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Knockdown of fascin-1 expression suppresses cell migration and invasion of non-small cell lung cancer by regulating the MAPK pathway

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ABSTRACT

Fascin-1 is a cytoskeletal protein and it can specifically bind to F-actin, it can be abnormally expressed in a variety of solid tumors. Fascin-1 was identified as a factor for poor prognosis in non-small cell lung cancer (NSCLC). However, the relevant molecular mechanisms are not yet fully understood. In this study, the fascin-1 knockdown cells were produced by lentivirus infection, and then cell proliferation, invasion and cell migration assay were used to investigate the role of fascin-1 in NSCLC cells. The MAPK pathway related proteins were determined by western blot. In the current study, lentivirus-mediated fascin-1 knockdown significantly decreased the proliferation of NSCLC cells. Furthermore, fascin-1 silencing partly inhibited cell invasion and migration. Inhibition of fascin-1 decreased the activity of the MAPK pathway. Therefore, targeting fascin-1 may inhibit the growth and metastasis of NSCLC cells, which is a potentially effective therapeutic strategy for treating NSCLC.

1. Introduction

Lung carcinoma is one of the most common malignancies of the respiratory system, with an incidence that has been steadily rising each year. In the United States, the number of new cases is estimated to be 222,500 in 2017, with 155,870 cases leading to death [1]; these numbers are similar to those reported in 2016 [2]. The situation in China is even worse [3]. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of total cases of lung carcinoma and the etiology and pathogenesis of lung carcinoma remain poorly defined. Recent studies have shown that genetic factors could be the major cause of NSCLC [4].

Fascin-1 is a cytoskeletal protein with a relative molecular mass of 55,000 Da [5] and it can specifically bind to F-actin [5]. The fascin-1 gene is located on chromosome 7 q22 [6]. Fascin-1 is usually expressed in neuronal, stromal and endothelial cells, but not in epithelial cells [7]. Fascin-1 protein can be abnormally expressed in a variety of solid tumors, such as laryngeal cancer, liver cancer, colorectal cancer, prostate cancer and breast cancer [8–12]. It is involved in the movement, metastasis and invasion of tumor cells. Fascin-1 is considered an important biomarker which influences tumor cell invasiveness.

In a previous study, we demonstrated that levels of fascin-1 is upregulated in NSCLC tissues relative to those in paracancerous tissues [13]. NSCLC patients with high expression of fascin-1 had a worse prognosis [13]. However, the relevant molecular mechanisms are not yet fully understood. Here, we examined the role of fascin-1 in the invasion and migration of NSCLC cells and analyzed the underlying mechanism of action.

2. Materials and methods

2.1. Cell culture

Two cell lines commonly used in NSCLC research (human lung adenocarcinoma A549 cell line and human lung squamous carcinoma H520 cell line) were purchased from the Institute of Medicinal Biotechnology (Chinese Academy of Sciences, Shanghai, China). Cells were cultured in a humidified atmosphere containing 5% CO_2 in 1640 medium (Gibco/Life Technologies, Carlsbad, CA, USA) containing 10% (V/V) fetal calf serum (Gibco), 50 U/ml penicillin (Invitrogen, Carlsbad, CA, USA) and 50 μg/ml streptomycin

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(Invitrogen). The cell lines were maintained in the exponential growth phase and provided fresh medium every 2–3 days.

2.2. Fascin-1 silencing

A hairpin sequence (Supplementary material 1) targeting fascin-1 was designed, synthesized (Adam & Stone Bio-Medicals Ltd. Co., Soochow, China) and sub-cloned into a PscioR vector (Addgene, Boston, MA, USA). The empty PscioR vector was used as a negative control. Lentivirus particles for fascin-1 knockdown were prepared as described previously [14,15]. In particular, the lentiviral particles were produced by transient co-transfection of human embryonic kidney 293T (HEK293T) cells with plasmids encoding the vesicular stomatitis virus G envelope, gag-pol, eGFP and abovementioned hairpin sequence targeting fascin-1 using Lipofectamine™2000 transfection reagent (Invitrogen). Media containing lentiviral particles was harvested 48 h after transfection, filtered (0.45 μm) and frozen until use. Both NSCLC cell lines were transfected using viral supernatants. Transfection efficiency was evaluated by examining GFP fluorescence with a fluorescence microscope (Carl Zeiss, Jena, Germany). The expression of fascin-1 was confirmed by western blotting.

2.3. Cell proliferation testing

The methyl thiazolyl tetrazolium (MTT) assay and count were used to assess cell proliferation activity. Cells were seeded in 96-well plates at a density of 4×10^3 cells per well and cell viability was determined after 48 h using the MTT assay. To each well, 20 μl of MTT stock solution (5 mg/ml) was added and 200 μl DMSO was added after 4 h incubation at 37 °C. Absorbance was measured at a wavelength of 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). For the cell counts, cells were serum free for 24 h. Then cells were trypsinized and equal number (2×10^5) of cells from each group was plated into 6-well culture plates in complete culture medium for 1, 2, 3 days. Then the cell number was determined by hemocytometer.

2.4. Transwell invasion assay

Boyden chamber assays were performed using modified two-chamber plates with a pore size of 8 μm. Cells in serum-free medium were seeded into the upper chamber of 24-well Transwell plates. Medium containing 10% fetal bovine serum was added to the bottom chamber. Cells that migrated to the bottom of the filter were fixed, stained with crystal violet and the number of cells in nine randomly selected fields were counted [16].

2.5. Wound healing assay

Cells were seeded into 6-well tissue culture plates. Viral infection was performed when cells reached 90–95% confluence as a monolayer. The monolayer was then slowly scratched with a new 0.2 ml pipette tip across the center of the well. After scratching, the wells were gently washed twice with medium to remove the detached cells. Cells were then cultured for 24 h and images were acquired (Olympus, Tokyo, Japan).

2.6. Western blotting

Western blot analyses were performed five days after lentivirus infection according to standard procedures [17]. Specifically, cells were harvested and lysed in 5 × SDS-PAGE loading buffer (250 mM Tris-HCl, 0.5% bromophenol blue, 50% glycerol, 10% SDS, 5% beta mercaptoethanol, pH 6.8). Equal amount of proteins (20 μg) were separated by 15% SDS-PAGE and transferred onto PVDF membranes (Millipore, Billerica, MA, USA) at 180 V for 50 min. The membranes were blocked with TBST containing 3% skim milk for 2 h at 20 °C and incubated with primary antibodies overnight at 4 °C. Secondary antibodies were then incubated with membranes for 2 h at 20 °C. Immuno-positive bands were visualized using a chemiluminescent method (G:BOX chemiXR5, SYNGEN, Sacramento, CA). The optical densities of the target protein bands visible on the X-ray film were determined by densitometry using Gel-Pro32 software [18]. Antibodies against fascin-1 (ab126772, rabbit monoclonal, Abcam, Cambridge, MA, USA), GAPDH (ab9485, rabbit polyclonal, Abcam), phos-MEK, MEK, phos-ERK (1/2), ERK (1/2), phos-MAPK, MAPK, phos-JNK and JNK (Abcam) were used at dilution ratios recommended by the manufacturer. The secondary antibody was anti-rabbit IgG (1:5000 dilution, Santa Cruz Biotechnology, Paso Robles, CA, USA).

2.7. Statistical analysis

Data were analyzed with SPSS v.19.0 software (SPSS Inc., Chicago, IL, USA) and are shown as the mean ± SD (standard deviation). The χ^2 and t-tests were used to evaluate differences between groups and values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Fluorescence microscopy

The expression of fascin-1 in A549 and H520 lung cancer cell lines was disrupted by lentiviral-delivered siRNA interference. After transfecting the cells with empty PscioR Vector and fascin-1-siRNA, green fluorescence was observed in the cytoplasm (Fig. 1A).

3.2. Western blot analysis of fascin-1 transfection efficiency

To assess whether fascin-1-siRNA effectively silenced the fascin-1 expression in A549 and H520 cells, proteins were extracted and the expression levels of fascin-1 protein in cell lysates were analyzed by western blot. As shown in Fig. 1 (B and C), a significant decrease in fascin-1 protein expression was observed after transfection with fascin-1-siRNA.

3.3. Silencing fascin-1 inhibits the proliferation, invasion and migration of NSCLC cells

To explore the biological function of fascin-1 in NSCLC, we performed tumor cell proliferation, invasion and migration assays. According to the results of MTT assays and cell counts, the proliferation of fascin-1-siRNA-transfected A549 and H520 cells was significantly suppressed ($P < 0.05$) compared to the control and PscioR Vector groups, as shown in Fig. 2. In the Transwell assay, the invasiveness of A549 and H520 cells in the fascin-1-siRNA group was significantly reduced ($P < 0.05$) compared to the corresponding controls, as shown in Fig. 3 (A and B). We studied the role of fascin-1 in NSCLC cell migration through scratch wound healing assays. The results after 24-h revealed that the migration of NSCLC cells in fascin-1-siRNA-treated groups decreased significantly compared to the control groups ($P < 0.05$), as shown in Fig. 3 (C and D). Therefore, silencing fascin-1 inhibits the proliferation, invasion and migration of NSCLC cells, all of which are the key features of tumorigenesis and metastasis.

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