Knockdown of integrin α3β1 expression induces proliferation and migration of non-small cell lung cancer cells

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Abstract. Integrin α3β1 is expressed on many types of cancer cells and can regulate tumor growth and progression. In the present study, we examined the roles and molecular mechanism of integrin α3β1 in modulating cell proliferation and migration of p53-deficient non-small cell lung cancer (NSCLC) cells. Reduced expression of integrin α3 by RNA silencing clearly induces cell proliferation and migration in H1299 cells, compared with those in control cells. Enhanced proliferation in integrin α3-silenced cells is mediated by upregulation and nuclear localization of cyclin-dependent kinases, and these effects require the activation of Akt and ERK as evidenced by treatment with LY294002 and PD98059, respectively. Furthermore, suppression of integrin α3 expression induces the expression of nuclear factor-κB and Bcl-2 as well as epidermal growth factor receptor, which are positively correlated with cell proliferation and survival. In contrast, increase in cell migration of integrin α3-silenced cells is found to be independent of Akt or ERK signaling pathways. Collectively, these findings suggest that integrin α3β1 plays pivotal roles in regulating cell proliferation and migration that enhance the invasive type of p53-deficient NSCLC cells.

Introduction

Integrins, cell surface receptors that mediate cell-extracellular matrix (ECM) and cell-cell interactions, function to modulate cell behaviors including cell adhesion, migration, proliferation, survival, invasion and angiogenesis (1-3). Integrin-mediated signaling cascades include the activation of a variety of protein kinases such as focal adhesion kinase, Src-family kinases, extracellular signal-regulated kinase (ERK), and phosphatidylinositol

3-kinase (PI3K)/Akt (1,3). These signaling events are complex and very similar to those triggered by growth factor receptors, demonstrating cross-talk between ECM- and growth factor-induced signal transduction pathways, which are involved in physiological and pathological processes (3-5). Integrin α3β1, a major receptor for laminin, is expressed on many types of cancer cells and plays the pivotal roles in regulation of cancer progression (3,6). Reduced integrin α3 expression in lung cancer is probably associated with increased aggressiveness and poor prognosis in lung cancer patients (7,8). Although significant advances have been made in understanding the function of integrin α3β1, the roles and molecular mechanisms in mediating the regulation of malignant cell behavior still remain unexplored.

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase which is highly expressed or activated in a variety of human cancers including lung, ovary, stomach, brain, breast and colon cancer, and it has therefore been known as the main therapeutic target for cancer treatment (9-11). EGFR-dependent signaling pathways include the activation of Akt and ERK, which are implicated in the cell proliferation, survival, migration and invasion (9). In addition, recent studies have established that nuclear factor-κB (NF-κB) is closely associated with regulation of cell proliferation and anti-apoptosis as well as inflammation and immune responses (12,13). NF-κB activation can either promote or suppress the proliferation and survival of cancer cells, depending on the cell and tissue types as well as the expression status of tumor suppressor proteins such as p53 or phosphatase and tensin homolog (12).

In the present study, we evaluated the biological effects and molecular mechanisms of integrin α3β1 on cell proliferation and migration in p53-deficient human lung cancer H1299 cells. Enhanced proliferation and migration in integrin α3-silenced cells are mediated by activation of Akt and ERK-dependent pathways and induction of cyclin-dependent kinases, EGFR, and NF-κB-inducible anti-apoptotic protein Bcl-2. These findings indicate that reduced integrin α3 expression in p53-deficient NSCLC cells results in multiple phenotypic changes that enhance the aggressiveness.

Materials and methods

Cell culture conditions. Human lung carcinoma cells (H1299) from American Type Culture Collection (Manassas, VA, USA)

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were grown in 10% fetal bovine serum-Dulbecco's modified Eagle's medium (FBS-DMEM) (HyClone Laboratories, Logan, UT, USA).

Reagents. PD98059 (MEK 1/2 inhibitor) and LY294002 (PI3K inhibitor) were obtained from Upstate Biotechnology (Lake Placid, NY, USA). The following antibodies were purchased from commercial sources: anti-phospho-ERK (T202/Y204), anti-phospho-Akt (Ser473), anti-lamin A/C (Cell Signaling, Beverly, MA, USA); anti-integrin β1 (BD Biosciences, Bedford, MA, USA); anti-integrin α3, anti-EGFR, anti-ERK, anti-Akt, anti-Cdk4, anti-Cdk2, anti-p27^{Kip1}, anti-NF-κB, anti-Bcl-2, anti-actin antibodies, and mouse, rabbit and goat IgG-horseradish peroxidase conjugates (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

RNA purification and RT-PCR. Total RNA was purified with easy Blue™ Total RNA extraction kit (iNtRON Biotechnology, Sungnam, Gyeonggi, Korea). Integrity of RNA was checked by agarose gel electrophoresis and ethidium bromide staining. RNA (1 μg) was used as template for each reverse-transcriptase (RT)-mediated polymerase chain reaction (PCR) by using the ImProm-II™ Reverse Transcription System (Promega, Madison, WI). Primers for PCR were synthesized by Bioneer Corporation (Daejeon, Korea). Primer sequences were: integrin α3, forward 5'-AAGCCAAGTCTGAGACT-3' and reverse 5'-GTAGTATTGGTCCCGAGTCT-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse 5'-GAAGATGGTGATGGGATTTC-3' (14).

siRNA preparation and transfection. For design of siRNA inserts, a cDNA sequence of integrin α3 AGCAACACAGACTACCTGGAG was selected according to the InvivoGen siRNAWizard program based on a BLAST search. As a control we used the scrambled oligonucleotide sequence AGCATATGTGCGTACCTAGCT, available prepackaged in the psiRNA-hH1zeo vector (InvivoGen, San Diego, CA, USA). Both the siRNA-targeting integrin α3 gene and scrambled sequences cloned into psiRNA-hH1zeo vector were designated α3 shRNA and control shRNA, respectively. These vector constructs were transfected into H1299 cells using LyoVec (InvivoGen) according to the manufacturer's instructions. After 48 h, the cells were selected with zeocin in 10% FBS-DMEM for 1-2 weeks until positive colonies formed (15).

Adhesion assay. Subconfluent cells were detached with trypsin and allowed to recover in 10% FBS-DMEM for 1 h at 37°C with gentle rocking. After recovery, the cells were collected by low-speed centrifugation and resuspended in fresh 10% FBS-DMEM. The cells were plated on non-coated, laminin-coated, or collagen-coated 96-well plates (1.5x10⁴ cells/well), and further incubated for 2 h at 37°C. Following incubation unattached cells were removed by washing the wells three times with ice-cold phosphate buffered saline (PBS, pH 7.4). Attached cells were fixed with methanol, and then stained with 0.04% Giemsa staining solution (Sigma-Aldrich Co., St. Louis, MO, USA). The cells were photographed and counted. The results (mean ± standard deviation) are presented as the fold-increase of the untreated adherent cells (16).

Cell growth assay. Subconfluent control shRNA or integrin α3 shRNA-transfected H1299 cells, plated on 6-well plates (2x10⁴ cells/well), were serum-starved for 48 h to synchronize cells in G_1/G_0 phase of cell cycle, and further incubated with 10% FBS-DMEM for the indicated time points in the presence or absence of MEK or PI3K inhibitor. The cells were then washed in ice-cold PBS, detached with trypsin, and counted using trypan blue exclusion method. The results from triplicate determinations (mean ± standard deviation) are presented as the numbers of cells per culture or the fold-increase of the untreated controls.

Migration assay. Cell migration was quantified in the *in vitro* wound-healing assay as previously described (17). After cells were plated on 48-well plates, grown to confluence, and a single wound was created in the center of the cell monolayer by the gentle removal of the attached cells with a sterile plastic pipette tip. Cells were pretreated with or without MEK or PI3K inhibitor for 30 min, followed by serum stimulation for 18 h. Cells were fixed with methanol, and then stained with 0.04% Giemsa staining solution. The migration of the cells into the wound was observed with still images taken at the indicated time point.

Western blot analysis. Subconfluent cells in 100-mm dishes (BD Biosciences) were serum-starved for 48 h in DMEM and replaced with fresh media, followed by treatments for different time points, as indicated. Cells were rinsed twice with ice-cold PBS and lysed by incubation in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EDTA, 100 μg/ml 4-(2-aminoethyl)benzenesulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml pepstatin A, 0.5 μg/ml leupeptin, 80 mM β-glycerophosphate, 25 mM NaF and 1 mM sodium orthovanadate for 30 min at 4°C. Cell lysates were clarified at 13,000 x g for 20 min at 4°C, and the supernatants were subjected to western blot analysis as described previously (18).

Subcellular fractionation. Following treatments as indicated, cells were rinsed twice with ice-cold PBS, and cytoplasmic and nuclear extracts were prepared using Nuclear/Cytosol Fractionation kit (BioVision Inc., Mountain View, CA, USA), according to the manufacturer's instructions.

Zymogram analysis. Activities of matrix metalloproteinases were measured by zymography (19). Aliquots of conditioned medium were diluted in sample buffer, applied to 10% polyacrylamide gels containing 1 mg/ml gelatin (Sigma-Aldrich Co.) as a substrate. After electrophoresis, the gels were incubated in 2.5% Triton X-100 for 1 h to remove SDS and allow re-naturalization of MMPs, and further incubated in developing buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM CaCl$_2$, and 150 mM NaCl for 20 h at 37°C. The gels were stained with 0.5% Coomassie brilliant blue R-250 in 30% methanol-10% acetic acid for 2 h and followed by destaining with 30% methanol-10% acetic acid. Gelatinolytic activities were detected as unstained bands against the background of the Coomassie blue-stained gelatin.

Statistical analysis. Statistical analysis was performed using Student's t-test, and was based on at least three different
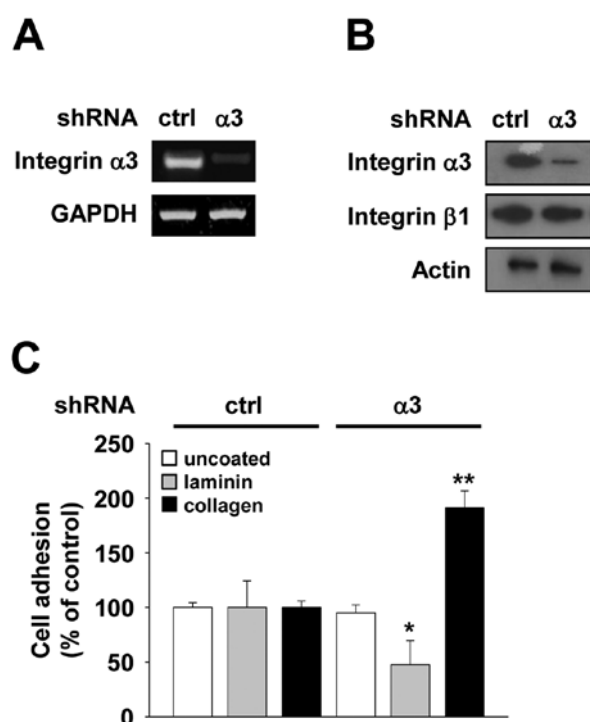
Figure 1. Adhesive behavior of integrin α3-silenced H1299 cells on ECM proteins. The levels of integrin α3 and β1 in H1299 cells were determined by RT-PCR (A) and western blot analysis (B) as described in Materials and methods. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and actin was used as internal control. Results shown are representative of at least three independent experiments. (C) Cells (1.5×10^4 cells/well) were plated on 96-well plates either uncoated or coated with proteins including laminin and collagen. The number of attached cells was determined by counting 10 random fields of cells (x100 field). Complete (100%) attachment of control cells to uncoated, laminin, and collagen type I was 264±11, 84±20, and 364±21, respectively. Cell adhesion results from six independent experiments (mean ± SD) are presented as the fold-increase of control shRNA-transfected cells. Statistical significance is indicated (*$p<0.05$, **$p<0.01$, compared with control cells).

experiments. The results were considered to be statistically significant at $p<0.05$.

Results

Integrin α3 silencing-induced cell proliferation is mediated by enhanced expression and nuclear localization of cyclin-dependent kinases. It has been reported that integrin α3β1 plays the pivotal roles in cell growth and migration (3,6). To investigate the biological roles of integrin α3β1 in p53-deficient NSCLC, H1299 cells were transfected with psiRNA-hH1zeo vector including siRNA-targeting integrin α3 or scrambled sequences (15,18), and the cells were designated α3 shRNA and control shRNA, respectively. RT-PCR and western blot analysis demonstrated that α3 shRNA dramatically suppressed expression of integrin α3 subunit in H1299 cells when compared with control cells transfected with control shRNA, and did not affect β1 subunit expression (Fig. 1A and B). Integrin α3β1 is abundantly expressed on the epithelial cell membranes and is known to be a major receptor for laminin-5 (3). To test whether integrin α3 functions to regulate the degree of cell adhesion, we first examined the adhesion of integrin α3-silenced H1299 cells to extracellular matrix proteins. As expected, reduction in
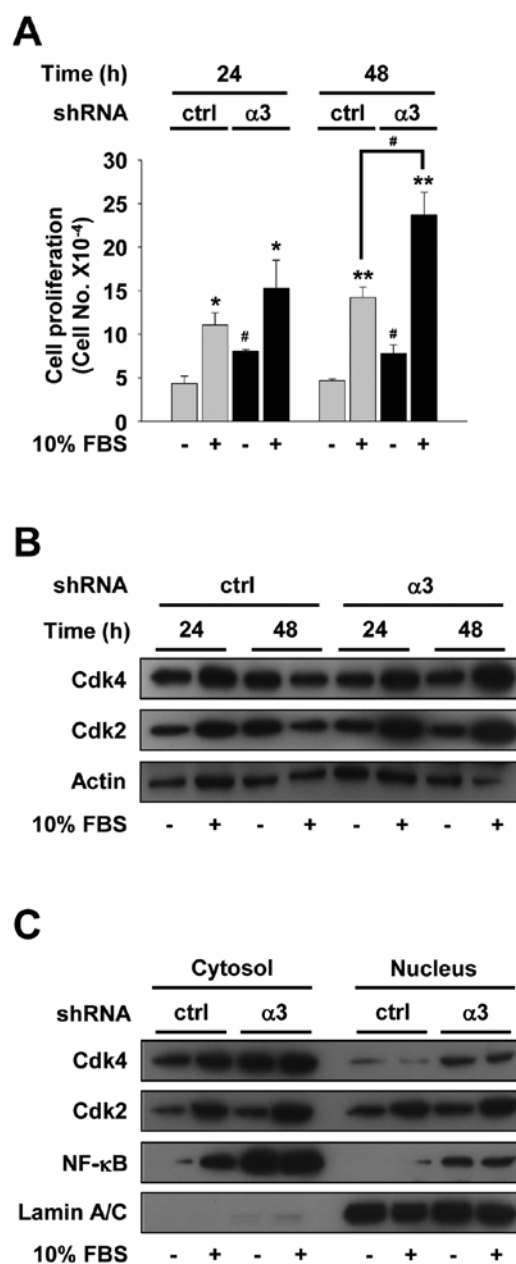

Figure 2. Integrin α3 silencing induces cell proliferation by upregulation and nuclear localization of Cdks and NF-κB. (A) Quiescent cells were treated with or without 10% serum for the indicated time points. Values represent the mean ± SD of three independent experiments. Statistical significance is indicated (*$p<0.05$, **$p<0.01$, compared with unstimulated cells; #$p<0.05$, compared with control shRNA-transfected cells). (B) Cell lysates were western-blotted with anti-Cdk4, anti-Cdk2, or anti-actin antibodies. Results shown are representative of at least three independent experiments. (C) Quiescent cells were stimulated with or without 10% serum for 24 h. The cells were subjected to subcellular fractionation into nuclear and cytosolic extracts as described in Materials and methods. The extracts were western-blotted with anti-Cdk4, anti-Cdk2, anti-NF-κB, or anti-lamin A/C antibodies. Lamin A/C was used as a marker for the nucleus. Results shown are representative of three independent experiments.

integrin α3 subunit expression markedly blocked the adhesion of cells to laminin, while did not alter the adhesion to tissue culture plastic (Fig. 1C). In contrast, integrin α3 silencing resulted in a two-fold increase in cell adhesion to collagen when compared with control shRNA (Fig. 1C), consistent with previous reports demonstrating that collagen receptors are
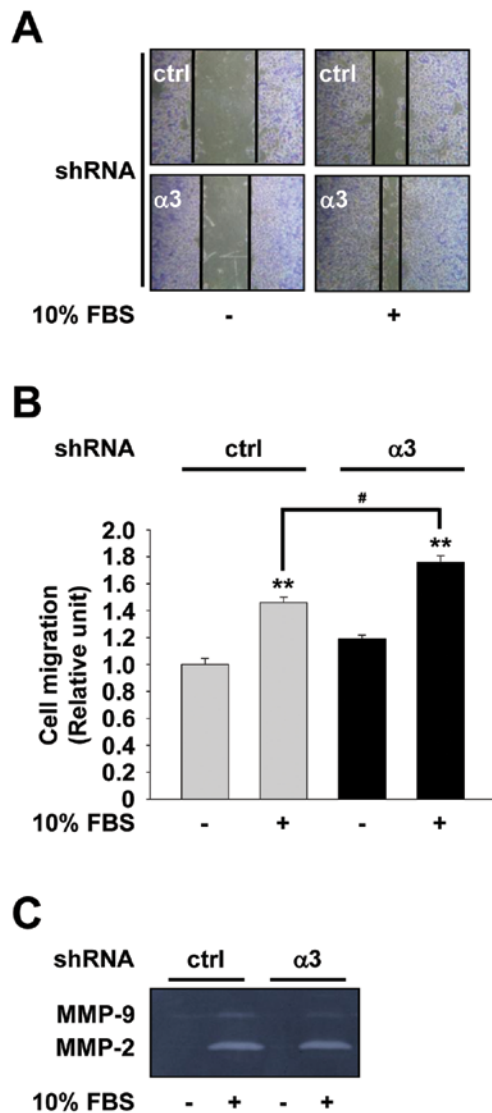
Figure 3. Enhanced migration in integrin α3-silenced cells does not require MMP activity. (A and B) Cell migration was quantified by measuring the migration distance of cells from wound edge as described in Materials and methods. Results from six independent experiments (mean ± SD) were represented as the fold-increase of unstimulated control shRNA-transfected cells. Statistical significance is indicated (**p<0.01, compared with unstimulated cells; #p<0.05, compared with control shRNA-transfected cells). (C) Gelatin zymogram analysis was carried out by use of conditioned media from control and integrin α3-silenced cells. Zymogram gel loading was normalized to total protein concentration. Results are representative of three independent experiments.

more active in integrin α3-deficient mice keratinocytes (20). In addition, microscopic analysis revealed that integrin α3 silencing did not affect morphological changes in H1299 cells (data not shown).

We next examined the effect and molecular mechanisms of integrin α3 silencing on cell proliferation. Control or integrin α3-silenced H1299 cells were serum-starved for 48 h to allow synchronization and growth arrest (G1/G0), and then stimulated with 10% FBS-DMEM for 24 or 48 h. After mitogenic stimulation for 48 h, integrin α3 silencing resulted in a significant increase (>70%) in cell proliferation, as compared with controls (Fig. 2A). Of note, integrin α3 silencing also induced cell proliferation in the absence of mitogenic stimuli. After

serum starvation for 48 h this increase was maintained for additional 48 h culture. These findings suggest that disruption of integrin α3 expression results in induction of cell proliferation and survival, enhancing the aggressiveness of lung cancer and leading to shorter survival of lung cancer patients (8,21). Based on these findings, we next analyzed the changes of cell cycle-related proteins in H1299 cells. It has been reported that cell cycle progression specifically requires activation of cyclin-dependent kinases (Cdks) through formation with cyclins, and is associated with subcellular localization of Cdks from the cytoplasm to nucleus (22). In control shRNA-transfected cells, mitogenic stimulation increased the protein levels of both Cdk4 and Cdk2 for 24 h, while showed little or no change of protein expression under 48 h cell culture conditions, compared with those in unstimulated controls (Fig. 2B). However, integrin α3 silencing dramatically induced the levels of Cdks until the end time point of this experiment. The levels of total Cdks in integrin α3-silenced cells were higher than those in control shRNA-transfected cells. These results are consistent with initial findings that integrin α3 silencing functions to increase cell proliferation (Fig. 2A). To investigate whether integrin α3 silencing mediates induction of cell proliferation through the changes of Cdk localization, Cdks in the nuclear compartments were directly examined by western blot analysis of nuclear and cytosolic extracts of H1299 cells with or without integrin α3 shRNA. As shown in Fig. 2C, reduction in integrin α3 expression markedly enhanced nuclear localization of Cdk4, regardless of mitogenic stimulation. Furthermore, p21[WAF1/Cip1], one of the best known Cdk inhibitors, was not detected in the nuclear compartments of integrin α3-silenced cells without mitogenic stimulation (data not shown). These changes of Cdk4 and p21[WAF1/Cip1] in the nuclear compartments of integrin α3-silenced cells are well correlated with previous observations on cell proliferation (Fig. 2A).

NF-κB is well known as a transcription factor that regulates the expression of a variety of genes in response to inflammation, adhesion, cell cycle progression, survival, and anti-apoptosis (12,13,23). Reduced expression of integrin α3 markedly induced the levels of NF-κB in the cytosolic compartments in both unstimulated and stimulated culture conditions (Fig. 2C). Moreover, nuclear localization patterns of NF-κB by integrin α3 silencing were similar to those of Cdk4. Collectively, these results demonstrate that knockdown of integrin α3 expression induces cell cycle progression and survival through enhanced expression and nuclear localization of Cdks and NF-κB.

Knockdown of integrin α3 enhances cell migration. Cell migration is controlled by the coordination of integrin-mediated interactions from the ECM to the intracellular components of the migrating cells, and plays the important roles in tumor invasion and metastasis (1,2,5). To study the effect of integrin α3 expression on cell migration, we next performed wound-healing assay using H1299 cells transfected with control or integrin α3 shRNA. As shown in Fig. 3A and B, integrin α3-silenced cells readily migrated into the wounded area within 18 h, consistent with previous reports (20). Expression and activation of matrix metalloproteinases (MMPs) are associated with enhanced cell migration and invasion by selective proteolysis of extracellular matrix components (24). Based on integrin α3 silencing-mediated induction of cell migration, we

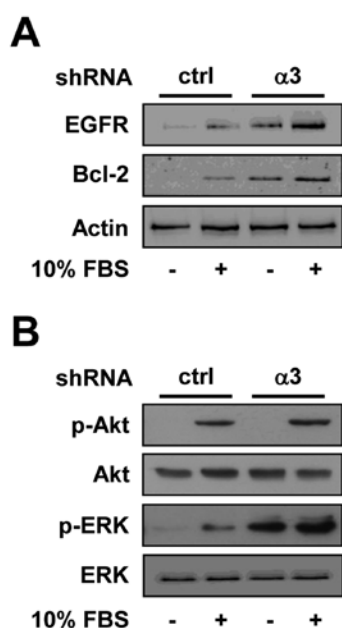Figure 4. Changes of EGFR expression and signaling pathways in integrin α3-silenced cells. (A and B) Quiescent cells were stimulated with or without 10% serum for 24 h. Cell lysates were western-blotted with anti-EGFR, anti-Bcl-2, anti-actin, anti-phospho-Akt, anti-Akt, anti-phospho-ERK, or anti-ERK antibodies. Results shown are representative of three independent experiments.

next analyzed the activities of MMP-2 and MMP-9 in H1299 cells. As shown in Fig. 3C, the conditioned media from cell cultures had high levels of MMP-2 activity relative to those of MMP-9. Knockdown of integrin α3 expression significantly reduced MMP-9 activity, but not MMP-2, similar to previous reports that integrin α3β1 expression is required for the production of MMP-9 in immortalized mouse keratinocytes (25). These observations suggest that integrin α3 silencing-mediated induction of H1299 cell migration may not require the expression and activity of MMPs.

Roles of ERK and Akt activation in integrin α3 silencing-induced cell proliferation and migration. To further investigate the molecular mechanism by which knockdown of integrin α3 expression induces cell proliferation and migration, we analyzed the changes in the expression of EGFR, which is overexpressed or constitutively activated in a variety of human cancers including lung cancer (9-11). As shown in Fig. 4A, the expression of EGFR was induced in integrin α3-silenced H1299 cells, as compared with control cells. Moreover, in integrin α3-silenced cells anti-apoptotic protein Bcl-2 was highly expressed, consistent with previous findings that NF-κB activation results in an increase in anti-apoptotic regulators such as Bcl-2, Bcl-x_L, Bfl-1/A1, and inhibitor of apoptosis (13,26,27). We next examined the changes in activation of PI3K/Akt and ERK, which are key down-stream molecules of EGFR signaling pathways (9,28). As shown in Fig. 4B, integrin α3-silenced cells showed little or no change in phosphorylation status of Akt as compared with control cells at the 24 h time point.

In contrast, activation of ERK in integrin α3-silenced cells was sustained up to 24 h in the absence or presence of mitogenic

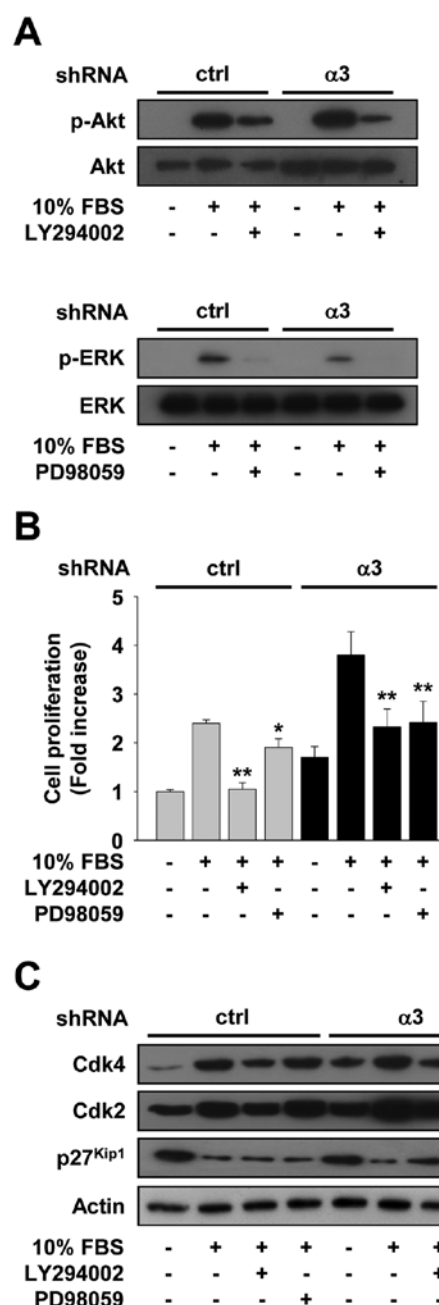Figure 5. Effects of Akt and ERK signaling pathways on the expression of Cdks and proliferation in integrin α3-silenced cells. (A) Quiescent cells were pretreated with LY294002 (10 μM) or PD98059 (25 μM) for 30 min, and then stimulated with 10% serum for 15 min (A) or 24 h (B and C). Cell proliferation and western blot analysis were performed as described in Materials and methods. Cell lysates were western-blotted with anti-phospho-Akt, anti-Akt, anti-phospho-ERK, anti-ERK, anti-Cdk4, anti-Cdk2, anti-p27^{Kip1} or anti-actin antibodies. Results shown are representative of three independent experiments. Statistical significance is indicated (*p<0.05, **p<0.01, compared with stimulated cells).

stimuli, indicating the cell mitogenesis and survival might be mediated through ERK rather than Akt activity. Pretreatment with PD98059, an inhibitor of ERK pathway or LY294002, an inhibitor of PI3K-Akt pathway, significantly inhibited the activation of ERK and Akt, and the proliferation of both control and integrin α3-silenced cells (Fig. 5A and B). Integrin α3-silenced cells are more responsive to PD98059 inhibition of cell proliferation as compared with control cells. Furthermore,
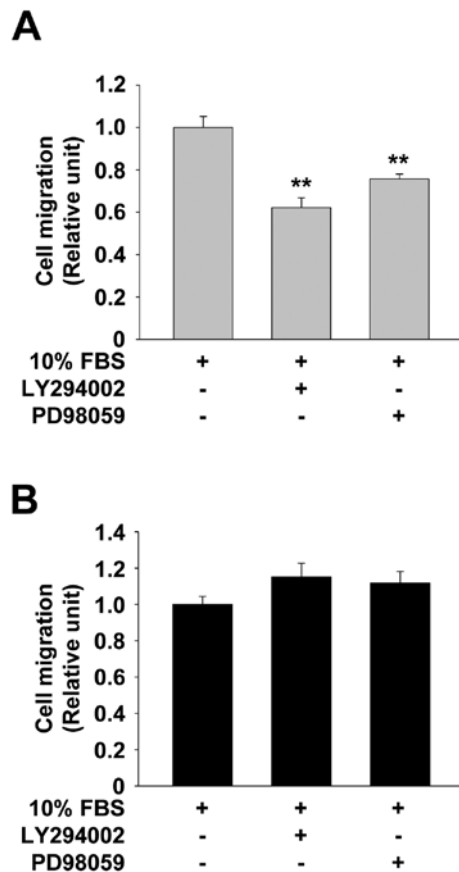
Figure 6. Enhanced migration of integrin α3-silenced cells is mediated by Akt- and ERK-independent mechanism. Quiescent control (A) and integrin α3-silenced (B) cells were pretreated with LY294002 (10 μM) or PD98059 (25 μM) for 30 min, and then stimulated with 10% serum for 18 h. Results from six independent experiments (mean ± SD) were represented as the percentage of maximally induced migration with 10% serum. Statistical significance is indicated (**p<0.01, compared with 10% serum-treated cells).

PD98059 treatment decreased the protein levels of both Cdk4 and Cdk2, and increased a Cdk inhibitor p27^{Kip1} levels in integrin α3-silenced cells, but not in control cells (Fig. 5C), in a good agreement with our findings on cell proliferation (Fig. 5B). Finally, we examined the roles of Akt and ERK in integrin α3 silencing-induced cell migration. Pretreatment with LY294002 or PD98059 significantly reduced the migration of control cells (Fig. 6A). In contrast, in integrin α3-silenced cells neither LY294002 nor PD98059 treatment blocked cell migration (Fig. 6B). Taken together, these observations suggest that knockdown of integrin α3 expression in p53-deficient NSCLC cells induces cell proliferation through Akt- and ERK-dependent pathways, whereas enhanced cell migration is independent of the activation of Akt and ERK.

Discussion

In the present study we demonstrate that reduced expression of integrin α3β1 induces the proliferation and migration of p53-deficient NSCLC cells. In addition, we show that upregulation of EGFR, Cdks, NF-κB, and Bcl-2 and sustained activation of ERK in integrin α3-silenced cells are responsible for the accelerated cell mitogenesis and survival. Our study is the first demonstration that p53 functions to regulate integrin

α3β1-EGFR-mediated signaling pathways and invasive phenotypes of NSCLC cells.

Integrin α3β1 is positively or negatively correlated with cancer invasion and metastasis (6,29). Expression of integrin α3β1 is highly induced in the process of brain metastasis of NSCLC (30). In contrast, reduced expression of integrin α3β1 has been reported to be associated with the highly invasive and metastatic behavior of small cell lung cancer (7) and poor prognosis in patients with NSCLC (8). These conflicting observations may be due to heterogeneity of cancer types and stages as well as differences in cancer microenvironment.

Overexpressed, constitutively activated EGFR, and deregulation of EGFR signaling are closely related to highly aggressive behaviors and therapeutic resistance of lung cancers (10,11), and these events are very complex. Increasing evidence indicates that cross-talk between integrin α3β1 and RTKs plays pivotal roles in angiogenesis and cancer progression (4,31,32). Although potential roles of integrin α3β1 or EGFR in cancer progression have been reported, however, no relationship between integrin α3β1 and EGFR in regulating NSCLC cell proliferation and migration has been clearly investigated to date. A recent study demonstrates that integrin β1 silencing inactivates the EGFR signaling pathways, resulting in suppression of tumorigenic properties of lung cancer cells (33). NF-κB has been reported to be activated by EGFR signaling events and positively associated with breast cancer cell proliferation and anti-apoptosis, indicating linkage between EGFR and NF-κB signaling (34). Mutations of the p53 tumor suppressor gene occur in a high percentage of lung cancer, resulting in cancer progression (35).

Our data show that integrin α3 silencing in p53-deficient H1299 cells induces the expression of EGFR and activation of ERK, and these events appear to be related to induction of Cdks, NF-κB, and Bcl-2, leading to cell proliferation and migration. These findings are correlated with a previous report that loss of p53 can drive NF-κB toward cancer-promoting activity by activation of signaling pathways including Akt and ERK, and induction of anti-apoptotic gene expression, leading to cancer growth, invasion and metastasis (12). In conclusion, our findings demonstrate that alterations in integrin α3β1 expression and subsequent signaling pathways may provide a molecular basis for the coordination of cell proliferation and migration in p53-deficient NSCLC, and suggest that expression of integrin α3β1 and regulation of EGFR-NF-κB signaling pathways result in suppression of invasive phenotypes of lung cancer.

Acknowledgements

References

1. Cox D, Brennan M and Moran N: Integrins as therapeutic targets: lessons and opportunities. Nat Rev Drug Discov 9: 804-820, 2010.
2. Hood JD and Cheresh DA: Role of integrins in cell invasion and migration. Nat Rev Cancer 2: 91-100, 2002.
3. Hynes RO: Integrins: bidirectional, allosteric signaling machines. Cell 110: 673-687, 2002.

4. Eliceiri BP: Integrin and growth factor receptor crosstalk. Circ Res 89: 1104-1110, 2001.
5. Desgrosellier JS and Cheresh DA: Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer 10: 9-22, 2010.
6. Kreidberg JA: Functions of α3β1 integrin. Curr Opin Cell Biol 12: 548-553, 2000.
7. Barr LF, Campbell SE, Bochner BS and Dang CV: Association of the decreased expression of α3β1 integrin with the altered cell: environmental interactions and enhanced soft agar cloning ability of c-myc-overexpressing small cell lung cancer cells. Cancer Res 58: 5537-5545, 1998.
8. Adachi M, Taki T, Huang C, et al: Reduced integrin α3 expression as a factor of poor prognosis of patients with adenocarcinoma of the lung. J Clin Oncol 16: 1060-1067, 1998.
9. Lemmon MA and Schlessinger J: Cell signaling by receptor tyrosine kinases. Cell 141: 1117-1134, 2010.
10. Sharma SV, Bell DW, Settleman J and Haber DA: Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer 7: 169-181, 2007.
11. Hynes NE and Lane HA: ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 5: 341-354, 2005.
12. Perkins ND: The diverse and complex roles of NF-κB subunits in cancer. Nat Rev Cancer 12: 121-132, 2012.
13. Kim YK, Lee EK, Kang JK, et al: Activation of NF-κB by HDAC inhibitor apicidin through Sp1-dependent de novo protein synthesis: its implication for resistance to apoptosis. Cell Death Differ 13: 2033-2041, 2006.
14. Cho Y-R, Choi S and Seo D-W: Sepiapterin regulates cell proliferation and migration: its association with integrin α3β1 and p53 in human lung cancer cells. Genes Genom 33: 577-582, 2011.
15. Seo D-W, Li H, Qu C-K, et al: Shp-1 mediates the antiproliferative activity of tissue inhibitor of metalloproteinase-2 in human microvascular endothelial cells. J Biol Chem 281: 3711-3721, 2006.
16. Kim S, Cho Y-R, Kim M-D, Kim H, Choi S and Seo D-W: Inhibitory effects of sepiapterin on vascular endothelial growth factor-A-induced proliferation and adhesion in human umbilical vein endothelial cells. Arch Pharm Res 34: 1571-1577, 2011.
17. Cho Y-R, Kim SH, Ko HY, Kim M-D, Choi SW and Seo D-W: Sepiapterin inhibits cell proliferation and migration of ovarian cancer cells via down-regulation of p70S6K-dependent VEGFR-2 expression. Oncol Rep 26: 861-867, 2011.
18. Seo D-W, Kim SH, Eom S-H, et al: TIMP-2 disrupts FGF-2-induced downstream signaling pathways. Microvasc Res 76: 145-151, 2008.
19. Hong SY, Cho JY and Seo D-W: Ginsenoside Rp1 inhibits proliferation and migration of human lung cancer cells. Biomol Ther 19: 411-418, 2011.
20. Hodivala-Dilke KM, DiPersio CM, Kreidberg JA and Hynes RO: Novel roles for α3β1 integrin as a regulator of cytoskeletal assembly and as a trans-dominant inhibitor of integrin receptor function in mouse keratinocytes. J Cell Biol 142: 1357-1369, 1998.
21. Gogali A, Charalabopoulos K and Constantopoulos S: Integrin receptors in primary lung cancer. Exp Oncol 26: 106-110, 2004.
22. Sherr CJ: The Pezcoller lecture: cancer cell cycles revisited. Cancer Res 60: 3689-3695, 2000.
23. Mayo MW, Denlinger CE, Broad RM, et al: Ineffectiveness of histone deacetylase inhibitors to induce apoptosis involves the transcriptional activation of NF-κB through the Akt pathway. J Biol Chem 278: 18980-18989, 2003.
24. Stetler-Stevenson WG and Seo D-W: TIMP-2: an endogenous inhibitor of angiogenesis. Trends Mol Med 11: 97-103, 2005.
25. DiPersio CM, Shao M, Di Costanzo L, Kreidberg JA and Hynes RO: Mouse keratinocytes immortalized with large T antigen acquire α3β1 integrin-dependent secretion of MMP-9/gelatinase B. J Cell Sci 113: 2909-2921, 2000.
26. Heckman C, Mehew J and Boxer L: NF-κB activates Bcl-2 expression in t(14;18) lymphoma cells. Oncogene 21: 3898-3908, 2002.
27. Lee HH, Dadgostar H, Cheng Q, Shu J and Cheng G: NF-κB-mediated up-regulation of Bcl-x and Bfl-1/A1 is required for CD40 survival signaling in B lymphocytes. Proc Natl Acad Sci USA 96: 9136-9141, 1999.
28. Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell 144: 646-674, 2011.
29. Stipp CS: Laminin-binding integrins and their tetraspanin partners as potential antimetastatic targets. Expert Rev Mol Med 12: e3, 2010.
30. Yoshimasu T, Sakurai T, Oura S, et al: Increased expression of integrin α3β1 in highly brain metastatic subclone of a human non-small cell lung cancer cell line. Cancer Sci 95: 142-148, 2004.
31. Seo D-W, Li H, Guedez L, et al: TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. Cell 114: 171-180, 2003.
32. Seo D-W, Saxinger WC, Guedez L, Cantelmo AR, Albini A and Stetler-Stevenson WG: An integrin-binding N-terminal peptide region of TIMP-2 retains potent angio-inhibitory and anti-tumorigenic activity in vivo. Peptides 32: 1840-1848, 2011.
33. Morello V, Cabodi S, Sigismund S, et al: β1 integrin controls EGFR signaling and tumorigenic properties of lung cancer cells. Oncogene 30: 4087-4096, 2011.
34. Biswas DK and Iglehart JD: Linkage between EGFR family receptors and nuclear factor kappaB (NF-κB) signaling in breast cancer. J Cell Physiol 209: 645-652, 2006.
35. Fuster JJ, Sanz-González SM, Moll UM and Andrés V: Classic and novel roles of p53: prospects for anticancer therapy. Trends Mol Med 13: 192-199, 2007.