Histol Histopathol (2022) 37: 269-285

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE **Open Access**

Downregulation of KIF15 inhibits the tumorigenesis of non-small-cell lung cancer via inactivating Raf/MEK/ERK signaling

Yingbin Luo*, Bo Zhang*, Lili Xu*, Minghua Li, Jianchun Wu, Yiyang Zhou and Yan Li

Department of Oncology, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, PR China

*These authors contributed equally to this work and should be considered as co-first authors

Summary. Background. Lung cancer is one of the most common causes of cancer-associated mortality worldwide. Upregulation of kinesin family member 15 (KIF15) expression has been observed in non-small cell lung cancer (NSCLC), and high expression levels of KIF15 are associated with a poor prognosis in patients with NSCLC. However, to the best of our knowledge, the mechanisms by which KIF15 regulates apoptosis, migration and invasion in NSCLC remain unclear.

Methods. Cell Counting Kit-8, flow cytometry and Transwell assays were performed to determine the proliferation, apoptosis and invasion of NSCLC cells, respectively. In addition, western blotting was used to detect the levels of phosphorylated (p-)c-Raf, p-ERK and p-MEK in NSCLC cells.

Results. Downregulation of KIF15 expression markedly inhibited the proliferation, migration and invasion of NSCLC cells through mediation of MMP2 and MMP9. In addition, downregulation of KIF15 markedly induced apoptosis and cell cycle arrest in NSCLC cells through regulation of active caspase 3, p27 Kip1 and cyclin D1. Furthermore, KIF15 knockdown notably decreased the levels of activating transcription factor 2, p-c-Raf, p-ERK and p-MEK in A549 and NCI-H460 cells. Finally, KIF15 knockdown notably inhibited the tumor growth of NSCLC *in vivo*.

Conclusion. In conclusion, the present study indicated that downregulation of KIF15 expression was able to inhibit the tumorigenesis of NSCLC by inactivating Raf/MEK/ERK signaling. These findings may help improve the diagnosis and treatment of NSCLC.

Key words: Non-small-cell lung cancer, Kinesin superfamily proteins, Apoptosis, Cell cycle arrest

Corresponding Author: Yiyang Zhou, Department of Oncology, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, No.274, Zhijiang Road, Jing'an District, Shanghai 200071, PR China. e-mail: yiyangzhou916@hotmail.com or Yan Li, Department of Oncology, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, No.274, Zhijiang Road, Jing'an District, Shanghai 200071, PR China. e-mail: liyan111lyly@126.com

DOI: 10.14670/HH-18-408

Introduction

Lung cancer is one of the most common causes of cancer-associated mortality worldwide (Liu et al., 2017). Aging and smoking are considered to be common risk factors for lung cancer (Bibaki et al., 2018). In addition, lung cancer has been divided into two subgroups: Small cell lung cancer (15%) and non-small cell lung cancer (NSCLC; 85%) (Liu et al., 2017). Lung adenocarcinoma (LUAD), lung squamous cell carcinoma and large-cell lung carcinoma are the most frequent subtypes of NSCLC (Liu et al., 2017). The identification of subtypes of NSCLC has become a determinant of therapy in NSCLC (Liu et al., 2017; Villalobos and Wistuba, 2017). Patients with NSCLC who are diagnosed at an early stage have a favorable prognosis following surgical resection, with overall 5-year survival rates of 70-90% (Blandin Knight et al., 2017). However, >70% of patients are diagnosed at an advanced stage, and the 5-year survival rate of these patients is ≤15% (Cagle et al., 2013; Zhang et al., 2017). Therefore, novel effective therapies for NSCLC are urgently required.

Kinesin superfamily proteins (KIFs) are ubiquitous in eukaryotes, and kinesin superfamily members share a highly conserved motor domain (Goulet et al., 2014). KIFs are involved in several essential cellular processes, including the transport of macromolecules, mitosis and meiosis (Liu et al., 2010; Song et al., 2018). In addition,

KIF genes serve important roles in the development and progression of human cancers, such as breast and pancreatic cancer (Liu et al., 2013; Wang et al., 2017; Qiao et al., 2018). Among the KIFs, kinesin family member 15 (KIF15) is a kinesin-12 motor that has been demonstrated to regulate mitosis (Wang et al., 2017; Qiao et al., 2018). Additionally, KIF15 expression has been reported to be upregulated in several types of human cancer, including gastric cancer, hepatocellular carcinoma and pancreatic cancer (Wang et al., 2017, 2019a,b; Ding et al., 2020). Qiao et al. (2018) reported that KIF15 expression is markedly upregulated in human lung tumor tissues, and high expression levels of KIF15 are associated with a worse prognosis in patients with LUAD. However, the mechanisms by which KIF15 regulates cell apoptosis, migration and invasion in NSCLC remains unclear.

The Raf/MEK/ERK signaling cascade belongs to MAPK signaling which is essential for cell inter- and intra-cellular communication, and it regulates fundamental cell functions such as growth, survival, and differentiation (Degirmenci et al., 2020). Meanwhile, it has been reported that activation of Raf/MEK/ERK signaling can promote the malignant behavior of NSCLC (Iezzi et al., 2018; Wang and He, 2020), and the Raf-MEK-ERK pathway may contribute to the growth, migration and invasion of NSCLC cells (Butt et al., 2021; Yuan et al., 2021). However, the correlation between KIF15 and Raf-MEK-ERK pathway remains unclear. Thus, we sought to investigate the function of KIF15 in this signaling pathway.

Therefore, the present study aimed to investigate the role of KIF15 in the tumorigenesis of NSCLC. We hope this research will shed new lights on exploring the new strategies for the treatment of NSCLC.

Materials and methods

Validation of gene expression based on The Cancer Genome Atlas (TCGA) database

Gene Expression Profiling Interactive Analysis (http://gepia.cancer-pku.cn/detail.php) is a tool for the analysis of RNA sequencing expression data of tumors and normal samples from TCGA and the Genotype-Tissue Expression project (Xie et al., 2016), and was used to validate the differential expression of KIF15 between LUAD/LUSC and adjacent normal samples. LUAD and LUSC are two main subtypes of NSCLC. In addition, TCGA dataset was downloaded and R analysis was performed as previously described (Colaprico et al., 2016).

Survival analysis

A TCGA dataset analyzed from Gene Expression Profiling Interactive Analysis (GEPIA) was utilized to determine whether the expression levels of KIF15 were associated with the survival of patients with LUAD and LUSC as previously described (Tang et al., 2017). Meanwhile, the Kaplan-Meier curves were analyzed from Gene Expression Profiling Interactive Analysis (GEPIA) as previously described (Tang et al., 2017).

Cell culture

The BEAS-2 human normal bronchial epithelial cell line, and A549, Calu-1 and NCI-H460 human NSCLC cell lines and 293T cells were obtained from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. The cells were maintained in DMEM supplemented with 10% FBS (Thermo Fisher Scientific, Inc.) and antibiotics (100 U/ml penicillin/streptomycin) in a humidified atmosphere containing 95% air and 5% CO_2 at 37°C.

Western blotting

BCA protein assay reagent (Beyotime Institute of Biotechnology) was used to determine the total protein concentration. Subsequently, proteins were subjected to 10% SDS-PAGE and then transferred onto an immunoblot PVDF membrane (EMD Millipore). Following blocking with 5% skimmed milk in TBS with Tween 20 (TBST) for 1 h, the membrane was incubated with primary antibodies overnight at 4°C. The antibodies were diluted 1:1,000, and included KIF15 (ab272615, Abcam), α-tubulin (ab7291, Abcam), active caspase 3 (ab2302, Abcam), pro-caspase 3 (ab32351, Abcam), activating transcription factor 2 (ATF2; ab239361, Abcam), phosphorylated (p-)c-Raf (ab230850, Abcam), p-MEK (ab96379, Abcam), p-ERK (ab201015, Abcam), cyclin D1 (ab16663, Abcam), p27 Kip1 (ab32034, Abcam) and ERK (ab109282, Abcam) antibodies. Subsequently, the membrane was washed three times with TBST and then incubated with HRP-conjugated secondary antibody (ab7090, Abcam) at room temperature for 1 h. Afterwards, the blots were developed using an enhanced chemiluminescent substrate kit (Thermo Fisher Scientific, Inc.).

Lentivirus production and cell transduction

The lentivirus-containing short hairpin RNAs (shRNAs) targeting KIF15 were purchased from Guangzhou RiboBio Co., Ltd., and are referred to as KIF15-shRNA1, KIF15-shRNA2 and KIF15-shRNA3. Additionally, scrambled control shRNA, which was used as a negative control (shRNA-NC), was purchased from Guangzhou RiboBio Co., Ltd. shRNA-NC, KIF15-shRNA1, KIF15-shRNA2 and KIF15-shRNA3 plasmids were transfected into 293T cells using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.). KIF15 shRNA1, shRNA2 or shRNA3 was packaged into lentiviruses. Then the lentiviral vector DNAs were then transfected into 293T cells including lenti-KIF15 shRNAs and negative control. After transfection, the cells were incubated at 37°C, and then the supernatant was

collected. After that, supernatants of three KIF15 shRNAs and negative control were filtered into particles. Finally, all NSCLC cells were infected with lentiviral particles according to the manufactures' protocol. After 48 h of incubation, stable NSCLC cells were then selected by puromycin (2.5 µg/mL, Sigma Aldrich, St. Louis, MO, USA). The sequences of shRNAs and negative control were as follows: shRNA NC: 5'-UUCUCCGAACGUGUCACGUTT-3'; KIF15 shRNA1: 5'-GGAAUGUGACAAAUUCUCATT-3'; KIF15 shRNA2: 5'-GACUUUUGUAUUUGAUUAUTT-3'; KIF15 shRNA3: 5'-CAUAAUCUGAGAGGAAU AATT-3'.

To transduce the A549, NCI-H460 and Calu-1 cells, the viral supernatant was added to the plate to infect A549, NCI-H460 and Calu-1 cells. After 72 h of transduction, the infected cells were selected using puromycin (Amresco, LLC). Cells in the control group were cultured in normal medium with 10% FBS.

For KIF15 overexpression, NSCLC cells were transfected with pcDNA3.1 or pcDNA3.1-KIF15 by using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's instruction. pcDNA3.1 and pcDNA3.1-KIF15 were purchased from Guangzhou RiboBio Co., Ltd.

Reverse transcription-quantitative PCR (RT-qPCR)

Total RNA was extracted from NSCLC cells using the TRIpure Total RNA Extraction reagent [ELK (Wuhan) Biotechnology Co., Ltd.]. Total RNA was converted into first-strand cDNA using an EntiLink™ 1st Strand cDNA Synthesis kit [ELK (Wuhan) Biotechnology Co., Ltd.]. Subsequently, qPCR was performed using EnTurbo™ SYBR Green PCR SuperMix reagent [ELK (Wuhan) Biotechnology Co., Ltd.] and the StepOne™ Real-Time PCR system (Thermo Fisher Scientific, Inc.) according to the following conditions: 60°C for 1 min, 90°C for 15 min, followed by 40 cycles of application at 90°C for 15 sec and 55°C for 60 sec. The relative expression levels of KIF15 were calculated according to the $2^{-\Delta\Delta Cq}$ method (Livak and Schmittgen, 2001), and GAPDH was used as an internal control. The primers used were as follows: KIF15 forward, 5'-CTCTCACAGTTGAATGTCCTTG-3' and reverse, 5'-CTCCTTGTCAGCAGAATGAAG-3'; and GAPDH forward, 5'-TGACTTCAACAGC GACACCCA-3' and reverse, 5'-CACCCTGTTGCT GTAGCCAAA-3'.

Cell Counting Kit-8 (CCK-8) assay

The viability of NSCLC cells was analyzed using a CCK-8 assay (Dojindo Molecular Technologies, Inc.). Cells were plated in a 96-well culture plate at a density of $5x10^3$ cells/ml, and incubated overnight at 37°C. Subsequently, cells were incubated for 0, 24, 48 and 72 h at 37°C, followed by incubation with 10 µl CCK-8 reagent for another 2 h at 37°C. Afterwards, the absorbance at a wavelength of 450 nm was measured using a microplate reader (Thermo Fisher Scientific, Inc.).

Flow cytometry

Cell apoptosis was detected using an Annexin V-FITC apoptosis detection kit (Thermo Fisher Scientific, Inc.). NSCLC cells were washed twice with PBS, resuspended in binding buffer, and then stained with 5 µl annexin V-FITC (BD Biosciences) and 5 µl PI (BD Biosciences) for 15 min at room temperature in the dark. Subsequently, cell apoptosis was analyzed using a flow cytometer (BD FACSAria III; BD Biosciences). The data were quantified by Flowjo software (BD Biosciences).

For cell cycle analysis, cells were washed twice with pre-cooled PBS, fixed for 30 min, centrifuged at 1000 r/min for 5 min and then stained with propidium (PI) staining buffer (BD Biosciences). Afterwards, cells were stained with PI in the dark for 30 min at 4°C. Subsequently, cell cycle distribution was analyzed using a flow cytometer (BD FACSAria III; BD Biosciences). The data were quantified by Flowjo software (BD Biosciences).

Wound healing assay

A549 and NCI-H460 cells were plated at a density of $2x10^5$ cells per well in a 12-well culture plate. When the cells reached 80% confluence, a wound area was scratched in the cell monolayer using a 20 µl pipette tip. Subsequently, cells were infected with KIF15 shRNA2 for 48 h at 37°C. Images were captured at 0 and 48 h under a fluorescence microscope (Leica Microsystems, Inc.). Quantitative analysis of cell migration was conducted using ImageJ software (National Institutes of Health).

Transwell invasion assay

Cell invasion was analyzed using 24-well Transwell chambers (0.8 µm; Corning, Inc.). A549 or NCI-H460 cells ($4x10^4$) in 200 µl serum-free medium were seeded into the upper chamber, which was pre-coated with Matrigel matrix (BD Biosciences). Afterwards, the lower chamber was filled with 600 µl DMEM containing 10% FBS. Subsequently, the cells in the lower chamber were fixed with 4% paraformaldehyde, and stained with 0.2% crystal violet at 24 h. Images of the invading cells were captured under a fluorescence microscope.

Immunofluorescence

A549 and NCI-H460 cells were fixed in 4% paraformaldehyde and permeabilized with 0.3% Triton X-100 for 15 min at room temperature. Subsequently, cells were incubated with rhodamine-conjugated phalloidin (Abcam, 1:1000) for 1 h. The nuclei were

counterstained with DAPI. The cells were observed under a fluorescence microscope.

Tumor xenograft study

All procedures performed in this study involving animals were in accordance with the National Institutes of Health guide for the care and use of Laboratory animals. The animal study was approved by Animal Care and Use Committee of Shanghai Municipal Hospital of Traditional Chinese Medicine. NCI-H460 cells (1×10^7 cells) were subcutaneously injected into the right flanks in 7-week-old BALB/c nude mice (n=9, Vital River, Beijing, China) as previously described (Shi et al., 2021). When tumor volumes reached 150 mm3, the mice were randomly divided into two groups: control and KIF15 shRNA. The mice in KIF15 shRNA group were intra-tumor injected with KIF15 shRNA twice a week. In addition, mice were intraperitoneally injected with 50 mg/kg pentobarbital sodium 10 min before administration. Tumor volume was measured every 4 days with calipers. The tumor volumes were calculated by the following formula: volume = (length×width^2)/2. At day 44, all mice were euthanized using CO_2 at a displacement rate of 30% of the chamber volume/min (CO_2 flow rate, 2.5 l/min). Tumors were resected, photographed and weighted immediately after sacrifice.

Statistical analysis

All statistical analyses were performed using GraphPad Prism software (version 7.0; GraphPad Software, Inc.). All figures were generated using GraphPad Prism software. One-way ANOVA followed by Tukey's post hoc test was performed for comparisons among multiple groups. All experiments were repeated

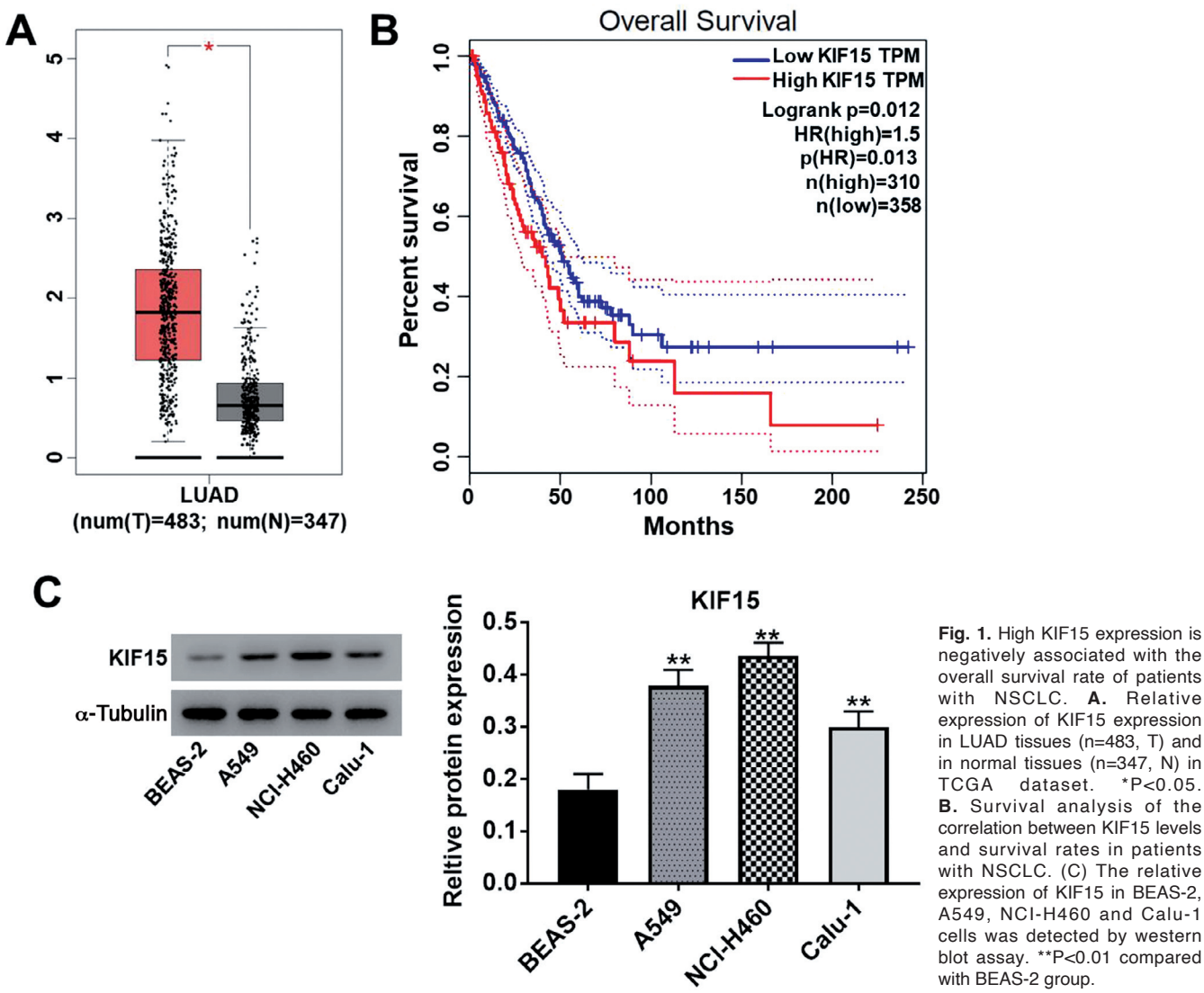

Fig. 1. High KIF15 expression is negatively associated with the overall survival rate of patients with NSCLC. **A.** Relative expression of KIF15 expression in LUAD tissues (n=483, T) and in normal tissues (n=347, N) in TCGA dataset. *P<0.05. **B.** Survival analysis of the correlation between KIF15 levels and survival rates in patients with NSCLC. (C) The relative expression of KIF15 in BEAS-2, A549, NCI-H460 and Calu-1 cells was detected by western blot assay. **P<0.01 compared with BEAS-2 group.

in triplicate. Data are presented as the mean ± SD. $P<0.05$ was considered to indicate a statistically significant difference.

Results

High KIF15 expression is negatively associated with the overall survival rate of patients with NSCLC

To determine the expression levels of KIF15 in NSCLC, a TCGA dataset was used. The data revealed that KIF15 expression in LUAD tissues was higher than that in normal tissues (Fig. 1A). In addition, high KIF15 expression was associated with poor overall survival rates in patients with NSCLC in the dataset from TCGA (Fig. 1B). Furthermore, it was observed that KIF15 expression was upregulated in A549, Calu-1 and NCI-H460 cells compared with in BEAS-2 normal human lung cells (Fig. 1C). These data indicated that KIF15 expression was upregulated in NSCLC, and high KIF15 expression was associated with a poor overall survival rate of patients with NSCLC.

Downregulation of KIF15 expression inhibits the proliferation of NSCLC cells via induction of apoptosis

To determine the function of KIF15 in NSCLC, three different shRNAs (KIF15 shRNA1, KIF15 shRNA2 and KIF15 shRNA3) were used to downregulate KIF15 expression in A549 and NCI-H460 cells. RT-qPCR and western blotting indicated that the mRNA and protein expression levels of KIF15 were markedly downregulated following transfection of KIF15 shRNA plasmids in NSCLC cells (Fig. 2A-D). Notably, the data revealed that KIF15 shRNA2 had the most efficacious inhibitory effect on KIF15 expression in NSCLC cells (Fig. 2A-D). Therefore, the KIF15 shRNA2 plasmid was utilized in the following experiments.

CCK-8 and flow cytometry assays were performed to investigate the effect of KIF15 on the proliferation and apoptosis of NSCLC cells. As shown in Fig. 3A,B, downregulation of KIF15 expression markedly inhibited the proliferation of A549 and NCI-H460 cells compared with that of cells in the shRNA-NC group. In

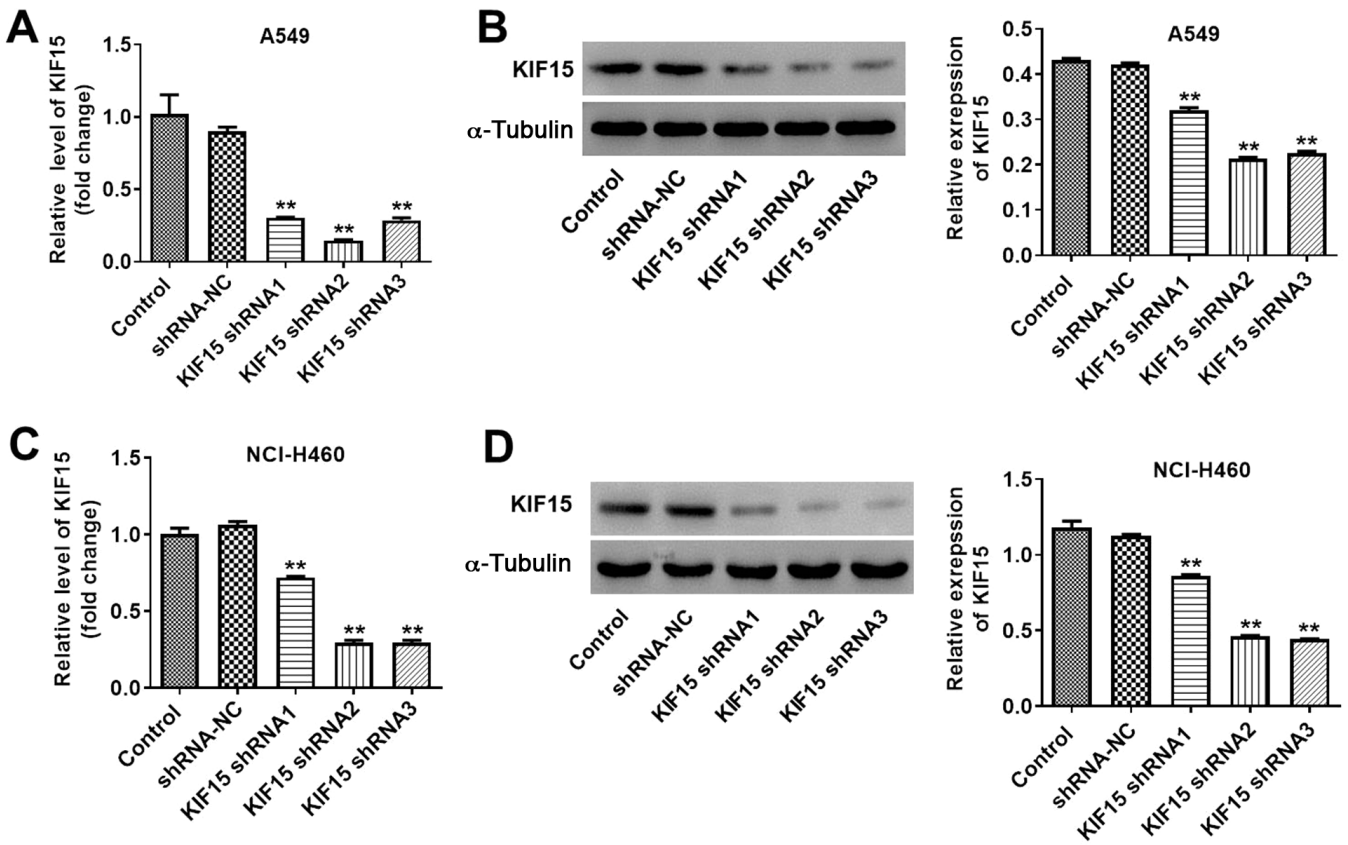

Fig. 2. KIF15 shRNA2 significantly downregulated the expression of KIF15 in NSCLC cells. A549 and NCI-H460 cells were infected with KIF15 shRNA1, KIF15 shRNA2, or KIF15 shRNA3 for 72 h. **A.** The level of KIF15 in A549 cells was analyzed by RT-qPCR. **B.** Expression level of KIF15 in A549 cells was detected with western blotting. The relative expression of KIF15 in A549 cells was quantified via normalization to α-Tubulin. **C.** The level of KIF15 in NCI-H460 cells was analyzed by RT-qPCR. **D.** Expression level of KIF15 in NCI-H460 cells was detected with western blotting. The relative expression of KIF15 was quantified via normalization to α-Tubulin. **P<0.01 compared with shRNA-NC group. NC, negative control.

addition, downregulation of KIF15 notably induced the apoptosis of A549 and NCI-H460 cells (Fig. 3C,D). These data indicated that downregulation of KIF15 inhibited the proliferation of NSCLC cells via induction of apoptosis.

Downregulation of KIF15 expression suppresses the migration and invasion abilities of NSCLC cells

The role of KIF15 in the regulation of migration and invasion in NSCLC cells was analyzed using wound healing

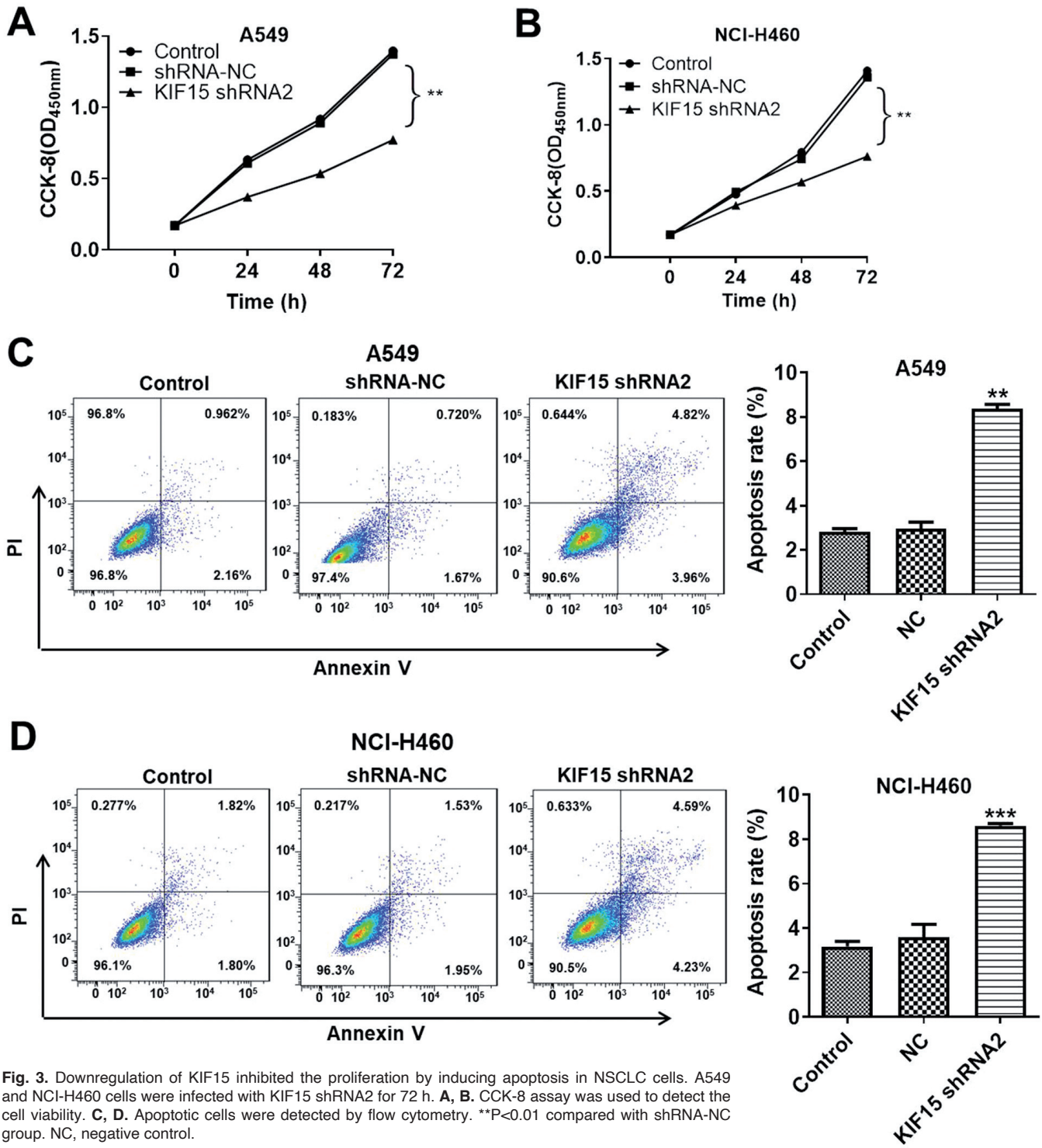

Fig. 3. Downregulation of KIF15 inhibited the proliferation by inducing apoptosis in NSCLC cells. A549 and NCI-H460 cells were infected with KIF15 shRNA2 for 72 h. **A, B.** CCK-8 assay was used to detect the cell viability. **C, D.** Apoptotic cells were detected by flow cytometry. **P<0.01 compared with shRNA-NC group. NC, negative control.

and Transwell invasion assays. As shown in Fig. 4A,B, knockdown of KIF15 notably suppressed the migration ability of A549 and NCI-H460 cells. In addition, a Transwell invasion assay indicated that the invasive abilities of A549 and NCI-H460 cells were decreased following KIF15 shRNA2 transfection (Fig. 4C). These data suggested that downregulation of KIF15 could suppress the migration and invasion abilities of NSCLC cells.

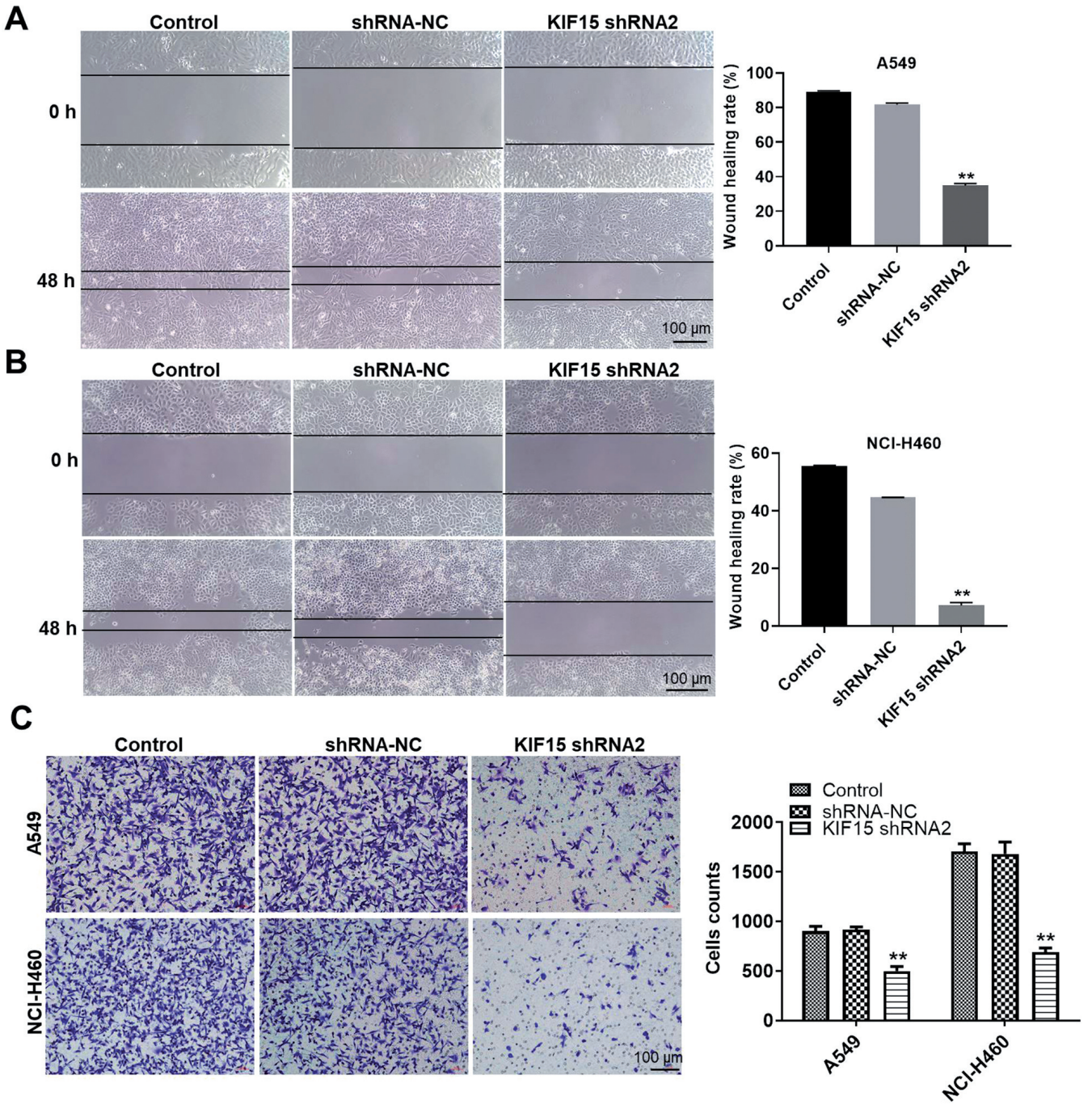

Fig. 4. Downregulation of KIF15 suppressed the migration and invasion abilities of NSCLC cells. **A, B.** A549 and NCI-H460 cells were infected with KIF15 shRNA2 for 48 h. Cell migration was detected using wound healing assay. **C.** A549 and NCI-H460 cells were infected with KIF15 shRNA2 for 24 h. Cell invasion was detected using transwell invasion assay. **P<0.01 compared with shRNA-NC group. NC, negative control.

Downregulation of KIF15 induced the apoptosis and inhibited the migration and invasion of NSCLC cells through mediation of active caspase 3, MMP2 and MMP9

To investigate the mechanism by which KIF15 mediates the growth, migration and invasion of NSCLC cells, western blot was performed. As revealed in Fig. 5A,B, the level of active caspase 3 in NSCLC cells was significantly upregulated by KIF15 shRNA2. In contrast, KIF15 knockdown notably inhibited the expressions of MMP2 and MMP9 in NSCLC cells (Fig. 5C-E). Meanwhile, the expression of KIF15 in NSCLC cells was significantly upregulated by pcDNA3.1-KIF15 (Fig.

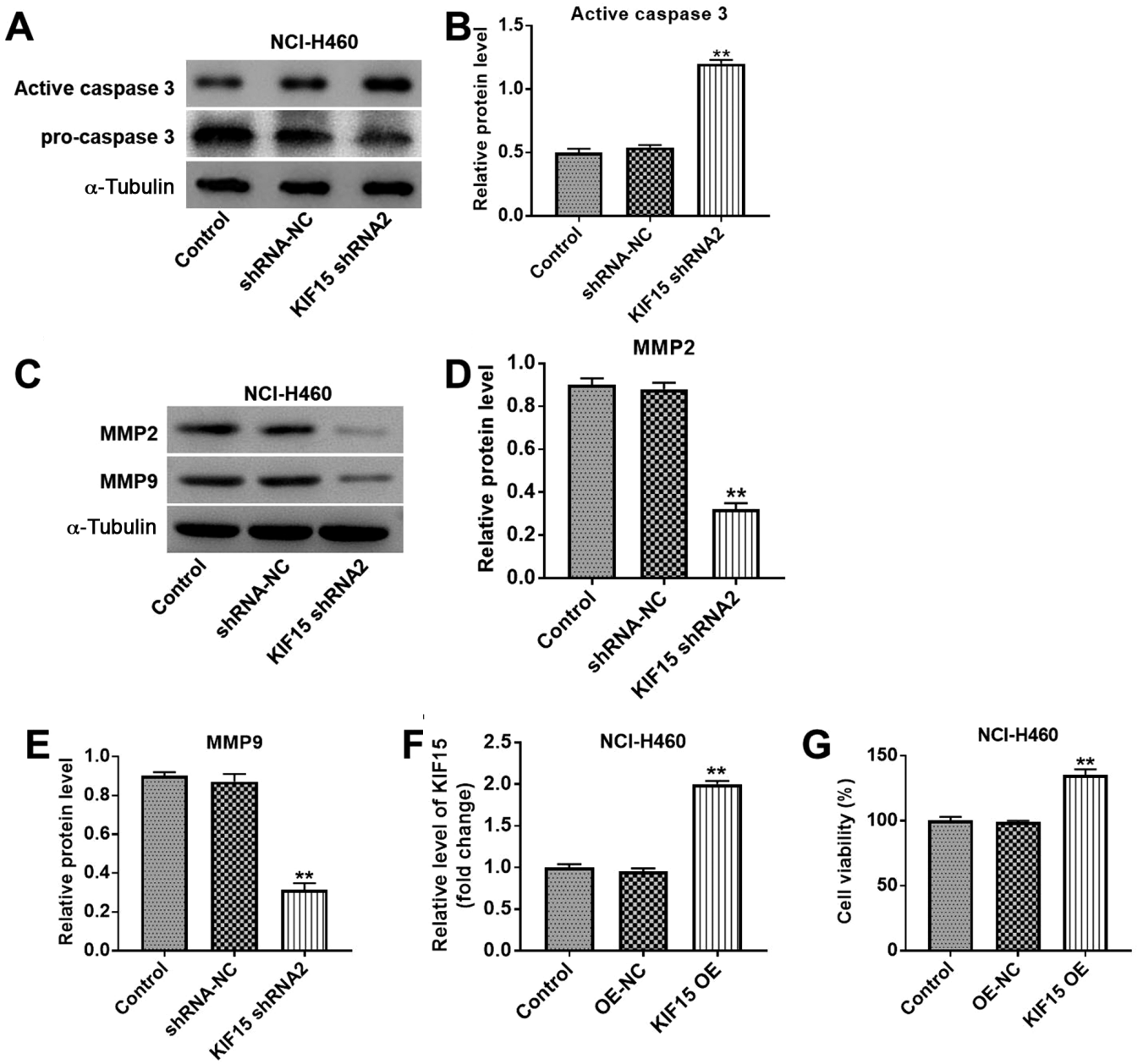

Fig. 5. Downregulation of KIF15 induced apoptosis and inhibited the migration and invasion of NSCLC cells through mediation of active caspase 3, MMP2 and MMP9. NSCLC cells were infected with KIF15 shRNA2 for 48 h. **A.** The protein level of active caspase 3 and pro-caspase 3 in NSCLC cells were detected with western blotting. **B.** The relative expression of active caspase 3 in cells was quantified via normalization to α-Tubulin. **C.** The protein levels of MMP2 and MMP9 in NSCLC cells were detected with western blotting. **D, E.** The relative expressions were quantified via normalization to α-Tubulin. **F.** NSCLC cells were transfected with pcDNA3.1-KIF15 for 24 h. Then, the efficiency of cell transfection was detected by RT-qPCR. **G.** The viability of NSCLC cells was tested by CCK-8 assay. **P<0.01 compared with shRNA-NC or OE-NC group. NC, negative control.

5F), and KIF15 overexpression notably increased the viability of NSCLC cells (Fig. 5G). In summary, Downregulation of KIF15 induced the apoptosis and inhibited the migration and invasion of NSCLC cells through mediation of active caspase 3, MMP2 and MMP9.

Downregulation of KIF15 expression induces cell cycle arrest of NSCLC cells

It has been demonstrated that the actin cytoskeleton serves an important role in cell remodeling in the first stages of apoptosis (Povea-Cabello et al., 2017).

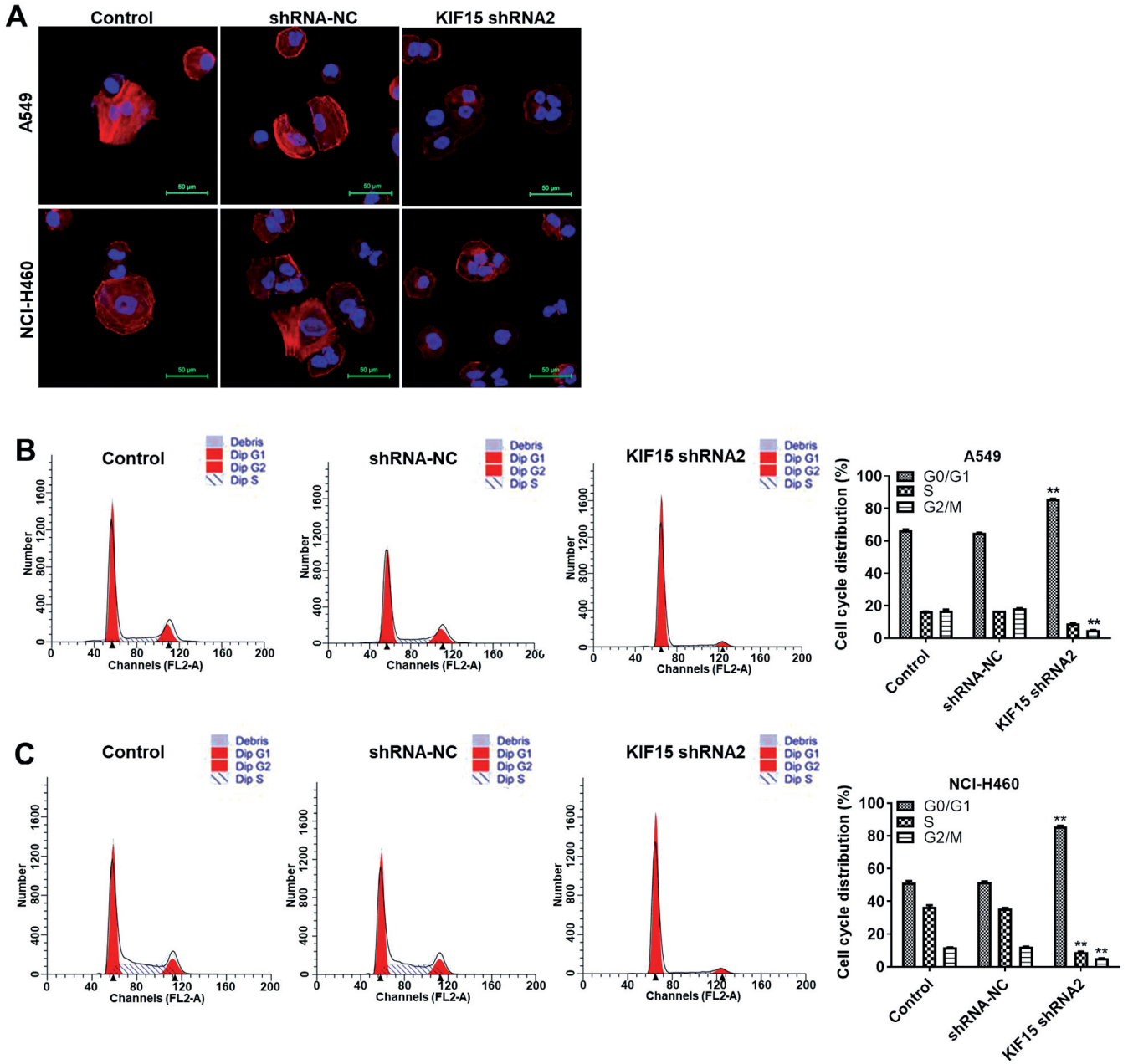

Fig. 6. Downregulation of KIF15 induced cell cycle arrest of NSCLC cells. A549 and NCI-H460 cells were infected with KIF15 shRNA2 for 72 h. **A.** The cytoskeleton and nuclei were stained with rhodamine-phalloidin and DAPI. **B, C.** Cell cycle distribution was analyzed by flow cytometry. **P<0.01 compared with shRNA-NC group. NC, negative control.

Therefore, in order to explore whether KIF15 knockdown in NSCLC cells induced morphological cell changes in the actin cytoskeleton, phalloidin (F-actin) staining was performed. In KIF15-knockdown A549 and NCI-H460 cells, weak F-actin staining was observed in the inner surface of the plasma membrane (Fig. 6A). In addition, the regular reorganization of actin filaments became disordered in KIF15-knockdown NSCLC cells (Fig. 6A). Furthermore, the percentages of NSCLC cells in each cell cycle phase (G_0/G_1, G_2/M and S) were analyzed using flow cytometry. As indicated in Fig. 6B, KIF15 knockdown markedly increased the percentage of A549 cells in G_0/G_1 phase; but decreased the percentage of cells in G_2/M phase. In addition, KIF15-knockdown NCI-H460 cells exhibited an increase in the percentage of G_0/G_1 phase cells, but a decreased proportion of cells in S and G_2/M phase (Fig. 6C). Mechanistically, downregulation of KIF15 notably inhibited the expression of cyclin D1 and upregulated the level of p27 Kip1 (Fig. 7A,B). These results suggested that downregulation of KIF15 could induce cell cycle arrest at the G0/G1 phase in NSCLC cells through regulation of p27 Kip1 and cyclin D1.

Downregulation of KIF15 expression inhibits the proliferation of NSCLC cells via downregulation of the Raf-MEK-ERK signaling pathway

To investigate the mechanism by which KIF15 knockdown inhibited the proliferation of NSCLC cells, western blotting was performed. As shown in Fig. 8A, B, Fig. 9A,B, downregulation of KIF15 expression

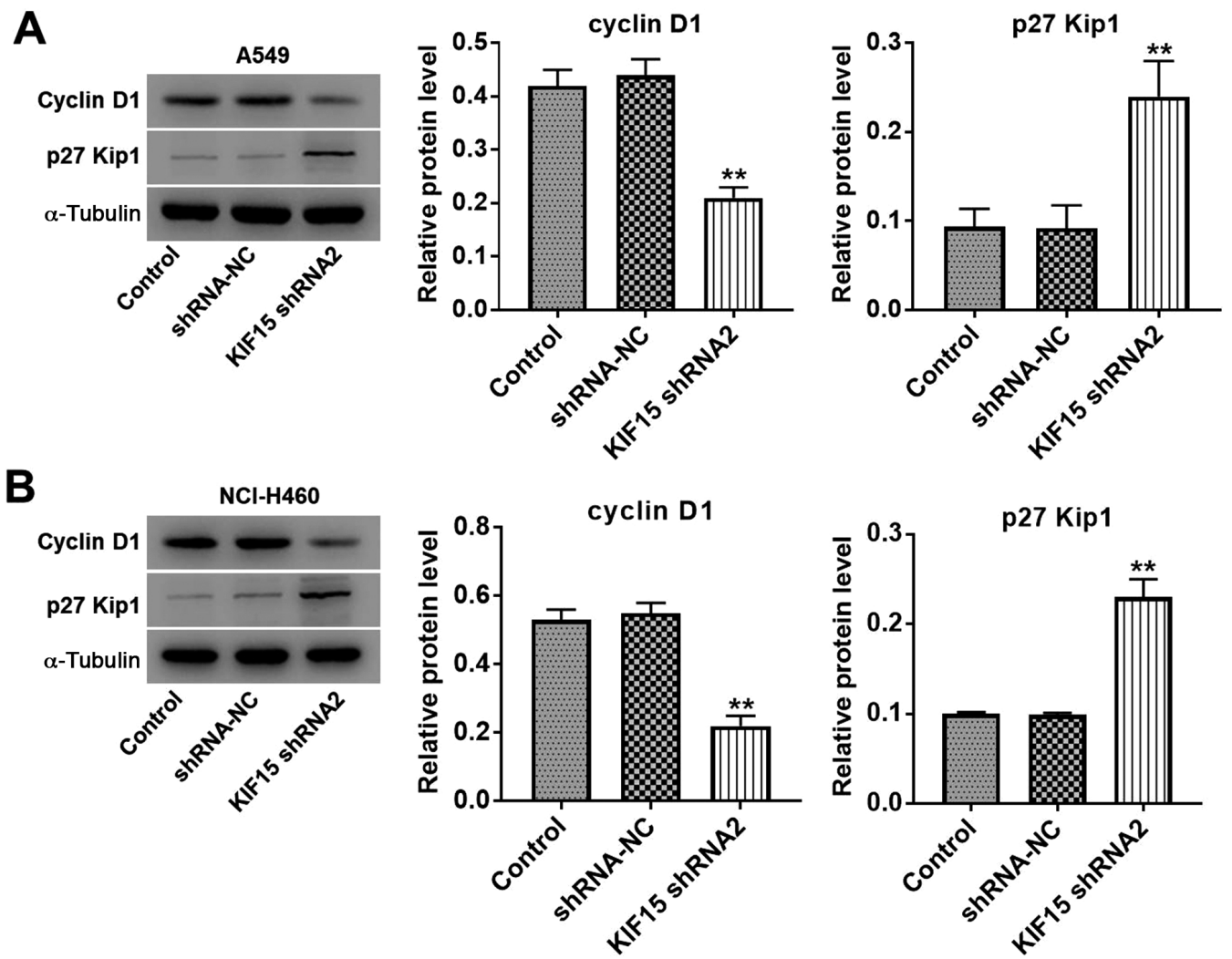

Fig. 7. KIF15 knockdown induced G1 arrest in NSCLC cells through mediation of p27 Kip1 and cyclin D1. **A.** The protein levels of p27 Kip1 and cyclin D1 in A549 cells were detected by western blotting. The relative expressions were quantified via normalization to α-Tubulin. **B.** The protein levels of p27 Kip1 and cyclin D1 in NCI-H460 cells were detected by western blotting. The relative expressions were quantified via normalization to α-Tubulin. **P<0.01 compared with shRNA-NC group. NC, negative control.

decreased the levels of ATF2, p-c-Raf, p-MEK and p-ERK in A549 and NCI-H460 cells. These data illustrated that downregulation of KIF15 expression might inhibit the proliferation of NSCLC cells via downregulation of the Raf-MEK-ERK signaling pathway.

Knockdown of KIF15 significantly inhibited the tumor growth of NSCLC in vivo

To further investigate the function of KIF15 in NSCLC, a xenograft mouse model was established. As shown in Fig. 10A,B, the tumor size of mice was significantly inhibited by KIF15 silencing. In addition, KIF15 knockdown notably decreased the protein levels of KIF15, p-ERK, ATF2, p-MEK and p-c-Raf in NSCLC cells (Fig. 10C,D). Taken together, knockdown of KIF15 significantly inhibited the tumor growth of NSCLC *in vivo*.

Knockdown of KIF15 significantly induced apoptosis in Calu-1 cells

To further confirm the role of KIF15 in LUSC (another subtype of NSCLC), TCGA database was used. As shown in Fig. 11A, the expression of KIF15 in LUSC tissues was significantly higher, compared with that in adjacent normal tissues, and the level of KIF15 in Calu-1 cells was obviously downregulated by KIF15 shRNA2 (Fig. 11B). In addition, downregulation of KIF15 expression markedly inhibited the proliferation of Calu-1 cells (Fig. 11C). Furthermore, downregulation of KIF15 notably induced the apoptosis of Calu-1 cells (Fig. 11D,E). Altogether, knockdown of KIF15 significantly induced apoptosis in Calu-1 cells.

Discussion

KIF family member genes have been identified to be

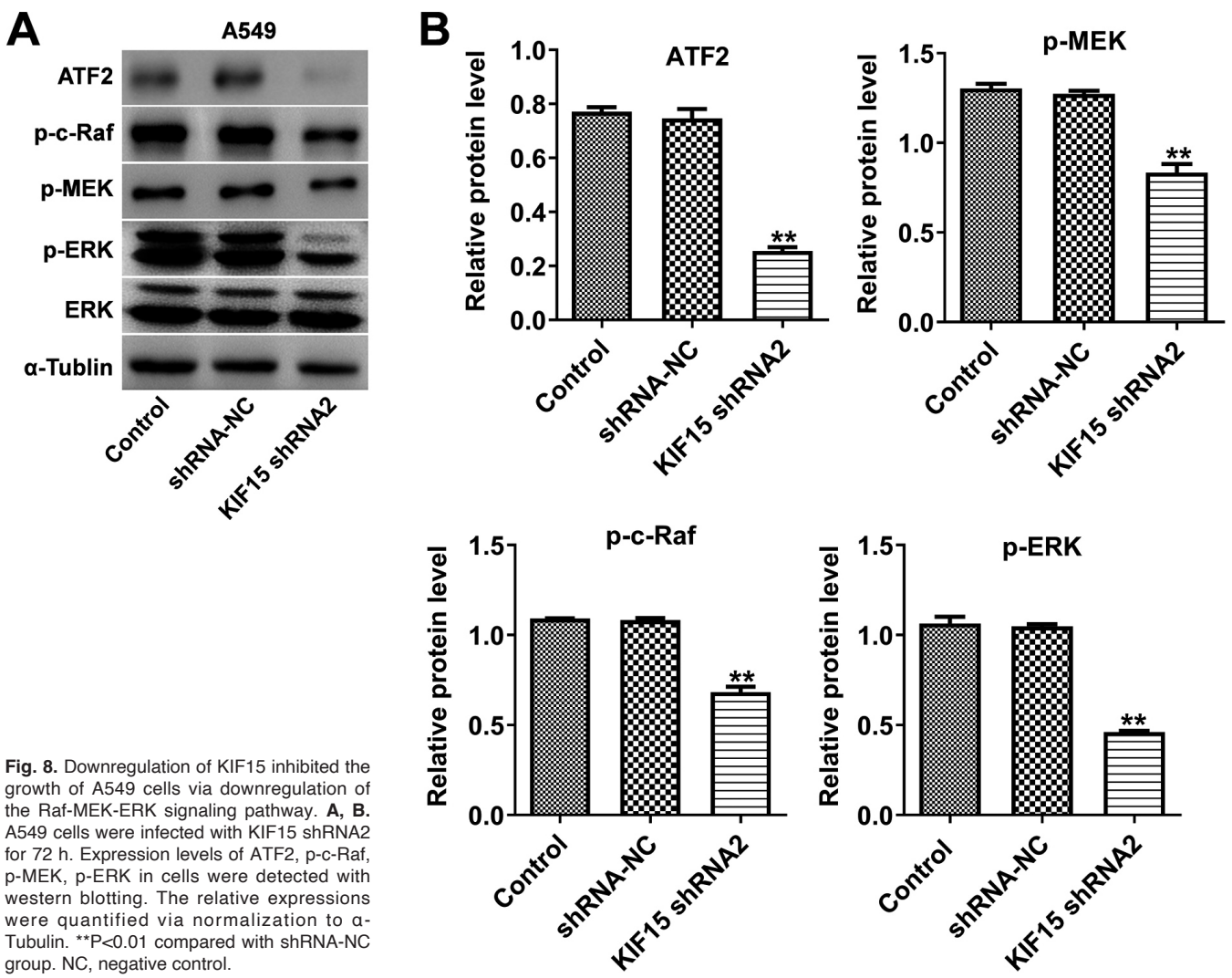

Fig. 8. Downregulation of KIF15 inhibited the growth of A549 cells via downregulation of the Raf-MEK-ERK signaling pathway. **A, B.** A549 cells were infected with KIF15 shRNA2 for 72 h. Expression levels of ATF2, p-c-Raf, p-MEK, p-ERK in cells were detected with western blotting. The relative expressions were quantified via normalization to α-Tubulin. **P<0.01 compared with shRNA-NC group. NC, negative control.

dysregulated in multiple types of human cancer (Rath and Kozielski, 2012). Zhou et al. (2019) reported that KIF11 expression is upregulated in breast cancer, and high KIF11 expression is associated with a poor prognosis. Li et al. (2019) demonstrated that KIF23 was able to promote the proliferation of gastric cancer cells. Furthermore, high KIF-2C expression is associated with poor prognosis and affects the progression of esophageal squamous cell carcinoma (Duan et al., 2016). In addition, it has been demonstrated that KIF family proteins might be used as diagnostic and prognostic biomarkers for NSCLC (Zhang et al., 2019). Qiao et al. (2018) reported that KIF15 expression is markedly upregulated in LUAD tissues compared with normal tissues. However, the mechanisms by which KIF15 regulates apoptosis, migration and invasion in NSCLC remain unclear.

KIF15 serves an important role in several tumors. For example, Wu et al. (2019) revealed that downregulation of KIF15 was able to suppress invasion and migration, as well as induce apoptosis, in osteosarcoma cells. Sun et al. (2020) observed that KIF15 promotes the proliferation of hepatocellular carcinoma cells. Additionally, Gao et al. (2020) identified that KIF15 expression is upregulated in breast cancer, and downregulation of KIF15 can inhibit the proliferation and migration of breast cancer cells. Furthermore, KIF15 has been considered as a novel diagnostic and therapeutic target for melanoma (Yu et al., 2019). Additionally, knockdown of KIF15 inhibits tumor growth and induces apoptosis in gastric cancer cells (Ding et al., 2020). Consistently, the results of the present study indicated that downregulation of KIF15 markedly inhibited the proliferation, migration and invasion of A549 and NCI-H460 cells. In addition, downregulation of KIF15 notably induced the apoptosis of A549 and NCI-H460 cells. This evidence supported the present finding that KIF15 may act as an oncogene. On the other hand, cyclin D1 and p27 Kip1 play key roles in cell cyle progression (El-Daly et al., 2020; Walczak et al., 2020), our data were consistent with the background, suggesting that KIF15 knockdown might

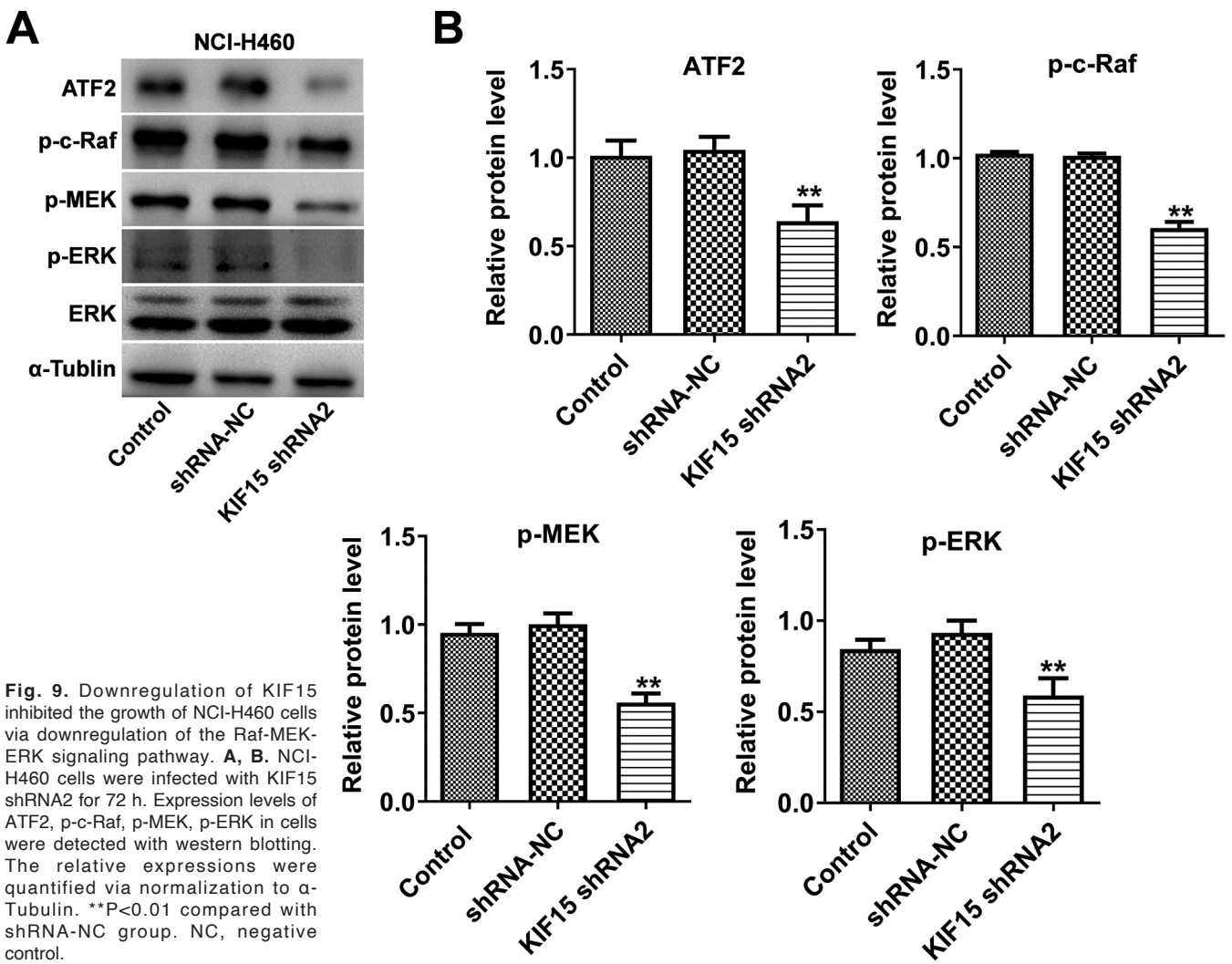

Fig. 9. Downregulation of KIF15 inhibited the growth of NCI-H460 cells via downregulation of the Raf-MEK-ERK signaling pathway. **A, B.** NCI-H460 cells were infected with KIF15 shRNA2 for 72 h. Expression levels of ATF2, p-c-Raf, p-MEK, p-ERK in cells were detected with western blotting. The relative expressions were quantified via normalization to α-Tubulin. **P<0.01 compared with shRNA-NC group. NC, negative control.

induce G1 arrest in NSCLC cells through mediation of cyclin D1 and p27 Kip1. Moreover, upregulation of active caspase 3 can induce cell apoptosis (Jia et al., 2021). This background is consistent with our data. Thus, KIF15 knockdown induced NSCLC cell apoptosis through upregulation of active caspase 3. It has been reported that MMP2 and MMP9 might promote cancer cell migration and invasion (Zhang et al., 2021). Consistently, our results indicated that KLF15 silencing inhibited the migration and invasion of NSCLC cells through downregulation of MMP2 and MMP9. Taken together, the data indicated that knockdown of KIF15 induced apoptosis and inhibited the invasion of NSCLC cells via regulation of active caspase 3, MMP2 and MMP9.

It has been reported that increased KIF15 expression was associated with the poor prognosis of patients with NSCLC (Qiao et al., 2018). Based on the data of TCGA, KIF15 expression was negatively correlated with the overall survival rate of patients with NSCLC. In addition, animal experiments were supplemented in this study. As we expected, KIF15 knockdown notably inhibited the tumor growth of NSCLC *in vivo*. Thus, KIF15 might be a crucial biomarker in NSCLC and it might be of great significance in clinical treatment of NSCLC.

KIF15 is known to be a mitotic kinesin motor, and serves an important role during mitosis (Sebastian, 2017). Additionally, KIF15 serves a crucial role in cell cycle progression, and overexpression of KIF15 can lead to tumor progression in LUAD (Bidkhori et al., 2013). Tao et al. (2020) revealed that KIF15 can promote cell mitosis and is involved in cell signaling transduction. Wang et al. (2017) revealed that upregulation of KIF15 was able to promote the proliferation of pancreatic cancer cells by promoting the G1/S transition. In addition, knockdown of KIF15 might inhibit the proliferation of glioma cells, as well as arrest cells in the G2 phase (Wang et al., 2020). Qiao et al. (2018) indicated that KIF15 knockdown markedly induces G1/S phase cell cycle arrest in LUAD cells. The present study revealed that downregulation of KIF15 expression induced cell cycle arrest at G0/G1 phase in NSCLC cells, which was consistent with previous studies. In addition, the actin cytoskeleton is reorganized during mitosis (Heng and Koh, 2010). Furthermore, the dynamics of the actin cytoskeleton contribute to cell cycle progression (Jones et al., 2019). Additionally, Wang et al. (2019a,b) found that G protein-coupled estrogen receptor induces re-organization of the F-actin cytoskeleton and promotes the migration of breast cancer cells. The present study revealed that downregulation of KIF15 impaired the actin

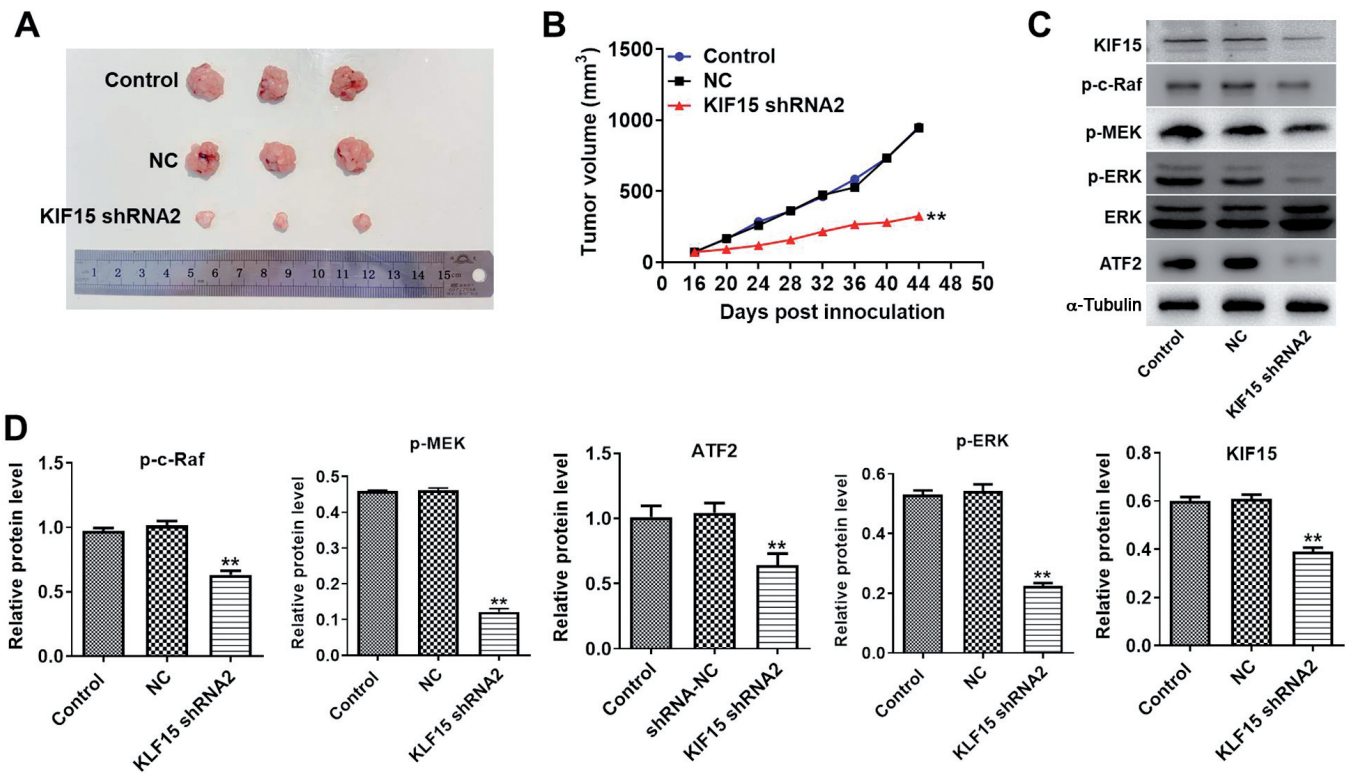

Fig. 10. Knockdown of KIF15 significantly inhibited the tumor growth of NSCLC *in vivo*. **A.** At the end of the study, tumor tissues were collected and pictured. **B.** Tumor volume of mice was measured every 4 days. **C.** The protein levels of ATF2, KIF15, p-c-Raf, p-MEK, p-ERK and ERK in tumor tissues were detected with western blotting. **D.** The relative expressions were quantified by normalization to α-Tubulin. **P<0.01 compared with NC group. NC, negative control.

cytoskeleton in NSCLC cells, as demonstrated by the degradation of actin filaments. These data indicated that KIF15 knockdown might affect cytoskeleton reorganization in NSCLC cells.

The Raf/MEK/ERK signaling pathway serves a vital role in regulating various cellular processes, including cell proliferation, survival and motility (Fan et al., 2000; Giordano et al., 2015). Previous evidence has suggested that the Raf-MEK-ERK signaling pathway might induce activation of ATF2 (Ouwens et al., 2002). Inhibition of MEK-ERK signaling might be considered to be a reasonable option for the treatment of NSCLC (Hayashido et al., 2014; Kim and Giaccone, 2018). Wang et al. (2017) revealed that upregulation of KIF15 activates MEK-ERK signaling in pancreatic cancer cells. Zhao et al. (2019) indicated that KIF15 promotes the proliferation of bladder cancer cells by activating the MEK-ERK signaling pathway. According to the previous references (Han et al., 2021; Liang et al., 2021), activation of Raf/MEK/ERK signaling might contribute to the progression of NSCLC. However, the correlation between KIF15 and Raf/MEK/ERK signaling remains unclear. Thus, we sought to investigate the function of KIF15 in this signaling pathway. The results of the present study indicated that knockdown of KIF15 markedly downregulated the levels of p-c-Raf, p-MEK, p-ERK and ATF2 in A549 and NCI-H460 cells. These data indicated that downregulation of KIF15 expression inhibited the proliferation of NSCLC cells by inactivating Raf/MEK/ERK signaling.

Frankly speaking, it has been reported that KIF15 is involved in NSCLC (Qiao et al., 2018). Based on the previous study (Qiao et al., 2018), our research firstly revealed the relation between KIF15 and Raf/MEK/ERK signaling in NSCLC, which supplemented the detailed

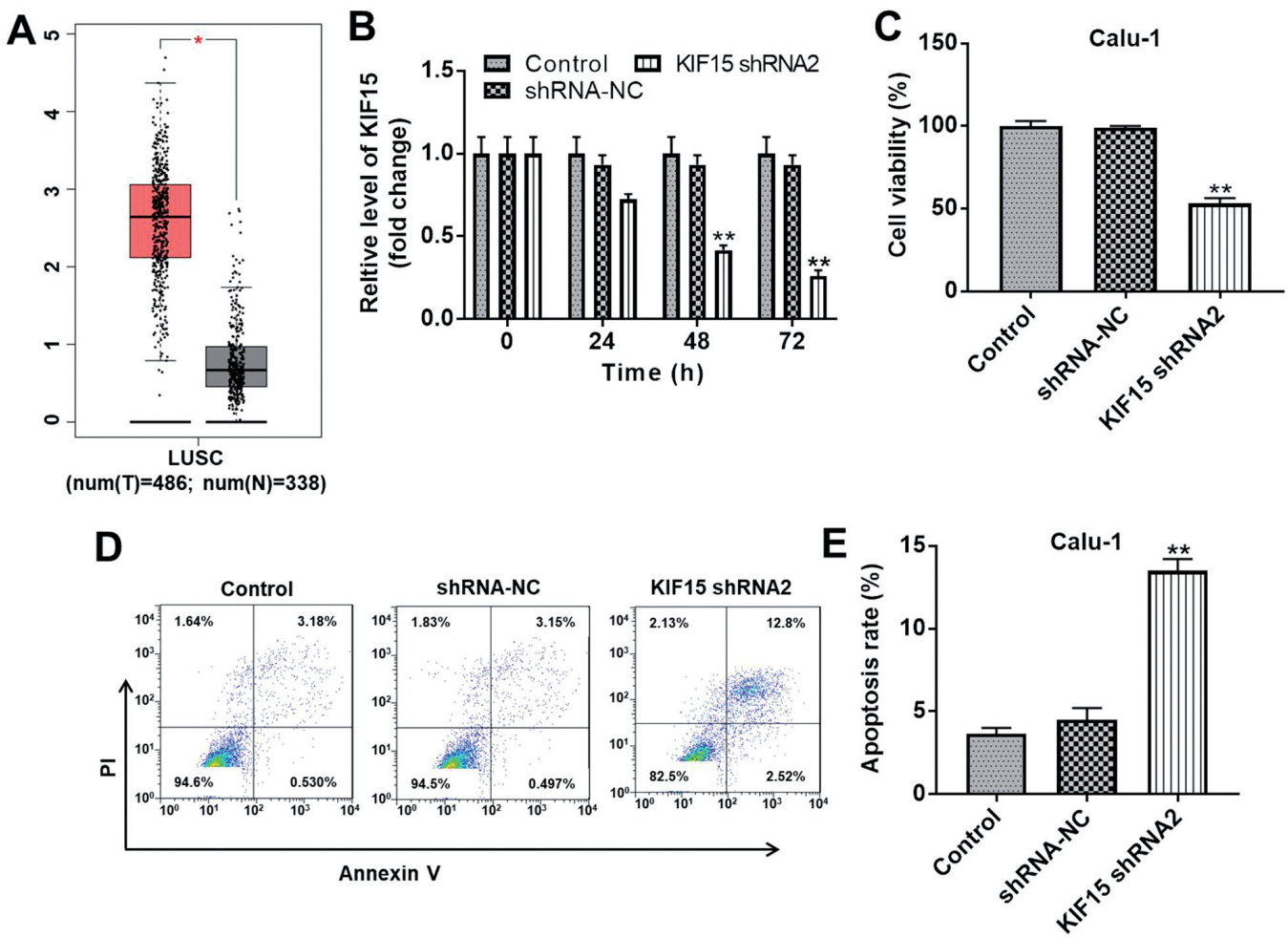

Fig. 11. KIF15 knockdown significantly induced apoptosis in Calu-1 cells. **A.** Relative expression of KIF15 expression in LUSC tissues (n=486, T) and in normal tissues (n=338, N) in TCGA dataset. *P<0.05. **B.** Calu-1 cells were transfected with KIF15 shRNA2 for 24, 48 or 72 h. Then, the efficiency of cell transfection was tested by RT-qPCR. **C.** The viability of Calu-1 cells was tested by CCK-8 assay. **D, E.** The apoptosis of Calu-1 cells was detected by flow cytometry. **P<0.01 compared with shRNA-NC group. NC, negative control.

function of KIF15 in NSCLC. To make our study more rational, another cell line (Calu-1) and animal study were performed. The data revealed that KIF15 knockdown significantly inhibited the growth of Calu-1 cells. In addition, KIF15 knockdown notably inhibited the tumor growth of NSLCL in mice.

There are some limitations in this research as follows: 1) more mechanisms by which KIF15 mediates the tumorigenesis of NSCLC need to be explored; 2) the detailed effect of KIF15 overexpression on Raf/MEK/ERK signaling needs to be further confirmed; 3) the correlation between KIF15 and Raf-MEK-ERK pathway remains unclear; 4) lung squamous cell carcinoma cell lines need to be used for further investigating the function of KIF15; 5) the function of KIF15 in EGFR mutation cells or different mutations NSCLC patients needs to be explored. Thus, more investigations are needed in future.

In conclusion, downregulation of KIF15 inhibited cell proliferation, migration and invasion and induced apoptosis of NSCLC cells by inactivating Raf/MEK/ERK signaling. These results suggested an important role of KIF15 as an oncogene in NSCLC.

Acknowledgements. Not applicable.

Availability of data and materials. The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions. Yingbin Luo and Bo Zhang made major contributions to the conception, design and manuscript drafting of the present study. Minghua Li, Jianchun Wu and Yiyang Zhou were responsible for data acquisition, data analysis and data interpretation. Yingbin Luo, Bo Zhang and Lili Xu performed the experiments and analyzed the data. Yiyang Zhou and Yan Li made substantial contributions to conception and design of the study and revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Funding. 1. Shanghai Municipal Natural Science Foundation (No. 17ZR1428000). 2. Projects within the budget of Shanghai University of Traditional Chinese Medicine (No. 2019LK09). 3. National Natural Science Foundation of China (General Program, No. 81603590). 4. Clinical Research Plan of SHDC (No. SHDC2020CR4052). 5. Shanghai Further Accelerate the Project of the Three-year Action Plan For the Development of TCM (2018-2020) (No. ZY(2018-2020)-CCCX-4001-01).

Ethics approval and consent to participate. All procedures performed in this study involving animals were in accordance with the National Institutes of Health guide for the care and use of Laboratory animals. The animal study was approved by Animal Care and Use Committee of Shanghai Municipal Hospital of Traditional Chinese Medicine

Conflicts of interest. The authors declare no competing financial interests.

References

Bibaki E., Tsitoura E., Vasarmidi E., Margaritopoulos G., Trachalaki A., Koutoulaki C., Georgopoulou T., Spandidos D.A., Tzanakis N. and Antoniou K.M. (2018). miR-185 and miR-29a are similarly expressed in the bronchoalveolar lavage cells in IPF and lung cancer but common targets DNMT1 and COL1A1 show disease specific patterns. Mol. Med. Rep. 17, 7105-7112.

Bidkhori G., Narimani Z., Hosseini Ashtiani S., Moeini A., Nowzari-Dalini A. and Masoudi-Nejad A. (2013). Reconstruction of an integrated genome-scale co-expression network reveals key modules involved in lung adenocarcinoma. PLoS One 8, e67552.

Blandin Knight S., Crosbie P.A., Balata H., Chudziak J., Hussell T. and Dive C. (2017). Progress and prospects of early detection in lung cancer. Open Biol. 7, 170070.

Butt S.U., Mejias A., Morelli C., Torga G., Happe M., Patrikidou A. and Arkenau H.T. (2021). BRAF/MEK inhibitors for BRAF V600E-mutant cancers in non-approved setting: a case series. Cancer Chemother. Pharmacol. 87, 437-441.

Cagle P.T., Allen T.C. and Olsen R.J. (2013). Lung cancer biomarkers: present status and future developments. Arch. Pathol. Lab. Med. 137, 1191-1198.

Colaprico A., Silva T.C., Olsen C., Garofano L., Cava C., Garolini D., Sabedot T.S., Malta T.M., Pagnotta S.M., Castiglioni I., Ceccarelli M., Bontempi G. and Noushmehr H. (2016). TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. Nucleic Acids Res. 44, e71.

Degirmenci U., Wang M. and Hu J. (2020). Targeting aberrant RAS/RAF/MEK/ERK signaling for cancer therapy. Cells. 9, 198.

Ding L., Li B., Yu X., Li Z., Li X., Dang S., Lv Q., Wei J., Sun H., Chen H., Liu M. and Li G. (2020). KIF15 facilitates gastric cancer via enhancing proliferation, inhibiting apoptosis, and predict poor prognosis. Cancer Cell Int. 20, 125.

Duan H., Zhang X., Wang F.X., Cai M.Y., Ma G.W., Yang H., Fu J.H., Tan Z.H., Fu X.Y., Ma Q.L., Wang X.Y. and Lin P. (2016). KIF-2C expression is correlated with poor prognosis of operable esophageal squamous cell carcinoma male patients. Oncotarget 7, 80493-80507.

El-Daly S.M., Gamal-Eldeen A.M., Gouhar S.A., Abo-Elfadl M.T. and El-Saeed G. (2020). Modulatory effect of indoles on the expression of miRNAs regulating G1/S cell cycle phase in breast cancer cells. Appl. Biochem. Biotechnol. 192, 1208-1223.

Fan M., Goodwin M., Vu T., Brantley-Finley C., Gaarde W.A. and Chambers T.C. (2000). Vinblastine-induced phosphorylation of Bcl-2 and Bcl-XL is mediated by JNK and occurs in parallel with inactivation of the Raf-1/MEK/ERK cascade. J. Biol. Chem. 275, 29980-29985.

Gao X., Zhu L., Lu X., Wang Y., Li R. and Jiang G. (2020). KIF15 contributes to cell proliferation and migration in breast cancer. Human Cell 33, 1218-1228.

Giordano G., Febbraro A., Tomaselli E., Sarnicola M.L., Parcesepe P., Parente D., Forte N., Fabozzi A., Remo A., Bonetti A., Manfrin E., Ghasemi S., Ceccarelli M., Cerulo L., Bazzoni F. and Pancione M. (2015). Cancer-related CD15/FUT4 overexpression decreases benefit to agents targeting EGFR or VEGF acting as a novel RAF-MEK-ERK kinase downstream regulator in metastatic colorectal cancer. J. Exp. Clin. Cancer Res. 34, 108.

Goulet A., Major J., Jun Y., Gross S.P., Rosenfeld S.S. and Moores C.A. (2014). Comprehensive structural model of the mechanochemical cycle of a mitotic motor highlights molecular adaptations in the kinesin family. Proc. Natl. Acad. Sci. USA 111, 1837-1842.

Han J., Liu Y., Yang S., Wu X., Li H. and Wang Q. (2021). MEK inhibitors for the treatment of non-small cell lung cancer. J. Hematol.

Oncol. 14, 1.

Hayashido Y., Kitano H., Sakaue T., Fujii T., Suematsu M., Sakurai S. and Okamoto T. (2014). Overexpression of integrin αv facilitates proliferation and invasion of oral squamous cell carcinoma cells via MEK/ERK signaling pathway that is activated by interaction of integrin αvβ8 with type I collagen. Int. J. Oncol. 45, 1875-1882.

Heng Y.W. and Koh C.G. (2010). Actin cytoskeleton dynamics and the cell division cycle. Int. J. Biochem. Cell Biol. 42, 1622-1633.

Iezzi A., Caiola E., Scagliotti A. and Broggini M. (2018). Generation and characterization of MEK and ERK inhibitors- resistant non-small-cells-lung-cancer (NSCLC) cells. BMC Cancer 18, 1028.

Jia X.B., Zhang Q., Xu L., Yao W.J. and Wei L. (2021). Lotus leaf flavonoids induce apoptosis of human lung cancer A549 cells through the ROS/p38 MAPK pathway. Biol. Res. 54, 7.

Jones M.C., Zha J. and Humphries M.J. (2019). Connections between the cell cycle, cell adhesion and the cytoskeleton. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 374, 20180227.

Kim C. and Giaccone G. (2018). MEK inhibitors under development for treatment of non-small-cell lung cancer. Expert Opin. Investig. Drugs 27, 17-30.

Li X.L., Ji Y.M., Song R., Li X.N. and Guo L.S. (2019). KIF23 promotes gastric cancer by stimulating cell proliferation. Dis. Markers 2019, 9751923.

Liang Y., Zhang T., Jing S., Zuo P., Li T., Wang Y., Xing S., Zhang J. and Wei Z. (2021). 20(S)-ginsenoside Rg3 inhibits lung cancer cell proliferation by targeting EGFR-mediated Ras/Raf/MEK/ERK pathway. Am. J. Chin. Med. 49, 753-765.

Liu M., Nadar V.C., Kozielski F., Kozlowska M., Yu W. and Baas P.W. (2010). Kinesin-12, a mitotic microtubule-associated motor protein, impacts axonal growth, navigation, and branching. J. Neurosci. 30, 14896-14906.

Liu X., Gong H. and Huang K. (2013). Oncogenic role of kinesin proteins and targeting kinesin therapy. Cancer Sci. 104, 651-656.

Liu G., Pei F., Yang F., Li L., Amin A.D., Liu S., Buchan J.R. and Cho W.C. (2017). Role of autophagy and apoptosis in non-small-cell lung cancer. Int. J. Mol. Sci. 18, 367.

Livak K.J. and Schmittgen T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 25, 402-408.

Ouwens D.M., de Ruiter N.D., van der Zon G.C., Carter A.P., Schouten J., van der Burgt C., Kooistra K., Bos J.L., Maassen J.A. and van Dam H. (2002). Growth factors can activate ATF2 via a two-step mechanism: phosphorylation of Thr71 through the Ras-MEK-ERK pathway and of Thr69 through RalGDS-Src-p38. EMBO J. 21, 3782-3793.

Povea-Cabello S., Oropesa-Ávila M., de la Cruz-Ojeda P., Villanueva-Paz M., de la Mata M., Suárez-Rivero J.M., Álvarez-Córdoba M., Villalón-García I., Cotán D., Ybot-González P. and Sánchez-Alcázar J.A. (2017). Dynamic reorganization of the cytoskeleton during apoptosis: The two coffins hypothesis. Int. J. Mol. Sci. 18, 2393.

Qiao Y., Chen J., Ma C., Liu Y., Li P., Wang Y., Hou L. and Liu Z. (2018). Increased KIF15 expression predicts a poor prognosis in patients with lung adenocarcinoma. Cell Physiol. Biochem. 51, 1-10.

Rath O. and Kozielski F. (2012). Kinesins and cancer. Nat. Rev. Cancer 12, 527-539.

Sebastian J. (2017). Dihydropyrazole and dihydropyrrole structures based design of Kif15 inhibitors as novel therapeutic agents for cancer. Comput. Biol. Chem. 68, 164-174.

Shi J., Yang C., An J., Hao D., Liu C., Liu J., Sun J. and Jiang J. (2021). KLF5-induced BBOX1-AS1 contributes to cell malignant phenotypes in non-small cell lung cancer via sponging miR-27a-5p to up-regulate MELK and activate FAK signaling pathway. J. Exp. Clin. Cancer Res. 40, 148.

Song X., Zhang T., Wang X., Liao X., Han C., Yang C., Su K., Cao W., Gong Y., Chen Z., Han Q. and Li J. (2018). Distinct diagnostic and prognostic values of kinesin family member genes expression in patients with breast cancer. Med. Sci. Monit. 24, 9442-9464.

Sun Y.F., Wu H.L., Shi R.F., Chen L. and Meng C. (2020). KIF15 promotes proliferation and growth of hepatocellular carcinoma. Anal. Cell. Pathol. 2020, 6403012.

Tang Z., Li C., Kang B., Gao G., Li C. and Zhang Z. (2017). GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 45, W98-W102.

Tao J., Sun G., Li Q., Zhi X., Li Z., He Z., Chen H., Zhou A., Ye J., Xu G., Guan W. and Zhang W. (2020). KIF15 promotes the evolution of gastric cancer cells through inhibition of reactive oxygen species-mediated apoptosis. J. Cell. Physiol. 235, 9388-9398.

Villalobos P. and Wistuba I.I. (2017). Lung cancer biomarkers. Hematol. Oncol. Clin. North Am. 31, 13-29.

Walczak K., Langner E., Szalast K., Makuch-Kocka A., Pozarowski P. and Plech T. (2020). A tryptophan metabolite, 8-hydroxyquinaldic acid, exerts antiproliferative and anti-migratory effects on colorectal cancer cells. Molecules 25, 1655.

Wang W. and He B. (2020). MiR-760 inhibits the progression of non-small cell lung cancer through blocking ROS1/Ras/Raf/MEK/ERK pathway. Biosci. Rep. 29.

Wang J., Guo X., Xie C. and Jiang J. (2017). KIF15 promotes pancreatic cancer proliferation via the MEK-ERK signalling pathway. Br. J. Cancer 117, 245-255.

Wang X., Wang M., Li X.Y., Li J. and Zhao D.P. (2019a). KIFC1 promotes the proliferation of hepatocellular carcinoma *in vitro* and *in vivo*. Oncology Lett. 18, 5739-5746.

Wang Z., Sun L., Liang S., Liu Z.C., Zhao Z.Y., Yang J., Wang D. and Yang D.Q. (2019b). GPER stabilizes F-actin cytoskeleton and activates TAZ via PLCβ-PKC and Rho/ROCK-LIMK-Cofilin pathway. Biochem. Biophys. Res. Commun. 516, 976-982.

Wang Q., Han B., Huang W., Qi C. and Liu F. (2020). Identification of KIF15 as a potential therapeutic target and prognostic factor for glioma. Oncology Rep. 43, 1035-1044.

Wu Z., Zhang H., Sun Z., Wang C., Chen Y., Luo P. and Yan W. (2019). Knockdown of kinesin family 15 inhibits osteosarcoma through suppressing cell proliferation and promoting cell apoptosis. Chemotherapy. 64, 187-196.

Xie K., Liang C., Li Q., Yan C., Wang C., Gu Y., Zhu M., Du F., Wang H., Dai J., Liu X., Jin G., Shen H., Ma H. and Hu Z. (2016). Role of ATG10 expression quantitative trait loci in non-small cell lung cancer survival. Int. J. Cancer 139, 1564-1573.

Yu X., He X., Heindl L.M., Song X., Fan J. and Jia R. (2019). KIF15 plays a role in promoting the tumorigenicity of melanoma. Exp. Eye Res. 185, 107598.

Yuan C., Wang M.H., Wang F., Chen P.Y., Ke X.G., Yu B., Yang Y.F., You P.T. and Wu H.Z. (2021). Network pharmacology and molecular docking reveal the mechanism of Scopoletin against non-small cell lung cancer. Life Sci. 270, 119105.

Zhang M., Li G., Wang Y., Wang Y., Zhao S., Haihong P., Zhao H. and Wang Y. (2017). PD-L1 expression in lung cancer and its correlation with driver mutations: a meta-analysis. Sci. Rep. 7, 10255.

Zhang L., Zhu G., Wang X., Liao X., Huang R., Huang C., Huang P., Zhang J. and Wang P. (2019). Genome-wide investigation of the

clinical significance and prospective molecular mechanisms of kinesin family member genes in patients with lung adenocarcinoma. Oncol. Rep. 42, 1017-1034.

Zhang L., Meng S., Yan B., Chen J., Zhou L., Shan L. and Wang Y. (2021). Anti-proliferative, pro-apoptotic, anti-migrative and tumor-inhibitory effects and pleiotropic mechanism of theaflavin on B16F10 melanoma cells. Onco. Targets Ther. 14, 1291-1304.

Zhao H., Bo Q., Wu Z., Liu Q., Li Y., Zhang N., Guo H. and Shi B. (2019). KIF15 promotes bladder cancer proliferation via the MEK-ERK signaling pathway. Cancer Manag. Res. 11, 1857-1868.

Zhou J., Chen W.R., Yang L.C., Wang J., Sun J.Y., Zhang W.W., He Z.Y. and Wu S.G. (2019). KIF11 functions as an oncogene and is associated with poor outcomes from breast cancer. Cancer Res. Treat. 51, 1207-1221.

Accepted December 15, 2021