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Original Article

Reduction in integrin α3β1 modulates lung cancer motility and invasion through p70^{S6K}-dependent E-cadherin localization

Young-Rak Cho[1,2], Eun-Kyung Ahn[2], Kyu-Bong Kim[1,3], Choong-Hyun Lee[1,3], Yoon Gyoon Kim[1], Joa Sub Oh[1,3], Dong-Wan Seo[1,3*]

[1] Department of Pharmacy, College of Pharmacy, Dankook University, Cheonan 31116, Republic of Korea
[2] Biocenter, Gyeonggi Business & Science Accelerator, Suwon 16229, Republic of Korea
[3] Center for Human Risk Assessment, Dankook University, Cheonan 31116, Republic of Korea

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Abstract

In the current study, we investigated the effects and action mechanism of integrin α3β1 in modulating non-small cell lung cancer (NSCLC) growth and progression. Reduced expression of integrin α3 by RNA silencing in p53 wild-type A549 NSCLC cells inhibits cell migration and invasion, compared with those in control cells. These anti-migratory and anti-invasive properties in integrin α3-silenced cells were associated with epithelial cadherin (E-cadherin) distribution at cell-cell contacts, and these effects require the activation of p70 S6 kinase (p70^{S6K}) as evidenced by treatment with rapamycin. Disruption of E-cadherin or blockade of p70^{S6K} activation abrogated the ability of integrin α3-silencing to inhibit cell migration and invasion. In contrast, enhanced proliferation in integrin α3-silenced cells was not affected by the changes in E-cadherin expression. These findings demonstrate the ability of integrin α3β1 to differentially regulate NSCLC cell growth and progression depending on the p53 status, and suggest that integrin α3β1-p70^{S6K}-p53 network may be a promising target for the treatment of NSCLC.

Keywords: Integrin α3β1; p70^{S6K}; E-cadherin; p53; Non-small cell lung cancer

1. Introduction

Lung cancer is the leading cause of cancer-related deaths, along with the fact that the 5-year relative survival rate is about 23% [1]. The highest mortality rates of lung cancer may be due to an absence of highly sensitive diagnostic methods or effective treatments for recurrent and aggressive phenotypes. Understanding the changes in gene expression and its regulatory mechanisms underlying lung cancer growth and progression may provide revelatory insights into the identification of therapeutic targets and effective strategies in the treatment of lung cancer.

Integrins, cell surface receptors are composed of α and β subunits, mediate the interactions of cell-cell and cell-extracellular matrix (ECM), leading to the modulation of diverse cellular events such as adhesion, migration, invasion, survival, and proliferation [2, 3]. Previous investigations indicate that expression and activation of integrins are highly related to the invasive and metastatic properties in various cancer types, leading to the development and clinical evaluation of integrin regulators as potential cancer therapeutics [4-6]. Among the integrin family, integrin α3β1 has been found to contribute positively or negatively to various types of cancer growth and progression [7-9]. It has been reported that enhanced expression of integrin α3β1 correlates with p53-mutated NSCLC cell metastasis to the brain [10]. In contrast, reduced expression of integrin α3β1 induces proliferative and migratory activities of p53-deficient NSCLC cells [11]. These conflicting investigations may be due in part to heterogeneous types and stages of cancer in the tumor microenvironment. Thus, sophisticated investigations of integrin α3β1 underlying cancer growth and progression may provide effective strategies for the treatment of various types of cancers.

Epithelial-to-mesenchymal transition (EMT) is involved in morphological changes, and the loss of intercellular and cell-ECM adhesion, resulting in acquisition of enhanced migration and invasion [12]. During EMT, expression of epithelial markers such as epithelial cadherin (E-cadherin), claudins and desmosomes is decreased, whereas that of mesenchymal markers including vimentin, neural cadherin and matrix metalloproteinases (MMPs) is increased [13, 14]. Although the changes in cadherins

* Corresponding author.
E-mail address: dwseomb@dankook.ac.kr (D-W. Seo).
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in the EMT process have been widely studied, functional relationship between integrin α3β1 and cadherin in regulating cellular behaviors including migration and invasion is not clearly understood.

Aberrant expression and activation of p70 S6 kinase ($p70^{S6K}$) have been correlated with various types of cancer progression [15]. A previous study demonstrates that $p70^{S6K}$ promotes invasiveness through Snail-dependent E-cadherin repression in p53-null or mutated ovarian cancer cells, suggesting that therapeutic targeting of $p70^{S6K}$ may be a promising strategy to block cancer progression associated with invasion and metastasis [16]. In contrast, the present study shows that $p70^{S6K}$ mediates the anti-migratory and anti-invasive activities in integrin α3-silenced, p53 wild-type A549 NSCLC cells, involving the elevated distribution of E-cadherin at cell-cell contacts. These findings suggest that pharmacological inhibition of $p70^{S6K}$ activity might enhance EMT, leading to aggressive potential of p53 wild-type NSCLC with low integrin α3β1 expression.

2. Materials and methods

2.1. Cell culture conditions

Human non-small cell lung cancer cells (A549) from the American Type Culture Collection (Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT, USA).

2.2. RNA interference

Both the shRNA-targeting integrin α3 gene and scrambled sequences cloned into psiRNA-hH1zeo vector (InvivoGen, San Diego, CA, USA) were designated α3 shRNA and control shRNA, respectively. These vector constructs were transfected into A549 cells as described previously [17]. For transient transfection of A549 cells, the integrin α3, E-cadherin and control siRNA were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Cells were transfected with siRNA (integrin α3 siRNA; E-cadherin; control siRNA, each 50 nM) using Lipofectamine RNAiMAX and Opti-minimum essential medium (MEM) I reduced serum media (Invitrogen, Carlsbad, CA, USA). The concentrations of siRNAs were chosen based on dose-response experiments.

2.3. Reagents

The following antibodies and agents were obtained from commercial sources: anti-phospho-$p70^{S6K}$ (T421/S424), anti-phospho-Akt (S473), anti-phospho-ERK (T202/Y204) and MEK inhibitor PD98059 (Cell Signaling Technology, Beverly, MA, USA); anti-integrin β1 and anti-E-cadherin (BD Biosciences, Bedford, MA, USA); anti-integrin α3, anti-$p70^{S6K}$, anti-Akt, anti-ERK, anti-Cdk2, anti-Cdk4, anti-cyclin D, anti-cyclin E, anti-$p27^{Kip1}$, anti-$p21^{Waf1/Cip1}$ and anti-actin (Santa Cruz Biotechnology); anti-mouse and anti-rabbit IgG-horseradish peroxidase secondary antibodies (Thermo Fisher Scientific Inc., Waltham, MA, USA); mammalian target of rapamycin (mTOR)/$p70^{S6K}$ inhibitor rapamycin (Sigma-Aldrich Co., St. Louis, MO, USA); phosphatidylinositol 3-kinase (PI3K)/Akt inhibitor LY294002 (Merck Millipore, Billerica, MA, USA).

2.4. RNA isolation and RT-PCR

RNA was purified and measured by using PureHelix™ total RNA purification kit (Nanohelix Co., Daejeon, Republic of Korea) and the NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Inc.). cDNA synthesis reaction for each reverse-transcriptase (RT)-polymerase chain reaction (PCR) was performed by using 1st strand cDNA synthesis kit (BioAssay Co., Daejeon, Republic of Korea). PCR primers were synthesized by Bioneer Corporation (Daejeon, Republic of Korea). Primer sequences for integrin α3 were forward 5'-AAGCCAAGTCTGAGACT-3' and reverse 5'-GTAGTATTGGTCCCGAGTCT-3'; primer sequences for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were forward 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse 5'-GAAGATGGTGATGGGA TTTC-3'; and primer sequences for E-cadherin were forward 5'-GGGTGACTACAAAATCAATC-3' and reverse 5'-GGGGGCAGTAAGGGCTCTTT-3'.

2.5. Adhesion assay

Trypsin-EDTA-detached cells were recovered in 10% FBS-DMEM for 1 h with rocking gently. The cells were allowed to adhere to laminin-coated 96-well plates ($1.5x10^4$ cells/well) for 2 h at 37°C. After incubation for 2 h, adherent cells were fixed with methyl alcohol and stained with 0.04% Giemsa staining solution (Sigma-Aldrich Co.). Adhesion results (mean ± standard deviation) are presented as the percentage of wild-type adherent cells.

2.6. *In vitro* wound healing assay

Wound healing assay was performed as described previously [18]. A single wound of confluent cells in 48-well plates was created in the center of the cell monolayer by a plastic pipette tip. Cells were serum-starved for 2 h and then pretreated as indicated, followed by 10% FBS stimulation for 16 h. Following fixation with methyl alcohol, cells were stained with Giemsa staining solution.

2.7. *In vitro* transwell migration and invasion assay

Aliquots (100 µl) of cells ($8x10^5$ cells/ml) were added to the upper compartment of non-coated and Matrigel (1mg/ml, BD Biosciences)-coated transwell inserts (Costar, 6.5 mm diameter insert, 8 µm pore size) (Corning Inc., Corning, NY, USA) for transwell migration and invasion, respectively. Cells were serum-starved for 2 h and then pretreated as indicated, followed by 10% FBS stimulation for 16 h (migration) or 14 h (invasion). After the non-migratory or non-invasive cells were removed from the top of the insert membranes, changes in migration and invasion were quantified as described previously [19].

2.8. Cell proliferation assay

Cells were serum-starved for 24 h and further incubated with 10% FBS for the indicated time points. Changes in proliferation were determined by using trypan blue staining method. The results from triplicate determinations (mean ± SD) are shown as the fold-increase of control shRNA-transfected cells or the percentage of 10% FBS-stimulated cell proliferation in control shRNA- or integrin α3 shRNA-transfected cells.

2.9. Western blot analysis

Cells were serum-starved for 24 h, followed by treatments for indicated time points. Cells were washed with phosphate-buffered saline (PBS, pH 7.4) and lysed by incubation in 50 mM Tris-HCl (pH 7.4), 1% Triton X-100,

10% glycerol, 150 mM NaCl, 10 µg/ml aprotinin, 100 µg/ml 4-(2-aminoethyl) benzenesulfonyl fluoride, 1 µg/ml pepstatin A, 0.5 µg/ml leupeptin, 1 mM sodium orthovanadate, 25 mM sodium fluoride, 80 mM β-glycerophosphate and 1 mM EDTA for 30 min at 4°C. Cell lysates were subjected to Western blot analysis as previously described [18].

2.10. Immunofluorescence microscopy

Cells plated on coverslips in 24-well plates were serum-starved for 24 h, and then pretreated with rapamycin for 30 min, followed by 10% FBS stimulation for 3 h. Following fixation with 3.7% formalin and permeabilization with 0.1% Triton X-100, cells were blocked with PBS containing 5% BSA, incubated with anti-E-cadherin and subsequent Alexa Fluor 488-conjugated secondary antibody (#A28175) (Invitrogen), and then mounted with DRAQ5™ (BioStatus, Shepshed, Leicestershire, United Kingdom). Images were analyzed as previously described [17].

2.11. Zymogram analysis

Aliquots of conditioned media collected from control shRNA- or integrin α3 shRNA-transfected cells were applied to 10% polyacrylamide gels containing 1 mg/ml gelatin (Sigma-Aldrich Co.). After running gel electrophoresis, the gels were washed with 2.5% Triton X-100 for 1 h and then incubated in a 50 mM Tris-HCl (pH 7.5) containing 150 mM NaCl and 10 mM $CaCl_2$ for 20 h at 37°C. Gelatinolytic activities of MMPs were analyzed as previously described [19].

2.12. Statistical analysis

Statistical analysis of data resulting from at least three independent experiments was performed using Student's *t*-test of SigmaPlot software (Grafiti LLC, Palo Alto, CA, USA). Results are considered to be statistically significant if the p-value is less than 0.05.

3. Results

3.1. Integrin α3 silencing affects adhesive property and morphology of p53 wild-type A549 NSCLC cells

It has been reported that integrin α3β1 plays significant roles in cellular events including adhesion, migration, invasion and proliferation, which are closely involved in cancer growth and progression [7, 8, 20]. To investigate the biological roles of integrin α3β1 in p53 wild-type NSCLC cells, A549 cells were transfected with psiRNA-hH1zeo vector including shRNA-targeting integrin α3 or scrambled sequences [21], and the cells were designated as α3 shRNA and control shRNA, respectively. RT-PCR and Western blot analyses demonstrated that α3 shRNA dramatically suppressed expression of integrin α3 subunit in A549 cells when compared with wild type or control (scrambled) shRNA-transfected cells, and did not affect β1 expression (Figure 1A). Integrin α3β1 reportedly interacts with many constituents of the ECM, leading to modulation of diverse cellular functions [7, 8]. We first examined the adhesion of integrin α3-silenced A549 cells to laminin, a major ligand for integrin α3β1. As expected, integrin α3 shRNA-transfected cells failed to attach to laminin, as compared with wild-type or control shRNA-transfected cells (Figure 1B). In addition, microscopic analysis revealed that integrin α3 silencing promoted the changes of morphology in A549 cells. Integrin α3 shRNA-transfected cells grew more dense, were smaller in volume and exhibited intensive cell-cell contacts, when compared with wild-type or control shRNA-transfected cells (Figure 1C). These findings suggest that disruption of integrin α3 expression exerts changes in morphology as well as adhesive property, which may regulate cellular responses such as migration, invasion and proliferation.

3.2. Integrin α3 silencing inhibits cell migration and invasion

We next investigated the effects of integrin α3 silencing on cell migration and invasion in A549 cells. Wild-type or control shRNA-transfected cells showed highly migratory and invasive responses to mitogenic stimuli. In contrast, integrin α3-silenced cells were resistant to mitogen-induced migration and invasion (Figure 2A, B). Transient transfection of A549 cells with integrin α3 siRNA also inhibited the migratory and invasive responses (Figure 2C-E). Because both stably and transiently transfected cells exhibited similar effects on the regulation of migration and invasion in A549 cells, integrin α3 shRNA-transfected cells were used in most of the subsequent experiments. It has been reported that activation and expression of MMPs are related to increase in cell migration and invasion by proteolytic cleavage of ECM and cell adhesion molecules [22, 23]. Based on integrin α3 silencing-mediated inhibition of migration and invasion (Fig. 2A-E), the gelatinolytic activities of MMPs were measured in the conditioned media of A549 cells with control or integrin α3 shRNA. As shown in Figure 2F, integrin α3 shRNA did not significantly affect basal or mitogen-induced activity of MMP-2 and MMP-9 when compared with control shRNA-transfected cells, suggesting the existence of alternative mechanisms to regulate cell migration and invasion.

3.3. Integrin α3 silencing induces the distribution of E-cadherin at cell surfaces

Loss of E-cadherin from the cell surface often occurs in cancer progression, which induces diverse cellular responses such as disruption of cell-cell contacts, cell detachment, morphological change, cell migration and invasion [24]. The roles and molecular mechanisms of cadherin can be evaluated by the levels of cadherin detectable at cell surfaces or in the Triton-insoluble cell fraction [17, 25]. As shown in Figure 3A, integrin α3 shRNA did not alter

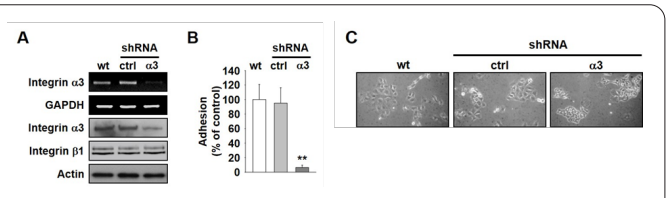

Fig. 1. Changes of adhesion and morphology in integrin α3-silenced A549 cells. (A) The levels of integrin α3 and β1 in p53 wild-type A549 cells were determined by RT-PCR and Western blot analyses as described in Materials and Methods. (B) Cells ($1.5x10^4$ cells/well) were plated on laminin-coated 96-well plates. The number of adherent cells was determined by counting ten different fields of cells. Adhesion results (mean ± SD) are shown as the percentage of wild-type cells. Statistical significance is indicated (**P < 0.01, compared with wild-type cells). (C) Microscopic images of wild-type, control shRNA- or integrin α3 shRNA-transfected cells.

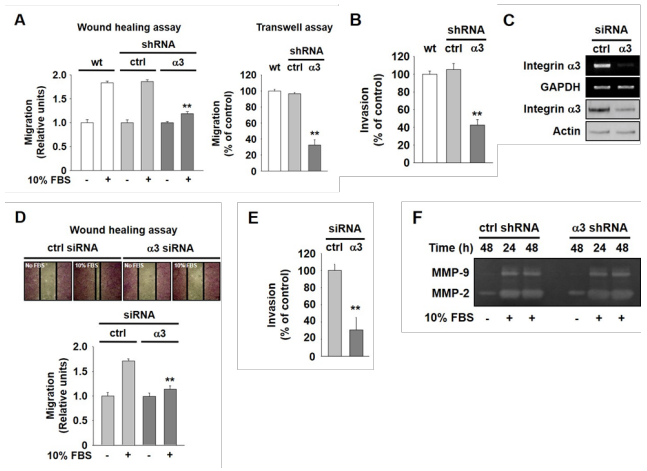

Fig. 2. Reduced migration and invasion in integrin α3-silenced A549 cells. Wound healing (A, D) and invasion (B, E) assays were performed as described in Materials and Methods. (A) Results from six different experiments (mean ± SD) are shown as the fold-increase of each of the unstimulated cells (left panel) or the percentage of wild-type cells (right panel). Statistical significance is indicated ((A, left panel) **P < 0.01, compared with 10% FBS-stimulated wild-type cells; (A, right panel) **P < 0.01, compared with wild-type cells). (B) The number of invasive cells was determined by counting six different fields of cells. Invasion results from six different experiments (mean ± SD) are shown as the percentage of wild-type cells. Statistical significance is indicated (**P < 0.01, compared with wild-type cells). (C) Following transient transfection of cells with control or integrin α3 siRNA, the levels of integrin α3 were measured by RT-PCR and Western blot analyses as described in Materials and Methods. (D, E) Results from six different experiments (mean ± SD) are shown as the fold-increase of each of the unstimulated cells (D) or the percentage of control siRNA-transfected cells (E). Statistical significance is indicated ((D) **P < 0.01, compared with 10% FBS-treated control siRNA-transfected cells; (E) **P < 0.01, compared with control siRNA-transfected cells). (F) Cells were serum-starved for 24 h, and incubated with 10% FBS for 24 or 48 h. Gelatinolytic activities of MMPs were analyzed using conditioned media collected from cell culture as described above.

the levels of E-cadherin in the Triton-soluble fraction. In contrast, integrin α3 shRNA increased the distribution of E-cadherin into the Triton-insoluble fraction. Transient transfection of cells with integrin α3 siRNA also showed increased E-cadherin distribution in the Triton-insoluble fraction (Figure 3B). Increase in E-cadherin in the Triton-insoluble fraction was detectable from 3 h and sustained up to 24 h until the end time point of this experiment (Figure 3A). These results suggest that enhanced localization of E-cadherin at cell surfaces might contribute to the reduction of cell migration and invasion in integrin α3 shRNA-transfected cells (Figure 2A, B). To confirm the functional significance of E-cadherin in integrin α3 shRNA-mediated regulation of cell migration and invasion, E-cadherin siRNA was transiently transfected in integrin α3-silenced A549 cells (Figure 3C). Disruption of E-cadherin expression abrogated the integrin α3 shRNA-mediated inhibition of cell migration and invasion, similar to the levels observed in control shRNA (Figure 3D, E).

On the other hand, integrin α3 shRNA significantly enhanced cell proliferation, as compared with control shRNA (Figure 4A). This effect was closely associated with down-regulation of cyclin-dependent kinase (Cdk) inhibitors such as $p27^{Kip1}$ and $p21^{Waf1/Cip1}$, and up-regulation of Cdk2 and cyclin D (Figure 4B). E-cadherin siRNA did not affect the ability of integrin α3 shRNA to enhance cell proliferation (Figure 4C). Collectively, these findings suggest that distribution of E-cadherin at integrin α3-silenced, p53 wild-type A549 cell surfaces is essential for inhibition of cell migration and invasion, but not proliferation.

3.4. E-cadherin mediates integrin α3 silencing-induced suppression of cell migration and invasion through $p70^{S6K}$-dependent mechanism

To elucidate molecular mechanisms by which integrin α3 silencing inhibits cell migration and invasion, we next examined the changes in activation of mitogenic pathway components including extracellular signal-regulated kinase (ERK), Akt and $p70^{S6K}$ in integrin α3 shRNA-transfected cells. The phosphorylation intensities of ERK, Akt and $p70^{S6K}$ in integrin α3 shRNA-transfected cells seemed to be slightly stronger than those in control cells (Figure

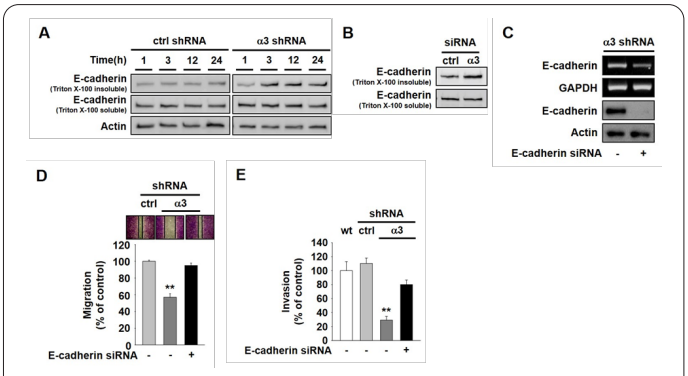

Fig. 3. Functional role of E-cadherin in integrin α3 silencing-mediated inhibition of migration and invasion. Distribution of cadherin in integrin α3 shRNA (A) or integrin α3 siRNA (B) cells was determined by the solubility of Triton X-100. The Triton-insoluble or soluble fraction was separated by SDS-PAGE and then Western blotted for detection of E-cadherin. (C) Transient transfection of integrin α3-silenced cells with E-cadherin siRNA was performed as described in Materials and Methods. The levels of E-cadherin were determined by RT-PCR and Western blot analyses. Wound healing (D) and invasion (E) results (mean ± SD) are shown as the percentage of control shRNA (D) or wild-type (E) cells. Statistical significance is indicated ((D) **P < 0.01, compared with control shRNA-transfected cells; (E) **P < 0.01, compared with wild-type cells).

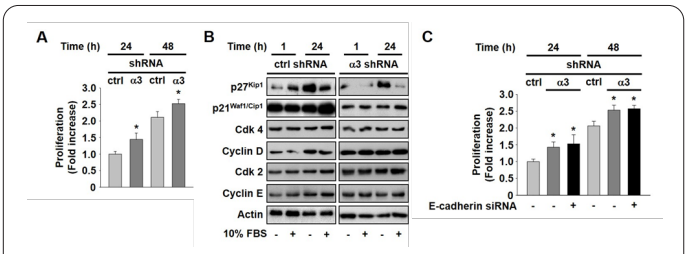

Fig. 4. Enhanced proliferation in integrin α3-silenced A549 cells. (A) Cells were serum-starved for 24 h, and followed by 10% FBS stimulation for 24 h and 48 h. Cell proliferation results (mean ± SD) are shown as the fold-increase of each of the control shRNA-transfected cells. (B) Cells were incubated for the indicated time points. Results are representative of at least three different experiments. (C) Following transient transfection of integrin α3-silenced cells with E-cadherin siRNA, cell proliferation assay was performed as in panel (A). Statistical significance is indicated (*P < 0.05, compared with control shRNA-transfected cells at the indicated time points).

5A). Treatment with PD98059 (an inhibitor of mitogen-activated protein kinase (MEK)/ERK), LY294002 (an inhibitor of phosphatidylinositol 3-kinase/Akt) or rapamycin (an inhibitor of mammalian target of rapamycin/p70^{S6K}) significantly blocked cell proliferation in both control and integrin α3 shRNA-transfected cells (Figure 5B). Suppression of proliferation in integrin α3 shRNA-transfected cells was more sensitive to PD98059 or LY294002, but not rapamycin, than that in control shRNA cells.

Treatment with PD98059 or LY294002 significantly inhibited mitogen-induced migration in control shRNA-transfected cells, but rapamycin did not reduce cell migration, consistent with previous reports (Figure 5C, left panel) [26]. In contrast, treatment with PD98059 or LY294002 did not affect the anti-migratory response in integrin 3 shRNA-transfected cells (Figure 5C, right panel). Rapamycin ablated the anti-migratory and anti-invasive activities of integrin α3 shRNA, and restored invasion to similar levels observed in mitogen-treated control shRNA-transfected cells (Figure 5C, D). As shown in Figure 5E, immunofluorescence microscopy revealed that integrin α3 silencing led to the enhanced E-cadherin distribution at cell-cell contacts, in agreement with previous findings from Western blotting for E-cadherin in Triton-insoluble fractions (Figure 3A). Treatment of integrin α3 shRNA-transfected cells with rapamycin significantly prevented the distribution of E-cadherin at cell-cell contacts (Figure 5E). Finally, these findings demonstrate a functional relationship between integrin α3 silencing-induced p70^{S6K} activity, enhanced distribution of E-cadherin at cell-cell contacts, and reduced migratory and invasive responses.

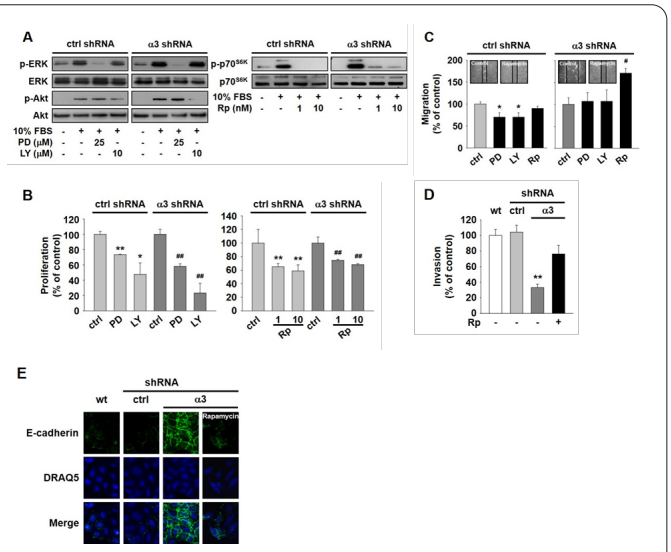

Fig. 5. Changes of E-cadherin distribution, cell migation and invasion in p70^{S6K} inhibitor-treated integrin α3-silenced cells. Cells were serum-starved for 24 h, pretreated with PD98059 (PD, 25 μM), LY294002 (LY, 10 μM) or rapamycin (Rp, 1-10 nM) for 30 min, and followed by 10% FBS stimulation for 15 min (A) or 24 h (B). Cell proliferation results (mean ± SD) are presented as the percentage of control shRNA or integrin α3 shRNA cells. Statistical significance is indicated (*P < 0.05, **P < 0.01, compared with control shRNA-transfected cells; $^{\#\#}$p < 0.01, compared with integrin α3 shRNA-transfected cells). Cells were pretreated with PD98059 (PD, 25 μM), LY294002 (LY, 10 μM) or rapamycin (Rp, 10 nM) for 30 min, and followed by 10% FBS stimulation for 16 h (C) or 14 h (D). (C) Cell migration results (mean ± SD) are shown as the percentage of control shRNA or integrin α3 shRNA cells. Statistical significance is indicated (*P < 0.05, compared with control shRNA-transfected cells; $^{\#}$P < 0.05, compared with integrin α3 shRNA-transfected cells). (D) Statistical significance is indicated (**P < 0.01, compared with wild-type cells). (E) Cells were treated with rapamycin (10 nM) for 30 min, and followed by 10% FBS stimulation for 3 h. The cellular distribution of E-cadherin was detected with anti-E-cadherin antibodies (green). DNA was stained with DRAQ5 (blue).

4. Discussion

EMT is one of the crucial responses associated with cancer progression, which includes enhanced cell migration and invasion through integrin-dependent mechanisms [12, 27]. Although the precise role and molecular mechanism of integrins in EMT-related cancer progression are not fully understood, due to complex interactions of integrins with ECM or cell surface molecules, integrins are widely considered as potential molecular targets for the treatment of cancer. The current study is the first description that disruption of integrin α3β1 expression regulates the migration and invasion of p53 wild-type A549 NSCLC cells by p70^{S6K}-dependent localization of E-cadherin at cell-cell contacts.

E-cadherin, a Ca^{2+}-dependent cell-cell adhesion protein, functions as tumor suppressor that is often inactivated or silenced in highly aggressive cancers [28, 29]. The function and expression of E-cadherin are tightly regulated by extracellular and intracellular communication networks-mediated phosphorylation, internalization and cleavage. Src-mediated tyrosine phosphorylation of E-cadherin leads to the destabilization of cadherin-catenin complex, whereas casein kinases- or glycogen synthase kinase-3β-mediated serine phosphorylation strengthens the interactions of E-cadherin with catenins, resulting in enhancing cell-cell adhesion [30-32]. In addition, MMP-mediated cleavage of E-cadherin disrupts cell-cell adhesion, and the cleaved E-cadherin fragments reportedly activate receptor tyrosine kinases, G protein-coupled receptors and Wnt/ β-catenin signaling pathways, involving the increase in cell migration and invasion [17, 33, 34]. These observations demonstrate the existence of a variety of factors affecting the levels and activity of E-cadherin in regulating EMT-associated responses.

It has been reported that reduction of integrin α3 subunit expression stimulates migration in p53-deficient H1299 NSCLC cells, this result might be associated with defective expression of E-cadherin as previously reported [11, 34, 35]. In contrast, the present study suggests that p53 functions as a critical modulator of integrin α3β1-mediated regulation of NSCLC progression. Integrin α3 silencing-mediated inhibition of p53 wild-type NSCLC cell migration and invasion requires p70^{S6K}-dependent localization of E-cadherin at cell-cell contacts as evidenced by an mTOR/p70^{S6K} inhibitor rapamycin or siRNA-targeting E-cadherin, inconsistent with previous report that p70^{S6K} enhances EMT by Snail-dependent suppression of E-cadherin expression in ovarian cancer cells [16]. These conflicting observatins may be due to differential gene expression and heterogeneity of cancer types. Our findings suggest that inactivation of integin α3β1 might be a potential therapeutic target for the regulation of p53 wild-type NSCLC cell migration and invasion.

5. Conclusion

In conclusion, current findings establish a novel insight into p70^{S6K}-dependent E-cadherin localization associated

with inhibition of integrin α3-silenced cell migration and invasion, and suggest that coordinated regulation of integrin α3β1 expression and $p70^{S6K}$ signaling pathways may be a potential strategy for the treatment of NSCLC differing in p53 status.

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Conflict of interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions
Y.R.C. and D.W.S designed and performed research and wrote the manuscript; E.K.A., K.B.K., C.H.L., Y.G.K., and J.S.O. analyzed and organized data; D.W.S. was responsible for supervising the entire project and writing the manuscript.

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