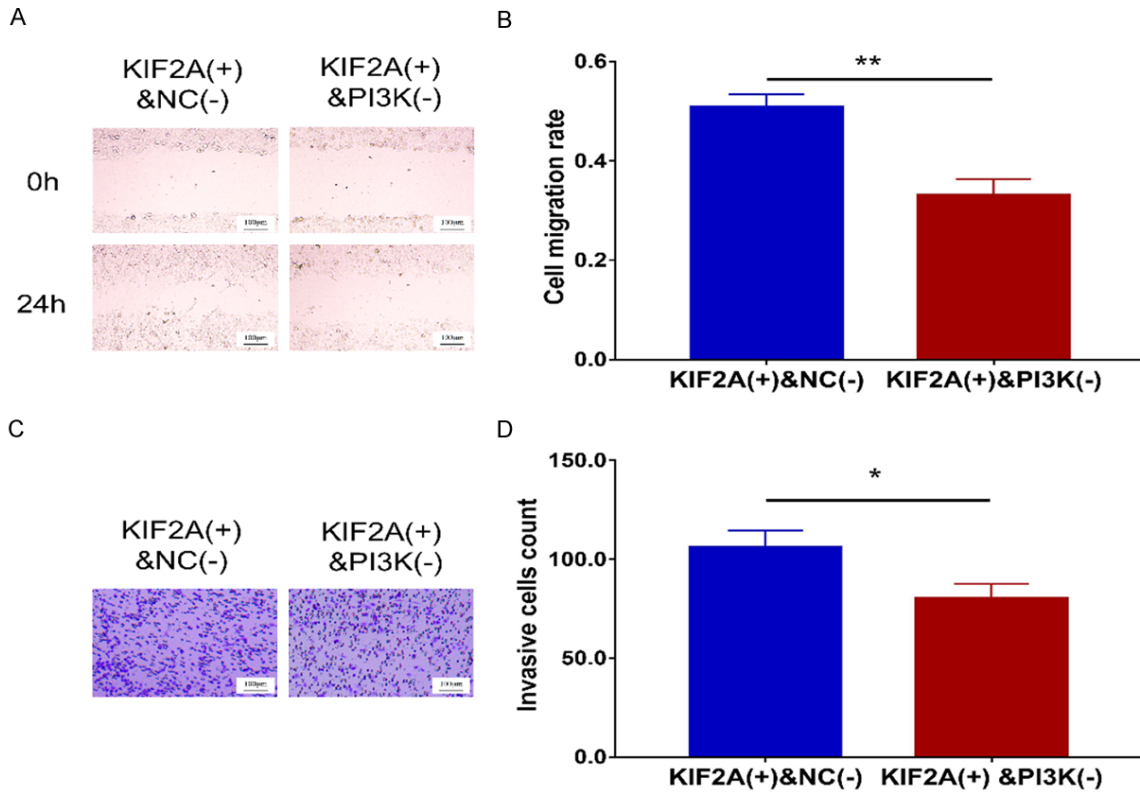
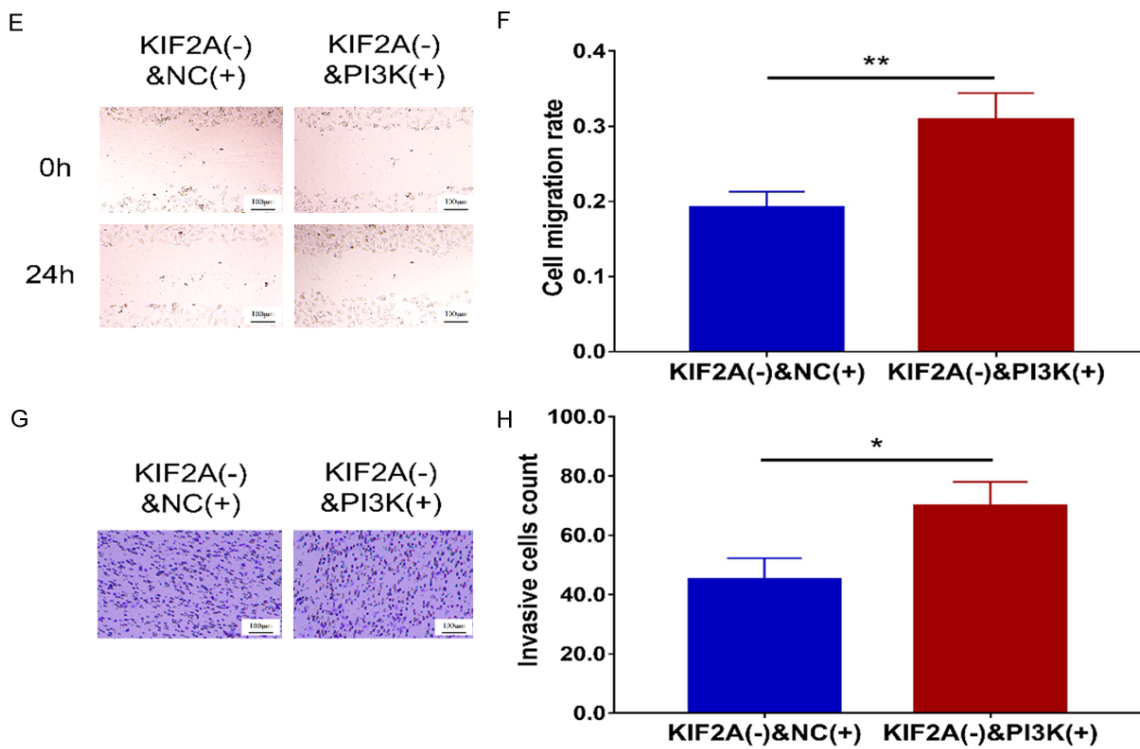


Figure 6. PI3K diminished the effect of KIF2A on proliferation and apoptosis in NSCLC cells. Cell proliferation (A) and cell apoptosis (B, C) in the KIF2A(+) & NC(-) group and KIF2A(+) & PI3K(-) group in A549 cells. Cell proliferation (D) and cell apoptosis (E, F) in the KIF2A(-) & NC(+) group and KIF2A(-) & PI3K(+) group in NCI-H1299 cells. PI3K, phosphatidylinositol-3-kinase; KIF2A, kinesin family member 2A; NSCLC, non-small cell lung cancer; NC, negative control; OD, optical density; CCK-8, Cell Counting Kit-8; PI, propidium iodide; AV, Annexin V-FITC.

A549 Cell Line



NCI-H1299 Cell Line



KIF2A in NSCLC

Figure 7. PI3K reversed the effect of KIF2A on migration and invasion in NSCLC cells. Cell migration (A, B) and cell invasion (C, D) in the KIF2A(+) & NC(-) group and KIF2A(+) & PI3K(-) group in A549 cells. Cell migration (E, F) and cell invasion (G, H) in the KIF2A(-) & NC(+) group and KIF2A(-) & PI3K(+) group in NCI-H1299 cells. PI3K, phosphatidylinositol-3-kinase; KIF2A, kinesin family member 2A; NSCLC, non-small cell lung cancer; NC, negative control.

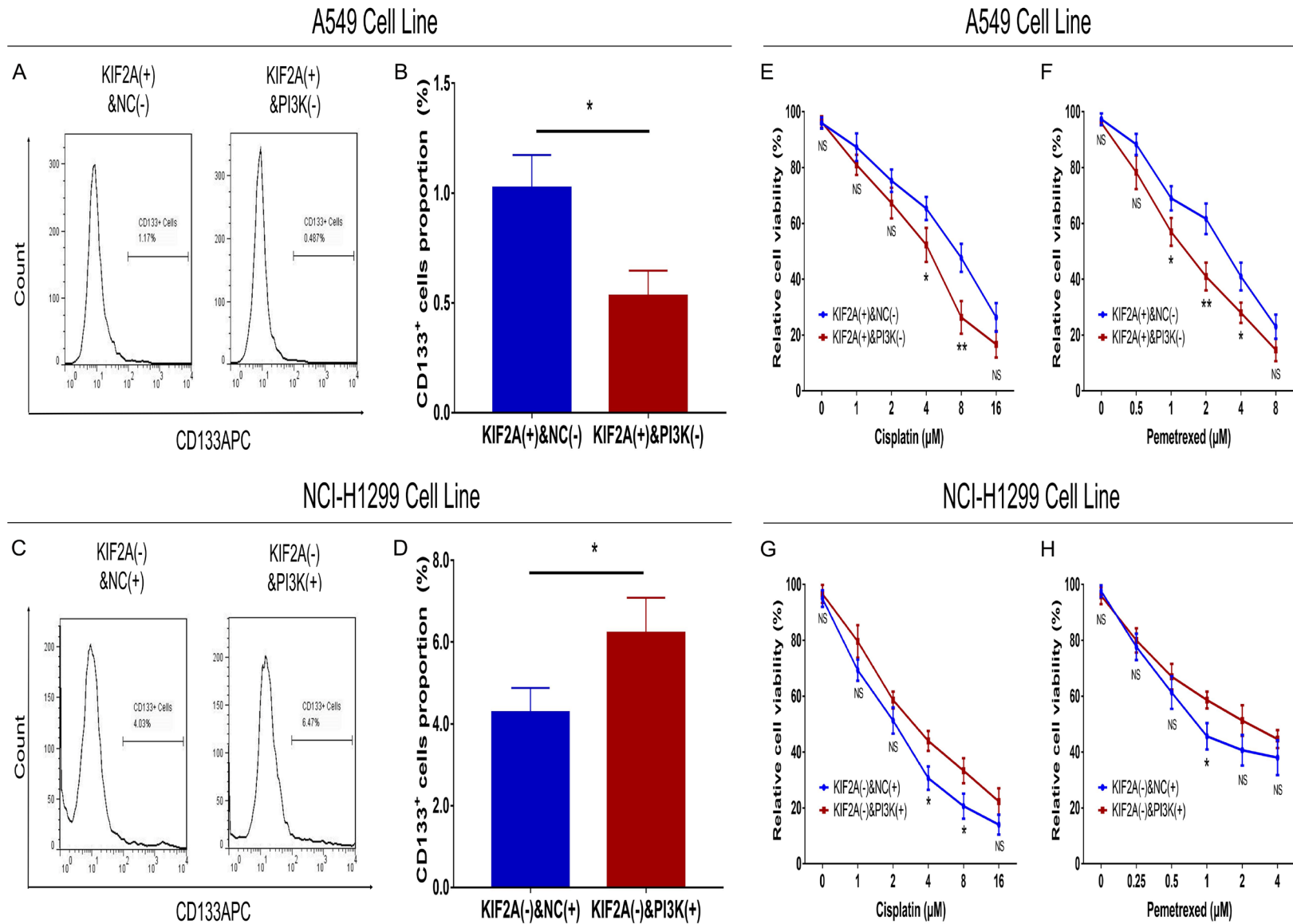


Figure 8. PI3K reduced the effect of KIF2A on stemness and sensitivity to cisplatin in NSCLC cells. CD133⁺ cell proportions in the KIF2A(+) & NC(-) group and KIF2A(+) & PI3K(-) group in A549 cells (A, B) and in the KIF2A(-) & NC(+) group as well as the KIF2A(-) & PI3K(+) group in NCI-H1299 cells (C, D). Relative cell viability between the KIF2A(+) & NC(-) group and KIF2A(+) & PI3K(-) group in cisplatin-treated A549 cells (E) and pemetrexed-treated A549 cells (F). Relative cell viability between the KIF2A(-) & NC(+) group and KIF2A(-) & PI3K(+) group in cisplatin-treated NCI-H1299 cells (G) and pemetrexed-treated NCI-H1299 cells (H). PI3K, phosphatidylinositol-3-kinase; KIF2A, kinesin family member 2A; NSCLC, non-small cell lung cancer; CD133⁺, CD133 positive; NC, negative control; NS, not significant.

according to these previous studies, KIF2A may be involved in the modulation of the PI3K/AKT signaling pathway in cancers. However, to the best of our knowledge, the correlation between KIF2A and the PI3K/AKT signaling pathway in NSCLC has not been investigated. In this study, we found that PI3K/AKT/VEGF signaling pathway rescued the effect of KIF2A on NSCLC cell functions and sensitivity to cisplatin, which unveiled a possible regulatory mechanism of KIF2A in NSCLC etiology.

In summary, this current study illustrates that KIF2A regulates NSCLC cell viability, mobility, stemness and sensitivity to cisplatin through activating PI3K/AKT/VEGF signaling pathway. These findings contribute to our understanding of the mechanistic role of KIF2A in the modulation of tumor growth, metastasis, stemness and chemosensitivity in NSCLC.

Disclosure of conflict of interest

None.

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