

# Knockdown of KIF3C Inhibits Epithelial-Mesenchymal Transition in Lung Cancer Cells A549

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**Abstract:** The objective of this study is to reveal the role of KIF3C gene in the proliferation of lung cancer cells, and the regulation of epithelial mesenchymal transition (EMT) of tumor cells. The plate clone formation assay and cell scratch assay were used in this study to detect the changes of cell proliferation and migration ability after siKIF3C interference, while EMT-related protein expression after KIF3C downregulation was detected by Western blot. The cell clone formation assay showed that the number of clones of lung cancer cells A549 was significantly reduced after transfected with siKIF3C ( $P < 0.05$ ); The scratch assay showed that the healing ability of cells was significantly reduced after transfected with siKIF3C ( $P < 0.05$ ); Western blot protein analysis revealed that the levels of EMT-related proteins, N-cadherin, Vimentin, Snail, and Slug were significantly down-regulated ( $P < 0.05$ ), however, E-cadherin protein levels were up-regulated after siKIF3C interference. In conclusion, KIF3C may promote the proliferation and invasive ability of lung cancer cells A549 through EMT.

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

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## 1. Introduction

Lung cancer is usually classified into small cell lung cancer and non-small cell lung cancer based on their histological type, with non-small cell lung cancer, accounting for a large proportion of the disease, and subpopulation classification based on driver molecules may be the key to precision tumor therapy <sup>[1]</sup>. Advance treatment such as targeted therapies and immunological treatments have transformed into clinical management strategies for advanced lung cancer, and many molecules may include in the standard care for targeted lung cancer in the future <sup>[2]</sup>.

Kinesin family member 3C (KIF3C) is a microtubule-associated protein that is able to hydrolyze ATP to generate energy involved in regulating a variety of intracellular biological processes such as cytoskeleton dynamics, transport of macromolecules, cell division, and cell migration <sup>[3,4]</sup>. KIF3C is a protein-coding gene that is often expressed at a higher level in neuronal cells and in astrocytes compared to members of subfamilies KIF3A and KIF3B, suggesting that multiple transport roles regulated by KIFs are more prevalent in neuronal cells <sup>[5]</sup>. There is also limited research on KIF3C in solid tumors as well as in neurological related diseases. Previous studies have shown that KIF3C specifically regulates microtubule cytoskeleton in neuronal growth cones, and involved in axonal growth and regeneration processes after

injury [6]. Previously, we demonstrated that KIF3C is an oncogenic factor in lung cancer through in vivo and in vitro experiments [7]. In this study, we focus on the changes in proliferation and migration ability of lung cancer cells A549 after KIF3C gene knock down, as well as the cell morphological changes and the activation status of EMT, which may provide new markers for the treatment and prognosis of lung cancer.

## **2. Materials and methods**

### **2.1. Materials**

Lung cancer cell line A549 were purchased from Shanghai Cell Bank, China. Fetal bovine serum (FBS) and RPMI-1640 medium were purchased from Procell, 6-well plates/24-well plates were purchased from Corning, KIF3C polyclonal antibody, N-cadherin, E-cadherin, Vimentin, Snail, Slug, GAPDH antibodies were purchased from proteintech. Phosphatase inhibitor, RIPA protein lysate, protease inhibition, Tris-buffered saline with Tween (TBST), and ECL chemiluminescence kits were purchased from Shanghai Biyuntian Biotechnology.

### **2.2. Cell culture and transfection**

The human lung cancer cell line A549 was grown in medium containing 10% FBS. KIF3C-siRNA and its control interfering sequence were transfected into A549 cells using Lipofectamine 3000 (Invitrogen) transfection reagent. Kif3c-siRNA and control sequence-siRNA were obtained from Reebok Biotechnology Ltd (Guangzhou, China). A549 cells were cultured in standard cell culture conditions at 37°C and 5% carbon dioxide (CO<sub>2</sub>), and cells were passaged every 2 d at a ratio of 1:2. Transfection of siKIF3C was performed when cells in 6-well plates reached 30%-50% of confluency.

### **2.3. Clone formation assay**

Cells were transfected with siKIF3C for 36 h. After trypsin digestion with EDTA, the cells were inoculated in two groups of 500 cells per well in 6-well plates and grown in complete medium for 10-14 days. After 2-3 washes in PBS, the cells were fixed in 4% paraformaldehyde for 15 min, followed by crystal violet staining for 20 min. The cells washed 2-3 time in PBS, and dried. Number of colonies with 50 or more cells were counted.

### **2.4. Cell scratch assay**

Cells were cultured into fused monolayers in 6-well plates. Cells were previously scribed and marked at the bottom of the 6-well plates and cells were scribed with a 200 µl pipette tip perpendicular to the 6-well plates with reference to the scribe mark. Following the scribing, the cells were washed 2-3 times with PBS to ensure that there were no dislodged cells in suspension and photographed under the microscope. The growth medium was then changed to 1% serum medium and incubation continued. The subsequent healing of the marked scratches observed in the same position.

### **2.5. Western blotting**

The cells suspension was centrifuged at 3000 rpm for 5 min to collect post-transfection cell precipitate. The cells were treated with a cold protein lysate solution for 20 min and centrifuged to extract the protein supernatant. The respective protein concentrations were determined simultaneously using an enzyme marker. Equal amounts of 35 µg of protein lysate were loaded to the lanes for gel electrophoresis. After completion of the gel electrophoresis, the protein was transferred from the gel to the membrane, and the membrane was incubated with 5% skimmed milk for 2 h. Primary antibodies were added to the membrane and incubated for overnight at 4°C. The dilution of primary antibodies used are 1:1000 for all primary antibodies except for GAPDH, a dilution of 1:5000 was used.

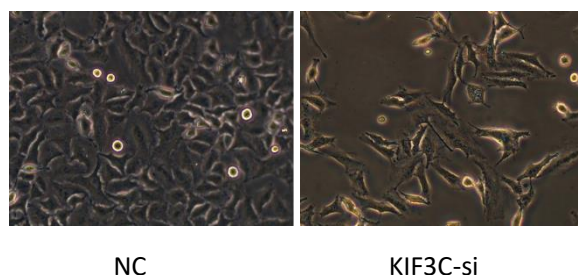
## 2.6. Statistical analysis

SPSS version 26.0 was used to analyze the experimental data and GraphPad Prism version 8.0 software was used for statistical graphing. The experimental data were expressed as mean  $\pm$  standard deviation (mean  $\pm$  SD). The difference was considered statistically significant at  $P < 0.05$ .

## 3. Results

### 3.1. Morphological observation of lung cancer cells A549 after knockdown of KIF3C

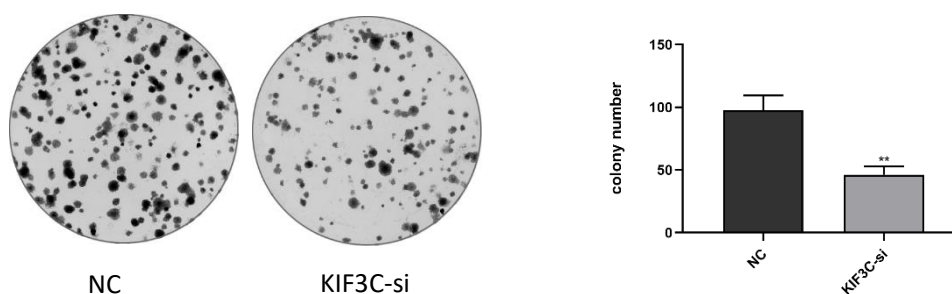
After transfection of A549 cells with KIF3C-siRNA and control-siRNA, the cells transfected with KIF3C-siRNA changed from epithelial-like cells to long spindle-shaped cells, with more cytoplasmic protrusions and reduced adhesion compared with the control group, as shown in **Figure 1**.



**Figure 1.** Morphological changes of A549 cells after transfection with KIF3C-siRNA and control-siRNA

### 3.2. Reduced number of clones A549 cells following knockdown of KIF3C

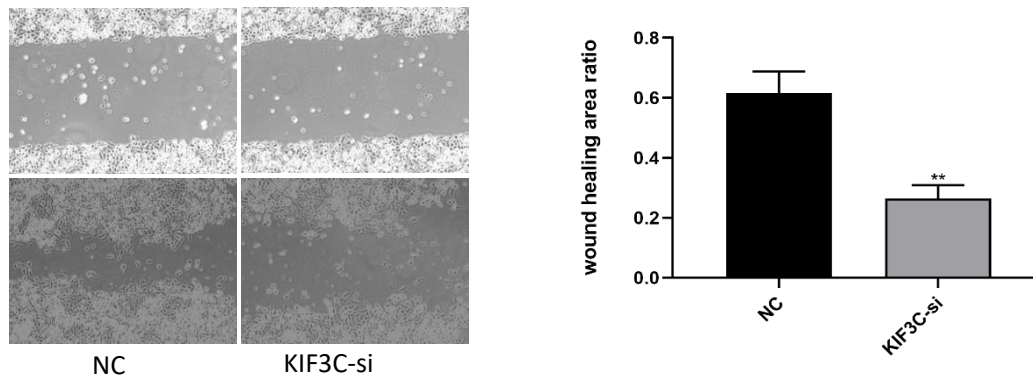
The results of the clone formation assay showed that A549 cells grown in complete medium for 10-14 days after transfected with siKIF3C. The number of clones of A549 cells was significantly lower than the control group by microscopic observation, and photographs were of the cell clones in 6-well plates were taken. Colonies with approximately 50 or more cells were counted. The number of colonies in the knock-down group of A549 cells ( $46 \pm 7$ ) was significantly lower than that in the control group ( $98 \pm 12$ ) ( $p < 0.05$ ) as shown in **Figure 2**.



**Figure 2.** Changes in clone-forming ability of cells in the KIF3C knockdown group compared to the control group (\*\* represents  $p < 0.01$ )

### 3.3. Effect of knockdown of KIF3C on the migration of A549 cells

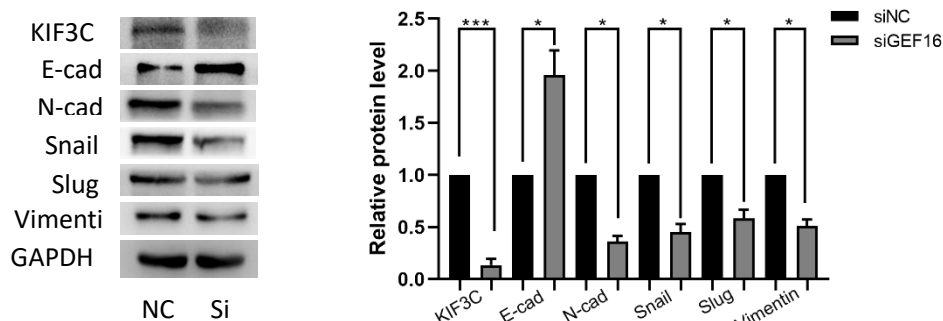
Scratch healing ability of cells was reduced after knockdown of KIF3C in A549 cells, indicated by the scratch assay (siKIF3C group:  $0.26 \pm 0.05$  vs. control group:  $0.62 \pm 0.07$ ,  $p < 0.01$ , **Figure 3**).



**Figure 3.** Changes in scratch healing ability of A549 cells after knockdown of KIF3C

### 3.4. Effect of knockdown of KIF3C on the expression of EMT-related proteins

Western blot was performed to detect changes in the expression of key EMT-related proteins. The results of the study showed that the expression of mesenchymal phenotypic proteins N-cadherin, Vimentin, Snail, and Slug in lung cancer A549 cells was significantly down-regulated ( $P < 0.05$ ) after transfected with siKIF3C compared to the protein expression in cells transfected with control interference sequences; however, detection of epithelial phenotypic molecule E-cadherin protein levels were found to be significantly up-regulated in the A549 cells transfected with siKIF3C, compared to their respective control. This suggests that KIF3C may influence the biological behavior of the cells by promoting the EMT process, which in turn may promote the invasion and metastasis of A549 cells (**Figure 4**).



**Figure 4.** Changes in key proteins of the EMT process after knockdown of KIF3C. \* represents  $p < 0.05$ , \*\*\* represents  $p < 0.001$

## 4. Discussion

Lung cancer is a common malignant epithelial tumor in a clinical setting. Numerous research data indicate that non-small cell lung cancer is the result of multi-gene involvement and multi-stage continuous progression, involving a complex signaling regulatory network formed by many genes. In addition to external factors such as air pollution and smoking, mutations or amplification of star genes play a key role in the malignant progression of lung cancer [8,9].

The kinesin superfamily proteins (Kifs) are proteins that act mainly through microtubules, while they regulate many biological processes closely related to tumorigenesis in an ATP-dependent manner, with significant pro-proliferative and pro-divisional characteristics such as intracellular transport of important molecules, cytoskeletal alterations, and cell division processes [10,11]. KIFs are microtubule-binding proteins

that move along microtubule tracks, and are involved in intracellular molecular transport and mitosis [12,13]. The KIFs family of proteins includes dozens of proteins that are divided into many subfamilies, all of which often have highly conserved structural domains [14]. KIFs have recently been discovered as oncogenes in a few cancers [15,16]. Kifc3/c1/1a/5a were found to mediate doxorubicin resistance in breast cancer cell lines [16]. Some scholars explored KIFs proteins association with prognosis of hepatocellular carcinoma through several tumor databases and in vitro experiments, and were surprised to find that high expression of eight KIFs (KIF2C, KIF4A, KIF10, KIF11, KIF14, KIF18B, KIF20A, and KIF23) was significantly associated with pathological stage and grading of HCC patients. Moreover, high expression of these proteins correlated with prognosis of HCC patients, as evidenced by a significant reduction in overall survival (OS) and disease-free survival (DFS). Down-regulation of all eight KIFs in cellular assays decreased the proliferation viability of hepatocellular carcinoma cells, and cell flow pattern showed G1 phase arrest [17].

MicroRNAs (miRNAs) are small non-coding RNA with 18-22 nucleotides in length, and their expression can be inhibited by targeting the genes. Studies have demonstrated that micorRNA-2053 negatively regulates tumor proliferation, invasion, and migration by targeting KIF3C in esophageal cancer [18]. Previously, we found that KIF3C mRNA levels were significantly upregulated in non-small cell lung cancer tissues through tumor database integration analysis, and correlated with poor prognosis. We also observed that KIF3C expression was upregulated in non-small cell lung cancer tissues and cells, and associated with an increase in 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 [7].

EMT is an important factor in tumors, which may contribute to cancer cell metastasis [19,20]. EMT usually refers to cells that lose their epithelial polar characteristics, lose some adhesion, and acquire mesenchymal cell properties. The process of EMT enables tumor cells to acquire stem cell properties, drug resistance, and greater tumorigenicity, thus adapting to changes in the surrounding environment [20]. High expression of KIF3C was found to play a role in the promotion of tumor recurrence and metastasis in breast cancer tissues. Tumor metastasis is closely related to EMT, and knockdown of the KIF3C gene may inhibit the TGF- $\beta$  signalling pathway. The expression of MMP2, MMP9, and vimentin, molecules which are associated with invasion and metastasis was also upregulated, and the expression of the epithelial phenotype molecule E-cad was downregulated after knockdown of KIF3C, indicating that EMT transformation was inhibited following knockdown of KIF3C [11].

There are relatively few studies on how KIF3C regulates the mechanism of EMT transformation. In this experiment, we demonstrated that KIF3C could promote the proliferation and migration of lung cancer A549 cells. After interfering with KIF3C, the original cell morphology was altered and we found similar results. Knockdown of KIF3C was able to reduce the transformation of EMT to mesenchymal phenotype as well as changes in the expression of related proteins, as shown by a down-regulation of N-cadherin, Vimentin, Snail, and Slug expression, and an up-regulation of E-cadherin.

In summary, this study reveals that KIF3C in non-small cell lung cancer affects the malignant phenotype and EMT transformation of cells through in vitro experiments. Changes in the levels of EMT-related proteins and alterations in the biological behavior and morphology of tumor cells were regulated at the cellular molecular level by transfection with KIF3C small interfering RNA. Our study of the mechanisms of tumor invasion and metastasis by linking KIF3C and EMT-related proteins may provide guidance on patient prognosis, as well as a theoretical basis for clinical gene targeting therapy.

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## Disclosure statement

The authors declare no conflict of interest.

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