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# KIF3C Promotes the Malignant Progression of Lung Cancer Cells A549

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**Abstract:** *Objective:* To investigate the role of KIF3C gene in promoting the malignant phenotype of lung cancer cells and in regulating PI3K/AKT signaling pathway. *Methods:* CCK-8 and transwell assays were used to detect the changes in cell proliferation and cell migration ability after being transfected with siKIF3C, as well as the protein expression levels of PI3K, p-PI3K, AKT, and p-AKT following the downregulation of KIF3C by Western blot. *Results:* The CCK-8 assay showed that the proliferation/viability of lung cancer cells A549 significantly reduced after being transfected with siKIF3C gene ($P < 0.05$); the migration ability of lung cancer cells A549 was significantly reduced after transfected with siKIF3C gene ($P < 0.05$); the levels of p-PI3K and p-AKT proteins were downregulated after KIF3C protein knockdown ($P < 0.05$); however, the detection of PI3K and AKT protein levels was not statistically significant. *Conclusion:* KIF3C may promote the proliferation and migration ability of lung cancer cells A549 through PI3K/AKT signaling pathway.

**Keywords:** KIF3C; A549; EMT; Lung cancer

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## 1. Introduction

According to global epidemiological surveys, lung cancer is the most common malignancy with a high mortality rate. Hence, early diagnosis and screening play important roles in clinical management [1]. Kinesin family member 3C (KIF3C) is a member of the kinesin superfamily and is thought to be involved in microscopic motility, transporting upstream or inside organelles along microtubules, with microtubule binding activity and microtubule motility activity. It is also part of the kinesin complex. KIF3C is a protein-coding gene. Pathways associated with KIF3C include responses to platelet cytoplasmic calcium ion ($Ca^{2+}$) elevation and Golgi to endoplasmic reticulum (ER) retrograde transport. Gene Ontology (GO) annotation indicates that KIF3C has adenosine triphosphate (ATP) hydrolytic activity and microtubule motility activity [2]. An important homolog of this gene is KIF3B, which encodes a protein that acts as a heterodimer with kinesin family member 3A to aid chromosome movement during mitosis and meiosis. Studies have shown that the overexpression of KIF3C can promote proliferation and metastasis in some tumors. However, a high expression of KIF3C protein in glioma cells is an indicator of good prognosis; clinical specimens have revealed that KIF3C expression is higher in low-grade gliomas than high-grade gliomas [3]. KIF3C also plays a role in paclitaxel resistance of breast cancer cells [4]. We have previously demonstrated that KIF3C is a tumor-promoting factor in lung cancer [4]. In this paper, we focus on the changes in the proliferation and migration ability of lung cancer cells A549 after knocking down the expression of KIF3C as well as

the activation status of PI3K/AKT signaling pathway, which may provide new concepts to the invasion and metastases of lung cancer cells A549 [5].

## 2. Materials and methods

### 2.1. Materials

Lung cancer cells A549 were purchased from Shanghai Cell Bank, China; fetal bovine serum (FBS) and Roswell Park Memorial Institute (RPMI)-1640 medium were purchased from Procell; transwell was purchased from Corning; KIF3C polyclonal antibody [6], PI3K, p-PI3K, AKT, p-AKT, and GAPDH antibodies were purchased from Proteintech. CCK-8 kit was purchased from Wuhan Doctor Bio [6]. TBST, radioimmunoprecipitation assay (RIPA) lysate, and enhanced chemiluminescence (ECL) kits were purchased from Shanghai Biyuntian Biotechnology Company [7].

### 2.2. Cell culture and transfection

Human lung cancer cell line A549 was cultured and passaged according to standard conditions. Lipofectamine 3000, a liposome transfection reagent, was purchased from Invitrogen. KIF3C-siRNA and control-siRNA were purchased from Ribo Biotechnology (Guangzhou, China). A549 cells were cultured in RPMI-1640 culture medium containing 10% FBS at 37°C and 5% carbon dioxide ($CO_2$), and transfected every 2–3 days at a ratio of 1:3. The day before siKIF3C transfection, the cells were inoculated in 6-well plate at 0.5–2 × 105 per well, and transient transfection was performed when 30–50% of cells were passaged [8]. The transfection procedure was performed by transfecting siKIF3C and control-interfering sequence into lung cancer cells A549 according to Lipofectamine 3000 instructions [8].

### 2.3. CCK-8 experiment

The cultured cells were divided into KIF3C-siRNA group and control-siRNA group. Single-cell suspension A549 cells were inoculated in 96-well plate at 3,000 cells/well. After the cells of both the groups were walled up and incubated, 10 μL of CCK-8 reagent was added to each well, and incubation was continued for 2 h at 37°C in an incubator to detect the growth of cells for 4 consecutive days [9]. The absorbance value of each well was detected at 450 nm by enzyme-linked immunosorbent assay (ELISA), and the growth curve of the tumor cells was plotted [10].

### 2.4. Transwell experiment

Cell invasion assays were performed using transwell chambers with 8 μm wells. Matrix gels were made based on a 1:3 dilution of Matrigel matrix gel with RPMI-1640 medium. 50 μL of mixed gel was added to the bottom of the transwell and incubated for 2 h to form a gel. The upper chamber was inoculated with 200 μL of cell suspension without serum culture medium per well. Serum-containing medium supplemented with 10% FBS was added to the lower chamber as a chemical elicitor. After incubating for 24 h, cells from the filter were fixed with 4% paraformaldehyde and stained with hematoxylin. Five randomly selected high-magnification fields were photographed and counted; the calculated mean value was taken as the final result of the experiment [11].

### 2.5. Western blotting

After transfection, cells from each group were collected, and the proteins were extracted by lysing the cells with RIPA. The concentration of the extracted proteins was determined by ultraviolet (UV) spectrophotometry. The proteins were electrophoresed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a membrane, closed with skimmed milk, and incubated dropwise with primary antibody overnight at 4°C; the membrane was then washed with TBST and exposed.

All primary antibody concentrations were 1:1,000, while the sheep anti-rabbit secondary antibody concentration was 1:10,000 [12].

### 2.6. Statistical analysis

The experimental data were analyzed using SPSS version 26.0 and GraphPad Prism version 8.0 for statistical graphing. The experimental data were expressed as mean ± standard deviation (mean ± SD). The difference was considered statistically significant at $P < 0.05$.

## 3. Results

### 3.1. Reduction in the viability of lung cancer cells A549 after KIF3C knockdown

Proliferation assays revealed changes in cell proliferation after the incubation of A549 cells for 0 h, 24 h, 48 h, and 72 h with 10 μL of CCK-8 reagent, see **Figure 1A**. CCK-8 results showed that the downregulation of KIF3C in transfected A549 cells resulted in reduced cell proliferation/viability compared to the control group. Analysis of data based on The Cancer Genome Atlas (TCGA) lung cancer tumor samples (http://ualcan.path.uab.edu/) revealed significantly higher mRNA levels of KIF3C in lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) tissues than in normal lung tissues, see **Figure 1B** and **Figure 1C** [13].

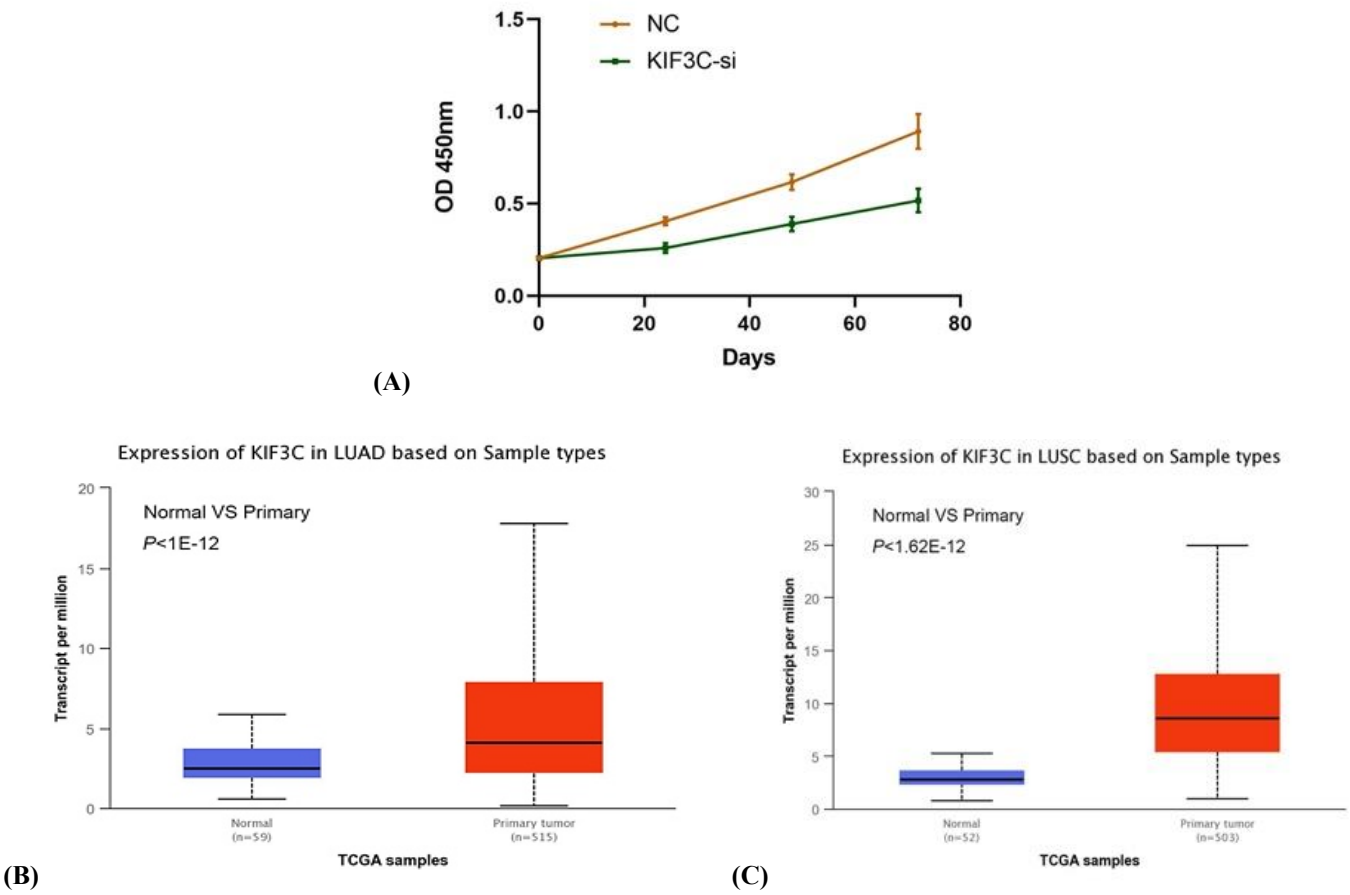


**Figure 1.** (A) CCK-8 results showing that the downregulation of KIF3C in transfected A549 cells decreased cell value-added viability. (B) The mRNA expression of KIF3C in lung adenocarcinoma tissues was higher than that in normal lung tissues. (C) The mRNA expression of KIF3C in lung squamous cell carcinoma tissues was higher than that in normal lung tissues

### 3.2. Reduction in the migration and invasion ability of A549 cells after KIF3C knockdown

The results of the transwell experiment showed that the migration ability of A549 cells was significantly weaker than that of the control group after the A549 cells were transfected with siKIF3C for 24 h [14]. The statistical analysis of the number of cells in the field of view of the collected images showed that the number of A549 cells migrating in the knock-down group (60 ± 6) was significantly lower than that of the control group (150 ± 19) ($P < 0.05$); the number of A549 cell invasion in the knockdown group (49 ± 10) was significantly lower than that in the control group (113 ± 13) ($P < 0.05$), see **Figure 2**.

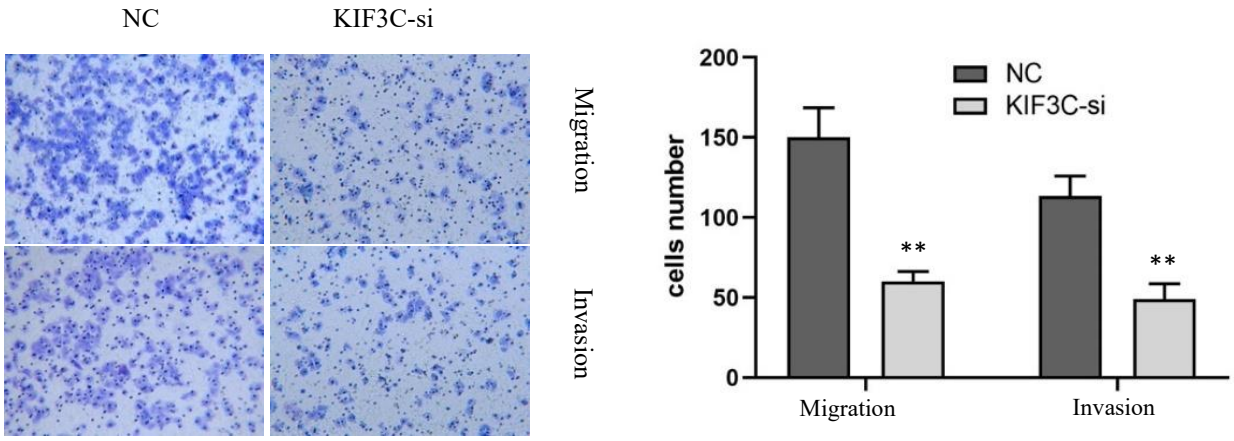


**Figure 2.** Changes in the migration and invasion ability of A549 cells in the KIF3C knockdown group compared to the control group (** represents $P < 0.01$)

### 3.3. Effect of KIF3C knockdown on PI3K/AKT signaling pathway activity

Western blotting was performed to detect the expression of the key proteins in PI3K/AKT signaling pathway. The results of the study showed that the expression of p-PI3K and p-AKT was significantly downregulated in A549 cells following transfection with siKIF3C compared with the expression of the transfected control interference series ($P < 0.05$); however, the detection of the total protein of PI3K and AKT was found to be statistically insignificant. This suggests that KIF3C may promote the proliferation, invasion, and metastasis of A549 cells through the regulation of PI3K/AKT signaling pathway [15].

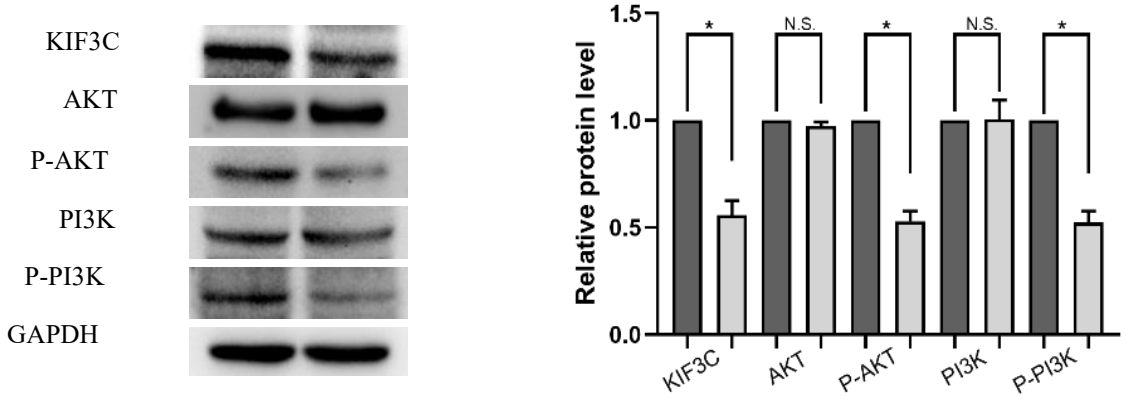


**Figure 3.** Changes in PI3K/AKT signaling pathway-related proteins after KIF3C knockdown (* represents $P < 0.05$; N.S. refers to no significance)

## 4. Discussion

Lung cancer is one of the most common malignancies seen in clinical practice. Evidence suggests that lung cancer is a complex disease whose pathogenesis involves mutation or activation of multiple oncogenes. In addition to external environmental factors, the abnormal regulation of key genes plays a significant role in the development of lung cancer. The kinesin superfamily proteins (KIFs) are highly conserved and are microtubule-dependent kinesins that convert chemical energy released during ATP hydrolysis into mechanical energy and are involved in regulating the transport of intracellular molecules [16]. The KIF3 protein family is a subfamily member of the KIF superfamily, which includes three species, KIF3A, KIF3B, and KIF3C. The molecular motor KIF3B is a key regulator of the dendritic structure of cortical neurons. Studies have shown that KIF3B is a key determinant of cortical neuronal morphology and has an inhibitory effect on structural plasticity [8]. KIF3C plays a role in a variety of biological processes, mainly in those related to neuronal development, differentiation, and axonal transport [17]. Through bioinformatics databases and glioma patient tissues, researchers have found higher levels of KIF3C expression in low-grade gliomas than high-grade gliomas, such as glioblastomas; patients with high KIF3C expression have also been found to have longer overall survival according to a survival analysis [3]. KIF3C expression is upregulated in breast cancer tissues and may be involved in tumor recurrence and metastasis. The knockdown of KIF3C gene significantly downregulates the level of phosphorylated Smad2, thus inhibiting TGF-β signaling pathway. Tumor metastasis is closely related to epithelial-mesenchymal transition (EMT). However, a study found that the expression of vimentin, metalloproteinase-2 (MMP2), and MMP9 was significantly upregulated by the downregulation of KIF3C expression, with a decrease in E-cad expression, thus suggesting that EMT was inhibited [18]. Through a tumor database integration analysis, we found that KIF3C mRNA levels were significantly upregulated in non-small cell lung cancer tissues, having a correlation with poor prognosis. We found that KIF3C expression was upregulated in non-small cell lung cancer cells and tissues and promoted the proliferation and metastasis of lung cancer cells. The expression of KIF3C was also found to be negatively regulated by miR-150-5p and miR-186-3p [19].

Dysregulation of PI3K/AKT signaling pathway has been demonstrated in both, tumor and non-tumor diseases. AKT only has altered phosphorylation levels after which the signaling pathway is fully activated, which in turn leads to the entry of a number of key downstream transcription factors into the nucleus, resulting in cell survival and proliferation as well as the inhibition of apoptosis. The overexpression of KIF2A promotes the malignant phenotype of A549 cells, including proliferation [20], migration, invasion, maintenance of tumor stem cell properties, and cisplatin resistance. Further mechanistic studies have revealed that the above malignant biological behaviors are acquired by KIF2A through mediating the activation of PI3K/AKT/VEGF signaling pathway [21].

There are relatively few studies on how KIF3C regulates the PI3K/AKT mechanism. The present experiment demonstrated that KIF3C could indeed affect the biological behavior of A549 cells. After interfering with KIF3C, the cell phenotype was significantly altered, including a decrease in cell proliferation/viability as well as cell invasion and migration ability. We also found similar results, in that KIF3C was able to activate PI3K/AKT signaling pathway, as evidenced by changes in the expression levels of p-PI3K and p-AKT, which may promote other intracellular mechanisms related to cellular transcriptional regulation.

In conclusion, this study reveals that KIF3C affects the malignant phenotype of cells and the regulation of signaling pathway mechanisms in non-small cell lung cancer through cellular experiments. The changes in the protein levels of PI3K/AKT and their role in the biological behavior of tumor cells have been verified at the cellular-molecular level by regulating the expression levels of KIF3C genes. Our study of the mechanisms of tumor invasion and metastasis by linking KIF3C and signaling pathway-related molecules may provide a theoretical basis for clinical gene-targeting therapy and some guidance in patient prognosis.

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**Disclosure statement**

The authors declare no conflict of interest.

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