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Original Article

KIF3a inhibits TGF-β1-induced epithelial-mesenchymal transition in lung cancer cells

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Abstract: KIF3a is a subunit of hetero-trimeric Kinesin-2, a microtubule (MT) plus-end directed motor protein and has been reported to be involved in tumorigenesis. However, the roles of KIF3a in lung cancer remain unknown. Hence, in the present study, we explored the regulatory effects of KIF3a during the transforming growth factor (TGF)-β1-induced epithelial-mesenchymal transition (EMT) in lung adenocarcinoma cells. The results showed that KIF3a was downregulated after induction with TGF-β1. Overexpression of KIF3a inhibited the TGF-β1-induced migration and invasion, as well as EMT phenotype of lung cancer cells. Furthermore, overexpression of KIF3a inhibited the TGF-β1-induced the expression of β-catenin, cyclin D1 and c-myc in A549 cells. Taken together, these results indicate overexpression of KIF3a might inhibit TGF-β1-induced EMT by blocking the Wnt signaling in A549 cells, providing potential targets to prevent and/or treat lung cancer cell invasion and metastasis.

Keywords: KIF3a, lung cancer, epithelial-mesenchymal transition (EMT), Wnt signaling

Introduction

Lung cancer is the most common reason for cancer-related deaths in the world. The high mortality rate of lung cancer is largely due to spread of disease to other organs [1]. Despite recent advances in early detection and targeted therapy [2, 3], metastatic lung cancer remains incurable and results in poor patient outcomes [4]. Therefore, dissecting the molecular mechanisms that regulate lung cancer invasion and metastasis may facilitate the advancement of clinical treatment.

Epithelial-mesenchymal transition (EMT) is a crucial developmental process implicated in cancer progression, invasion and metastasis. During EMT procedure, the actin cytoskeleton is reorganized and cells acquire increased cell-matrix contacts, leading to dissociation from surrounding cells and enhanced migratory and invasive capabilities [5]. A growing body of evidence demonstrated that the transforming growth factor (TGF)-β is a key mediator of progressive cancer. As a tumor promoter, TGF-β1 triggers EMT for cells to become invasive; concomitant with the loss of epithelial characteristics, cancer cells undergoing EMT acquire a mesenchymal phenotype characterized by migration and invasion [6-10].

KIF3a is a subunit of hetero-trimeric Kinesin-2, a microtubule (MT) plus-end directed motor protein. It has been shown to regulate early development and ciliogenesis [11]. Kif3a interacts with the Par3/Par6/aPKC complex to regulate ciliogenesis [12]. Recently, several reports showed that KIF3a was involved in the development of tumorigenesis. Liu *et al*. reported that exogenous expression of KIF3a promoted cell growth in the benign prostate cells, whereas silencing KIF3a decreased anchorage-independent cell growth, and cell migration/invasion [13]. However, the function of KIF3a in lung cancer is still unclear. In the present study, we explored the regulatory effects of KIF3a during the TGF-β1-induced EMT in lung adenocarcinoma cells. We found that overexpression of KIF3a inhibits TGF-β1-induced EMT via inhibition of Wnt signaling pathway.

Materials and methods

Cell culture and treatment

The human lung adenocarcinoma cell line (A549) was obtained from the American Type

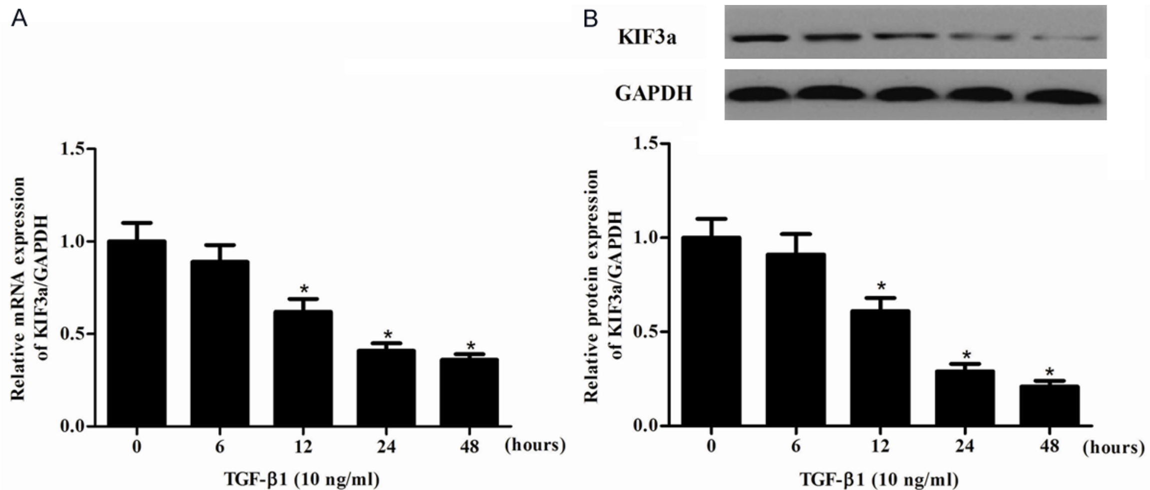

Figure 1. The expression of KIF3a in TGF-β1-induced lung cancer cells. (A) A549 cells were treated with TGF-β1 (10 ng/mL) for 6, 12, 24 and 48 h. The mRNA (A) and protein (B) levels of KIF3a were determined by real-time PCR and Western blotting, respectively. The values shown represent the mean ± SD, the symbols * indicates differences from control group at $P < 0.05$.

Culture Collection (ATCC, Manassas, VA, USA). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Hyclone, UT, USA) containing 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) in a humidified atmosphere of 5% CO_2 at 37°C. Then, A549 cells were treated with TGF-β1 (10 ng/mL) for 24 h.

Plasmid transfection

The KIF3a expressing construct was generated by inserting human KIF3a cDNA into pCMV-Tag2b vector (Stratagene, La Jolla, CA) with BamHI/HindIII restriction sites. A549 cells were transfected with overexpression-KIF3a or scramble using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

RNA extraction and quantitative real time (qRT)-PCR

Total RNA was extracted from cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the supplier's instructions. Reverse transcription of 5 μg of the total RNA into cDNA was performed by using M-MLV reverse transcriptase (Clon-tech, Palo Alto, CA, USA). QRT-PCR analysis was carried out using the Power SYBR Green PCR Master Mix (Applied Biosystems), according to manufacturer's instructions. PCR was performed under the following conditions: 95°C for 40 s, 56°C for 10 s, 72°C for 50 s, for 35 cycles. The relative expression levels were calculated by $2^{-\Delta\Delta Ct}$ method and the target gene was normalized to the internal reference gene.

Western blotting

Cells were harvested and lysed in cell lysis buffer (Beyotime, Haimen, China). Protein concentration was measured by Bradford method. The equal amount of protein samples was loaded and isolated using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then, proteins were electrophoretically transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). After being blocked by 5% non-fat milk, the membranes were incubated with primary antibodies at 4°C overnight. Next, the membrane was incubated with horseradish peroxidase (HRP) conjugated secondary antibodies for 1 h at room temperature. Finally, the protein binding was detected by an enhanced chemiluminescence (ECL) detection system (Amersham, Little Chalfont, UK). The following primary antibodies were used: anti-KIF3a, anti-E-cadherin, anti-N-cadherin, anti-vimentin, anti-β-catenin, anti-cyclin D1 and anti-c-myc (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Migration and invasion assays

For the migration assay, 1×10^5 cells transfected with overexpression-KIF3a were suspended in serum-free medium and plated on chambers

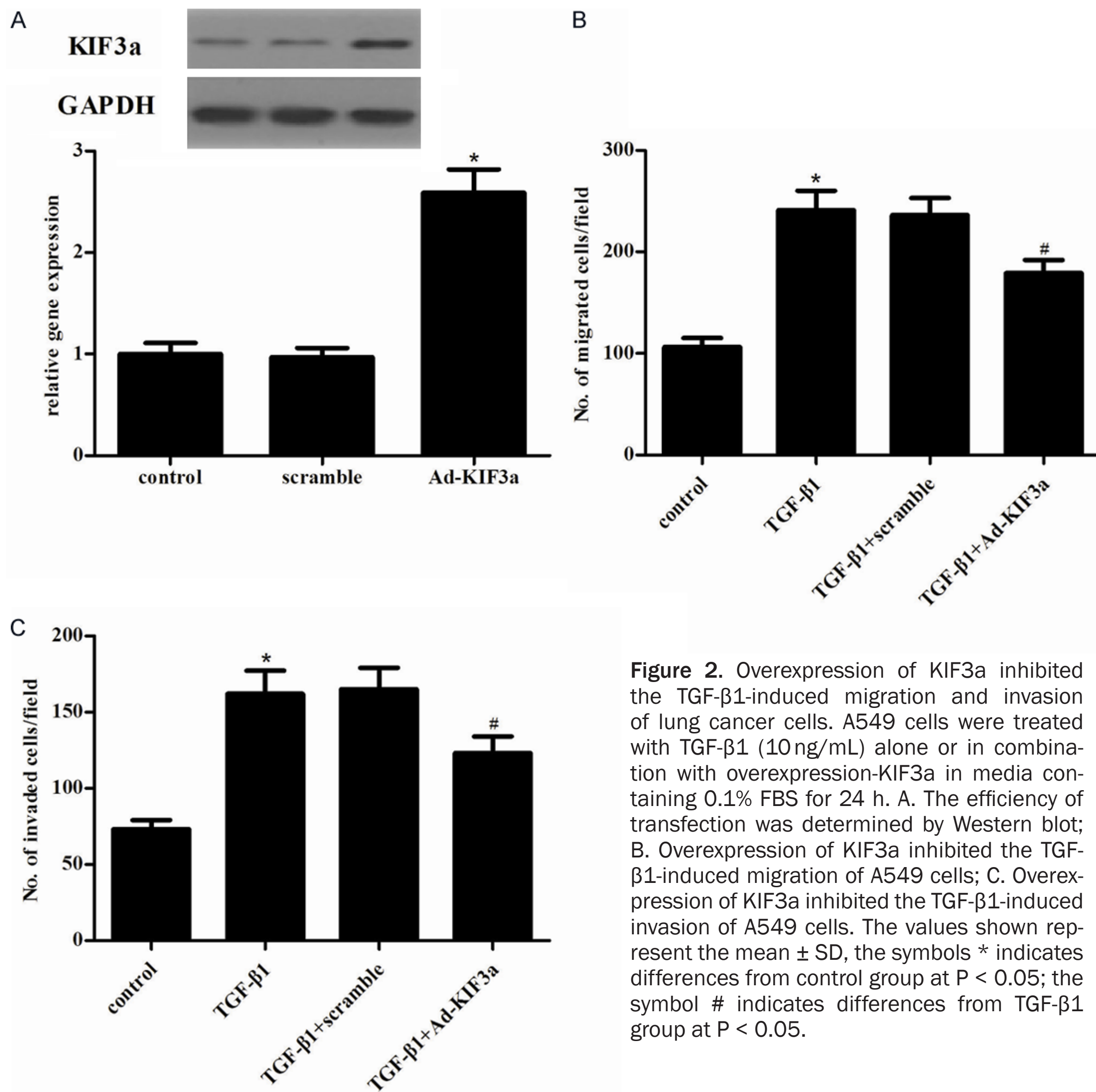

Figure 2. Overexpression of KIF3a inhibited the TGF-β1-induced migration and invasion of lung cancer cells. A549 cells were treated with TGF-β1 (10 ng/mL) alone or in combination with overexpression-KIF3a in media containing 0.1% FBS for 24 h. A. The efficiency of transfection was determined by Western blot; B. Overexpression of KIF3a inhibited the TGF-β1-induced migration of A549 cells; C. Overexpression of KIF3a inhibited the TGF-β1-induced invasion of A549 cells. The values shown represent the mean ± SD, the symbols * indicates differences from control group at P < 0.05; the symbol # indicates differences from TGF-β1 group at P < 0.05.

(Corning Costar, NY, USA) that were not coated with Matrigel. For the invasion assay, 1×10^5 cells transfected with overexpression-KIF3a were seeded into the upper chamber that was precoated with Matrigel (BD Bioscience, CA, USA). For both assays, the lower chamber contained medium supplemented with 10% FBS. After incubated for 24 h, cells on the membrane were scrubbed, washed with PBS and fixed in 100% methanol and stained with crystal violet solution. The number of cells per five high power fields was counted under a microscope.

Statistical analysis

All results are reported as means ± SD. Statistical analysis involved use of the Student's *t* test for the comparison of 2 groups or 1-way ANOVA for multiple comparisons. $P < 0.05$ was considered to be significant.

Results

The expression of KIF3a in TGF-β1-induced lung cancer cells

First, we examined the response of KIF3a to TGF-β1 in A549 cells. As shown in **Figure 1A**, TGF-β1 (10 ng/ml) greatly decreased the mRNA level of KIF3a in A549 cells in a time-dependent manner. Similarly, TGF-β1 also obviously inhibited the expression of KIF3a protein in A549 cells (**Figure 1B**).

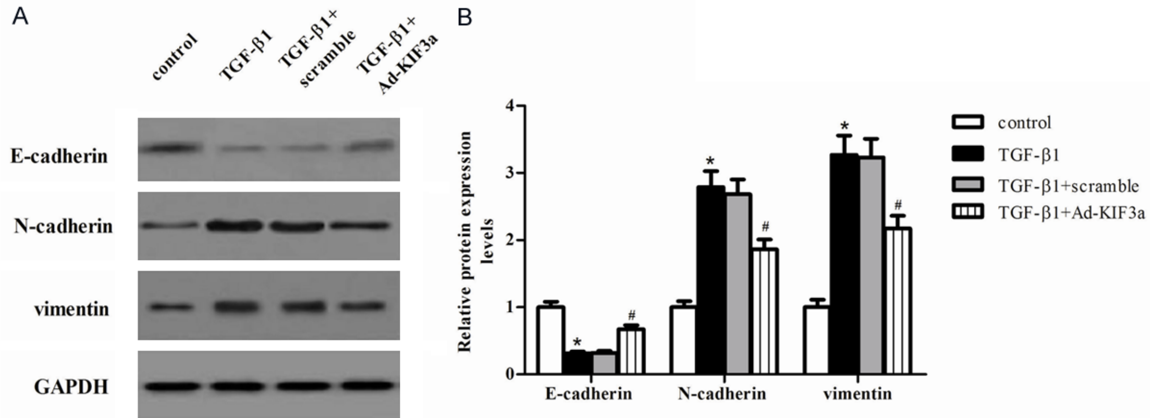

Figure 3. Overexpression of KIF3a inhibited the TGF-β1-induced EMT phenotype of lung cancer cells. A549 cells were treated with TGF-β1 (10 ng/mL) alone or in combination with overexpression-KIF3a in media containing 0.1% FBS for 24 h. (A) The protein levels of E-cadherin, N-cadherin and vimentin were determined by Western blotting, respectively. (B) Quantification of (A). The values shown represent the mean ± SD, the symbols * indicates differences from control group at $P < 0.05$; the symbol # indicates differences from TGF-β1 group at $P < 0.05$.

Overexpression of KIF3a inhibited the TGF-β1-induced migration and invasion of lung cancer cells

Given that KIF3a was decreased by TGF-β1, we asked whether ectopic expression of KIF3a could reverse TGF-β1-induced migration and invasion in A549 cells. To restore KIF3a expression in A549 cells, we overexpressed KIF3a in A549 cells by the transfection of recombinant adenovirus vector and the expression levels of KIF3a were detected by Western blot analysis. The results showed that KIF3a expression was obviously increased by Ad-KIF3a (**Figure 2A**), suggesting that the transfection was successful. Then, we examined the effect of KIF3a on the TGF-β1-induced migration and invasion of A549 cells. As shown in **Figure 2B**, the number of migrated cells was significantly increased by TGF-β1 treatment, whereas, overexpression of KIF3a significantly inhibited the TGF-β1-induced migration of A549 cells. Similarly, in the Boyden chamber assay, overexpression of KIF3a significantly inhibited the TGF-β1-induced invasion of A549 cells across the gelatin coated-membrane (**Figure 2C**).

Overexpression of KIF3a inhibited the TGF-β1-induced EMT phenotype of lung cancer cells

Next, we evaluated the effect of KIF3a on EMT phenotype in A549 cells. As shown in **Figure 3**, TGF-β1 treatment significantly decreased the expression of E-cadherin and increased the expression of N-cadherin and vimentin. Whereas, overexpression of KIF3a upregulated the expression of E-cadherin, as well as inhibited the expression of N-cadherin and vimentin in A549 cells.

Overexpression of KIF3a inhibited the TGF-β1-induced activation of Wnt pathway

To elucidate the mechanism of KIF3a inhibited-EMT in TGF-β1-induced lung cancer cells, we tested the effect of KIF3a on TGF-β1-induced activation of Wnt signaling. As shown in **Figure 4**, in the TGF-β-mediated EMT processes, the protein levels of β-catenin, cyclin D1 and c-myc were up-regulated in TGF-β1-induced A549 cells. Additionally, overexpression of KIF3a inhibited the TGF-β1-induced the expression of β-catenin, cyclin D1 and c-myc in A549 cells.

Discussion

Despite an increasing body of evidences is highlighting KIF3a as a cancer-related gene [14-16], little is known about its function. Here we focus on lung cancer, the most common reason for cancer-related deaths in the world, whose molecular mechanisms of invasion are still far from being completely elucidated. Our results demonstrated that KIF3a was downregulated after induction with TGF-β1. Overexpression of KIF3a inhibited the TGF-β1-induced migration and invasion, as well as EMT phenotype of lung cancer cells. Furthermore,

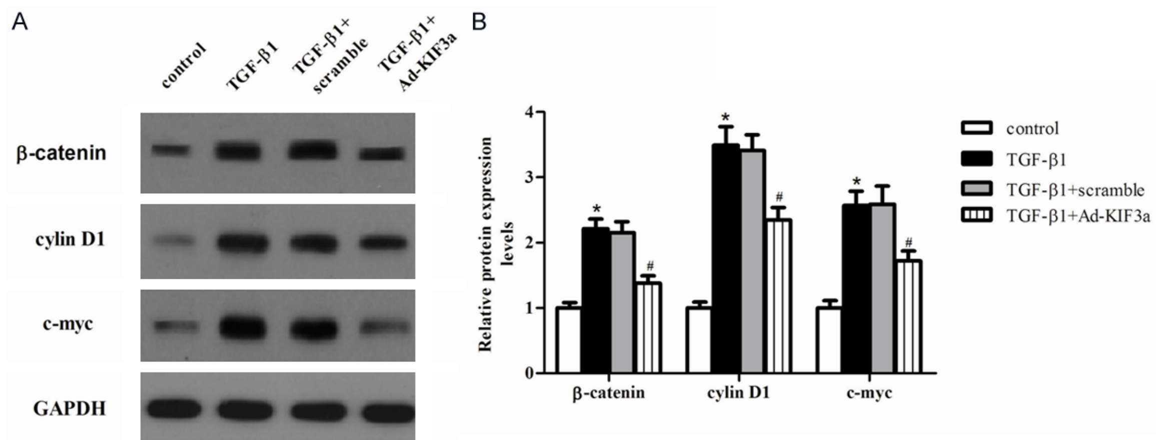

Figure 4. Overexpression of KIF3a inhibited the TGF-β1-induced activation of Wnt pathway. A549 cells were treated with TGF-β1 (10 ng/mL) alone or in combination with overexpression-KIF3a in media containing 0.1% FBS for 24 h. (A) The expression of β-catenin, cycline D1 and c-myc was determined by Western blotting. (B) Quantification of (A). The values shown represent the mean ± SD, the symbols * indicates differences from control group at $P < 0.05$; the symbol # indicates differences from TGF-β1 group at $P < 0.05$.

overexpression of KIF3a inhibited the TGF-β1-induced the expression of β-catenin, cyclin D1 and c-myc in A549 cells.

TGF-β1, a member of the TGF-β superfamily, is a multifunctional cytokine that regulates cell proliferation, differentiation, apoptosis, and migration [17]. The process of EMT is involved in tumor migration, invasion, and dissemination, thus facilitating tumor progression [18, 19]. Previous studies demonstrated that TGF-β1 treatment led to the marked reduction of E-cadherin, which is the prototype epithelial cell marker of EMT, and increased the expression of N-cadherin and vimentin, which are mesenchymal markers in A549 cells [18, 20]. Consistent with these results, in this study, we found that TGF-β1 significantly induced migration/invasion and EMT phenotype of lung cancer cells, whereas, overexpression of KIF3a inhibited these effects. These results suggest that KIF3a negatively regulates TGF-β1-induced EMT, consequently affects lung cancer cell migration and invasion *in vitro*.

A vast array of studies has indicated that the Wnt/β-catenin signaling pathway not only plays an important role in normal mammary development, but also plays a critical role in tumor progression, including lung cancer [21-24]. β-catenin is the central molecule that activates various downstream effectors responsible for cell proliferation, dedifferentiation, inhibition of apoptosis, and tumor progression. Several studies showed that nuclear β-catenin was highly expressed in lung cancer cells [25, 26]. During the TGF-β1-induced EMT, β-catenin maintains cell-cell adhesion, migration, and invasion by regulating the expression of EMT-related molecules. Moreover, it was reported that KIF3a functions as an agonist of the Wnt signaling pathway, and it increases CK1-dependent DVL2 phosphorylation and β-catenin activation in prostate cancer cells, leading to transactivation of the Wnt-signaling target genes such as cyclin D1, human enhancer of filamentation 1 (HEF1), and matrix metalloproteinase (MMP) 9 [13]; KIF3a also interacts with Wnt signaling component, adenomatous polyposis coli (APC), through an association with the kinesin superfamily-associated protein (KAP3) for regulating cell migration [27]. KIF3a also plays a critical role in the subcellular transport of β-catenin-cadherin(s) complex [28]. In the present study, we observed that overexpression of KIF3a inhibited the TGF-β1-induced the expression of β-catenin, cyclin D1 and c-myc in A549 cells. Taken together, these results suggest that overexpression of KIF3a might inhibit TGF-β1-induced EMT by blocking the Wnt signaling in A549 cells.

Taken together, our results demonstrate for the first time that KIF3a is a regulator of EMT in lung cancer cells, and that overexpression of KIF3a might inhibit TGF-β1-induced EMT by blocking the Wnt signaling in A549 cells. Therefore, KIF3a may be a potential therapeutic target for lung cancer.

Disclosure of conflict of interest

None.

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