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Targeting ILK and β4 integrin abrogates the invasive potential of ovarian cancer

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ABSTRACT

Integrins and integrin-linked kinase (ILK) are essential to cancerous invasion because they mediate physical interactions with the extracellular matrix, and regulate oncogenic signaling pathways. The purpose of our study is to determine whether deletion of β1 and β4 integrin and ILK, alone or in combination, has antitumoral effects in ovarian cancer. Expression of β1 and β4 integrin and ILK was analyzed by immunohistochemistry in 196 ovarian cancer tissue samples. We assessed the effects of depleting these molecules with shRNAs in ovarian cancer cells by Western blot, conventional RT-PCR, cell proliferation, migration, invasion, and *in vitro* Rac1 activity assays, and *in vivo* xenograft formation assays. Overexpression of β4 integrin and ILK in human ovarian cancer specimens was found to correlate with tumor aggressiveness. Depletion of these targets efficiently suppresses ovarian cancer cell proliferation, migration, and invasion *in vitro* and xenograft tumor formation *in vivo*. We also demonstrated that single depletion of ILK or combination depletion of β4 integrin/ILK inhibits phosphorylation of downstream signaling targets, p-Ser 473 Akt and p-Thr202/Tyr204 Erk1/2, and activation of Rac1, as well as reduce expression of MMP-2 and MMP-9 and increase expression of caspase-3 *in vitro*. In conclusion, targeting β4 integrin combined with ILK can instigate the latent tumorigenic potential and abrogate the invasive potential in ovarian cancer.

1. Introduction

Integrin signaling is regulated by complex interactions with a number of cytosolic proteins, including integrin-linked kinase (ILK). ILK is a ubiquitously expressed protein serine/threonine kinase that was initially discovered through its interactions with the β1 and β3 integrin subunits [1,2]. Although ILK initially named as a kinase, ILK acts as a central component of ILK–PINCH–Parvin complex at ECM adhesions mediating interactions with a large number of proteins via multiple sites including its pseudoactive site [3–6].

Now many studies have reported that ILK plays a role as an adaptor and signaling protein in various aspects of the oncogenic process through direct and indirect mechanisms during tumor progression [7,8]. Recent reports showed that aberrant ILK mediated signaling, due to overexpression or constitutive activation of the protein, leads to pathological alterations that ultimately result in malignant progression in a range of cancers [9,10].

ILK expression is increased in ovarian epithelial cancer relative to benign tumors and normal ovarian epithelium, correlates with increased tumor grade, and is stimulated by soluble factors in peritoneal tumor fluid through the activation of the downstream protein kinase B/Akt pathway [11,12]. In addition, a recent study showed that ILK directly mediated actin cytoskeletal rearrangements and cell migration and invasion through the concerted actions of phosphoinositide 3-kinase (PI3K)/Akt/Rac1 [13].

Meanwhile, β1 integrin expressed on metastatic ovarian cancer cells affects adhesion to the mesothelium. It has been proposed that ovarian cancer metastasis is regulated by β1 integrin binding to the fibronectin secreted by mesothelial cells [14]. More recent studies also demonstrated that high levels of α4β1 and or αvβ3 integrins were closely correlated with increased peritoneal metastasis and tumor proliferation in ovarian cancer, respectively [15,16].

Furthermore, the expression of α6 and β4 integrin subunits in serous ovarian carcinoma correlates with expression of the basement membrane protein laminin. In most solid ovarian tumors, expression of laminin is patchy or absent in the putative basement membrane zone surrounding the nest of epithelial tumor cells. In addition, neither laminin or the α6 or β4 integrin subunits are present on the surface of ovarian carcinoma cells isolated from the ascites fluid of most patients, regardless of whether they are present in the patient's solid tumor. Thus, it is possible that ovarian carcinoma epithelial cells may be released from the basement membrane of the ovary due to their deficit of α6 and β4 integrin

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subunits [17]. However, the role of α6 or β4 integrin subunits in modulating the phenotypic behavior of ovarian carcinoma cells has not been thoroughly investigated and is poorly understood.

Accordingly, we hypothesized that overexpression of ILK and integrin β subunits in highly oncogenic cancer cells is related to ovarian cancer progression. We determined if targeting these molecules has antitumor effects for ovarian cancer. Furthermore, we addressed the question of whether there is a complementary and synergistic advantage when these molecules were targeted alone or in combination in highly oncogenic human ovarian cancer cells.

2. Materials and methods

2.1. Preparation of ovarian cancer patient samples for immunohistochemistry

We examined medical records and archival slides from the collection of ovarian serous adenocarcinoma of the Gynecologic Oncology Files of Yonsei University College of Medicine in Korea. One hundred ninety-six samples of ovarian serous carcinomas were isolated between 1990 and 2003 and used to create tissue microarrays with 2-mm pores in 3.8-cm × 2.2-cm × 0.5-cm frames. The detailed immunohistochemistry procedures and antibody information are provided in Supplementary materials and methods. Immunostaining was graded and scored as follows: 0 signifies no staining; 1+ signifies weak, diffuse staining; and 2+ signifies strong, diffuse staining.

2.2. Cell lines and cell culture conditions

The TOV-112D and OV-90 cell lines were obtained from the American Type Culture Collection and cultured in a 1:1 mixture of MCDB 105 medium (Invitrogen) and Medium 199 (Hyclone) containing 15% fetal bovine serum (FBS; Gibco BRL), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Gibco BRL). The SK-OV-3, MCF-7, and MDA-MB-231 cell lines were obtained from the Korean Cell Line Bank (Seoul, Korea) and cultured in RPMI-1640 medium (Gibco BRL) containing 10% FBS (Gibco BRL), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Gibco BRL).

2.3. shRNA-mediated mRNA depletion

We used the SureSilencing shRNA plasmid for human ITGB1 (KH00650G for the GFP), ITGB4 (KH00680G for the GFP), and ILK (KH00737G for the GFP), and a scrambled sequence negative control plasmid (SABiosciences). The shRNA target sequences are listed in Supplementary Table S1. The cells were seeded and transfected using the Attractene Transfection Reagent (QIAGEN) according to the manufacturer's protocol. At 24, 48, 72, and 96 h after transfection with the shRNA plasmids, the cells were harvested and total RNA was extracted using an RNeasy Protect Mini Kit (QIAGEN). The SuperScript III Reverse Transcriptase kit (Invitrogen) was used to synthesize cDNA. Polymerase chain reactions (PCRs) were performed with HotStarTaq DNA polymerase (QIAGEN). Primers, product sizes, and PCR conditions are listed in Supplementary Table S2.

2.4. Western blot analysis

Equal amounts of cell extract were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Invitrogen). Blots were blocked with 5% non-fat dry milk freshly dissolved in 1× phosphate buffered saline with Tween 20 (PBS-T), and incubated for 1 h at room temperature with primary antibodies (provided in Supplementary materials and methods). The blots were probed with an enzyme (horseradish peroxidase)-linked secondary antibody in 1× PBS-T (1:1500–100,000) for 1 h at room temperature. Finally, chemiluminescent detection reagents were used to visualize the results. Western blot signal was analyzed by an image analysis program (Multi Gauge V3.0, FUJIFILM).

2.5. Flow cytometric analysis

Antibodies were added at the appropriate dilutions and incubated for 20 min on ice in the dark. Antibody information is provided in Supplementary materials and methods. Samples were analyzed on a BD FACSAria™ cell sorter (BD Biosciences).

2.6. Rac1 activity assay

Rac1 activation assays were performed 96 h after shRNA transfection using a Rac1 G-LISA™ Activation Assay kit (Cytoskeleton, Inc.) according to the manufacturer's instructions. We measured the level of active, GTP-loaded Rac1 protein in cell lysates by absorbance at a wavelength of 490 nm using a VERSAmax microplate reader (Molecular Devices).

2.7. Cell proliferation and viability analysis

Cell proliferation was measured 96 h after shRNA transfection using a Cell Counting Kit-8 (Dojindo Laboratories) according to the manufacturer's instructions. We measured sample absorbance at a wavelength of 450 nm using a VERSAmax microplate reader (Molecular Devices).

2.8. Cell migration and invasion assay

Cell migration and invasion were analyzed using an Oris™ cell migration and invasion assay kit (Platypus Technologies), following the manufacturer's instructions. The detailed procedures are described in Supplementary materials and methods. The migrated and invaded cells were stained with Calcein AM and detected with a Victor™ X5 Multilabel Plate Reader (PerkinElmer Life and Analytical Sciences) using 485/528 nm excitation/emission filters.

2.9. Xenograft tumorigenicity assay

Nude mice were purchased from Central Lab Animal, Inc. (Seoul, Korea) and maintained in accordance with the institutional guidelines of Yonsei University College of Medicine. All animal studies were performed according to approved experimental protocols. Tumor cells (1×10^6 cells in 0.2 ml PBS) were injected subcutaneously in the dorsal flank of 6-week-old female nude mice. Tumors were measured every 4 days. Tumor volume was calculated with an index of the growth rate using the following equation: volume = (width + length)/2 × width × length × 0.5236. The mice were sacrificed 60 days after inoculation of the cells, and metastatic lesions on the lungs and livers were counted macroscopically.

2.10. Statistical analysis

Student's *t*-test was used, and results were considered statistically significant if the null hypothesis was rejected with a *P*-value <0.05.

3. Results

3.1. ILK and β4 integrin are expressed in highly oncogenic human ovarian cancer cells

We evaluated the expression level of β1, β3, and β4 integrin subunits and ILK in 3 epithelial ovarian cancer cell lines of different histopathological types by Western blot analysis. We showed that β1 integrin was highly expressed in all cell lines, whereas β3 and β4 integrins and ILK were differentially expressed (Fig. 1A-a, *left panel*). Integrin subunit β3 was highly expressed in TOV-112D cell. In particular, ILK and β4 integrin were highly expressed in the highly oncogenic and invasive cancer cell line, SK-OV-3. ILK ($P < 0.05$) and β4 integrin ($P < 0.01$) expression was also significantly augmented in SK-OV-3 cells when compared with the other ovarian cancer lines, whereas β1 and β3 integrin expression was not (Fig. 1A-a, *right panel*).

In flow cytometric analysis, subunit β1 was moderately or highly expressed in most ovarian cancer cell lines, with the positive population comprising 85–99% of the total (Fig. 1A-b). In contrast, β3 integrin expression was low, with expression detected in only 0.5–8.2% of the cells of the ovarian cancer cell lines. In particular, β4 integrin was highly expressed in the SK-OV-3 cells (75.6% of which were positive for the integrin subunit), while it was expressed by only a small proportion (1.3–13.1%) of the cells of the other ovarian.

3.2. Expression of ILK and β4 integrin are related to Rac1 and Akt activation in highly oncogenic human ovarian cancer cells

To investigate Rac1 activity in 3 ovarian cancer cell lines, we performed Rac1 activity assay. Rac1 activity in SK-OV-3 cells was significantly higher than the other ovarian cancer cell lines (Fig. 1B-a; $P < 0.05$). In Western blot analysis, total Akt and p-Ser473 Akt expression were also highly expressed in SK-OV-3 cells (Fig. 1B-b). Furthermore, Rac1 activity was very similar to total Akt and p-Ser473 Akt expression in 3 ovarian cancer cell lines.

3.3. Expression of ILK and β4 integrin are related to the expression of cell migration- and invasion-related molecules in highly oncogenic human ovarian cancer cells

To investigate the expression of cell migration- and invasion-related molecules in 3 ovarian cancer cell lines, we evaluated expression level of Cdc42, RhoA, MMP-2, and MMP-9. Using Western blot analysis, we confirmed that SK-OV-3 cells expressed these

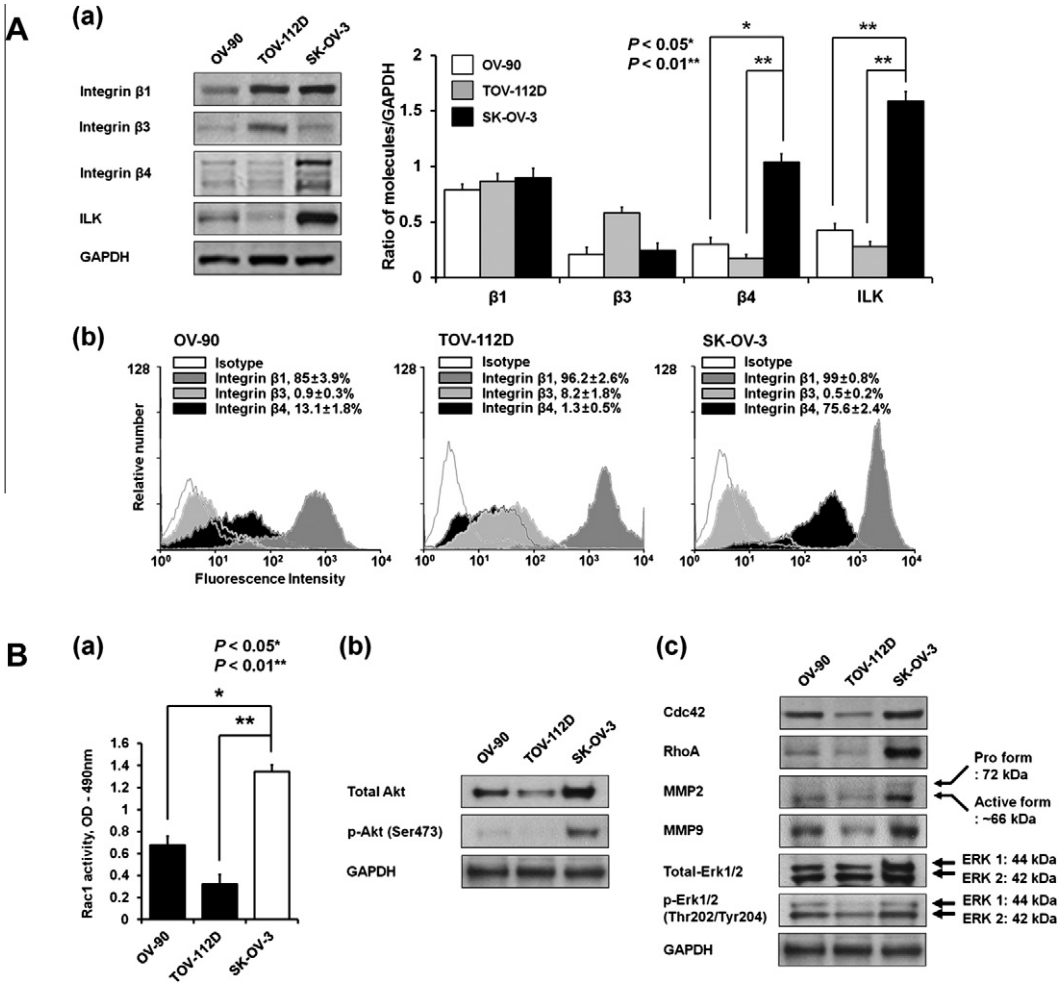

Fig. 1. Expression of cell survival signaling, migration, invasion, and proliferation-related molecules according to expression of ILK and integrin β subunits in ovarian cancer cell lines. (A) Expression of ILK and integrin β subunits in 3 ovarian cell lines. A-a: Protein expression levels of ILK and integrin β subunits (*left panel*). Relative expression levels are graphically represented (*right panel*), with GAPDH serving as a loading control. A-b: Analysis of expression levels of β1, β3, and β4 integrins in ovarian cancer cell lines by flow cytometry. Data are represented as mean ± SD of 3 independent experiments performed in triplicate. (B) Expression of cell survival, migration, invasion, and proliferation-related molecules in 3 ovarian cell lines. B-a: Rac1 activity. B-b: Expression of total Akt and p-Ser473 Akt. B-c: Expression of Cdc42, RhoA, MMP-2, MMP-9, total Erk1/2, and p-Thr202/Tyr204 Erk1/2. Columns, mean of 3 independent experiments; error bars, standard deviation (SD). $^{*}P < 0.05$, and $^{**}P < 0.01$, compared with SK-OV-3; *P*-values calculated using Student's *t*-test.

molecules at a clearly higher level than the other cell lines (Fig. 1B-c). Moreover, the expression pattern of these molecules was very similar to ILK and β4 integrin expression in SK-OV-3 cells (Fig. 1A).

3.4. ILK and β4 integrin were highly elevated in human ovarian cancer specimens

We performed immunohistochemistry to investigate the expression levels of β1 and β4 integrins and ILK in tissue microarrays, which consisted of 196 samples of human ovarian serous carcinomas. The percentage of ILK-positive samples at 1+ and 2+ levels was higher than those for β1 or β4 integrin (Supplementary Table S3). The percentages of tumors staining positively for each protein at 1+ and 2+ levels were as follows: integrin β1 (9.18%), integrin β4 (23.47%), and ILK (33.67%). The percentage of ILK-positive samples at the 2+ level (23.47%) was also higher than those for β1 (1.02%) or β4 integrin (2.55%). Meanwhile, the percentage of β4 integrin-positive samples (20.92%) at the 1+ level was higher than β1 integrin (8.16%) or ILK (10.20%).

Integrin subunit β1 staining was often highly positive along the mesenchymal cell membranes predominantly around the fibrovascular core, instead of the tumor cell borders, in serous carcinoma (Fig. 2A). Staining was intense along the basement membrane between tumor cells and stroma (Fig. 2B) and frequently noted in metastatic tumor node cells (Fig. 2C). ILK was highly expressed in serous carcinoma (Fig. 2D) and present in both the cytoplasmic membrane and cytoplasm of tumor cells (Fig. 2F). It was also overexpressed along the invading front margins of tumor cells (Fig. 2E). Integrin subunit β4 was overexpressed (Fig. 2G) and accentuated in the infiltrative nests and margins of tumor nests (Fig. 1H). It was also detected in lymph node metastases without exception (Fig. 2I). None of the tissues were positive for staining with IgG1 isotype control.

3.5. Effect of ILK and integrin β4 depletion by shRNA on cell migration and invasion in human ovarian cancer cell line SK-OV-3

We measured the depletion efficiency of shRNA plasmids, alone or in combination, and confirmed that each molecule was effectively depleted by more than 80% when compared with mock or negative control shRNA plasmid-transfected cells (Supplementary Fig. S1).

To evaluate the direct effect of β1 and β4 integrin and ILK shRNA-mediated depletion, alone or in combination, on cell migration and invasion process, we performed cell migration and cell invasion assays in SK-OV-3 cells. The data showed that the effects of silencing these targets alone or in combination with shRNAs on cell migration and invasion were very similar. The most effective combination treatment consisted of the depletion of β4 integrin/ILK, which decreased cell migration (Fig. 3A-a; Supplementary Fig. S2) and invasion (Fig. 3B-a) by 71.4% and 67.6%, respectively, compared with negative control shRNA-transfected cells ($P < 0.05$). The most effective single treatment consisted of the depletion of ILK, which decreased cell migration (Fig. 3A-a; Supplementary Fig. S2) and invasion (Fig. 3B-a) by 54.3% and 54.8%, respectively, compared with negative control shRNA-transfected cells ($P < 0.05$). In addition, of the combination treatments, only depletion of β4 integrin/ILK produced statistically significant differences in cell migration ($P = 0.034$) and invasion ($P = 0.028$) when compared with ILK shRNA single depletion.

3.6. Effect of ILK and β4 integrin depletion by shRNA on cell migration- and invasion-related molecules in human ovarian cancer cell line SK-OV-3

We performed Western blot analysis to evaluate the effect of shRNAs, alone or in combination, on cell migration- and

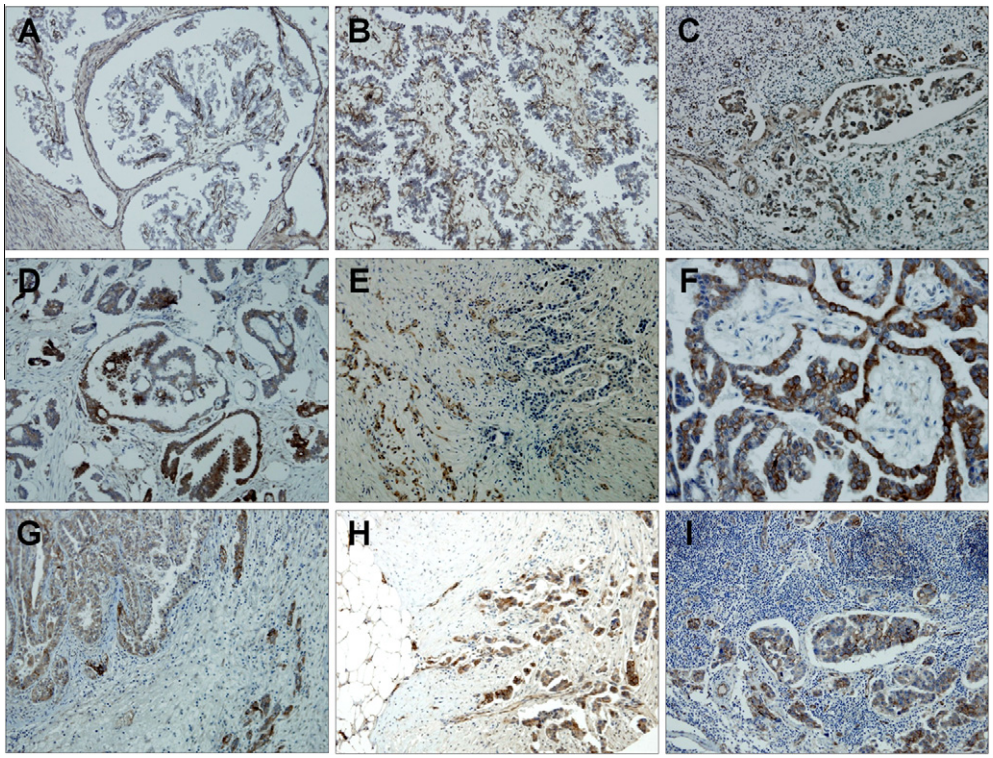

Fig.2. Immunohistochemistry of β1 and β4 integrin and ILK in human ovarian tumors. (A–C) Integrin subunit β1 in ovarian cancer, A: staining was mainly observed along the fibrovascular core attaching to the tumor cells, B: staining was intense along the basement membrane between tumor cells and stroma, C: notice β1 integrin positive carcinoma in the metastatic node. (D–F) ILK in ovarian cancer, D: ILK was detected in the tumor cytoplasm, E: staining along the edge of the invasive front, (F) ILK was localized to the cytosol and cytoplasmic membrane of tumor cells. (G–I) Integrin subunit β4 in ovarian cancer, G: staining was intense at the edges of nests and alongside infiltrative cords. H: Staining was strong in the infiltrative nests and cords. I: Integrin β4 was detected in the metastatic foci.

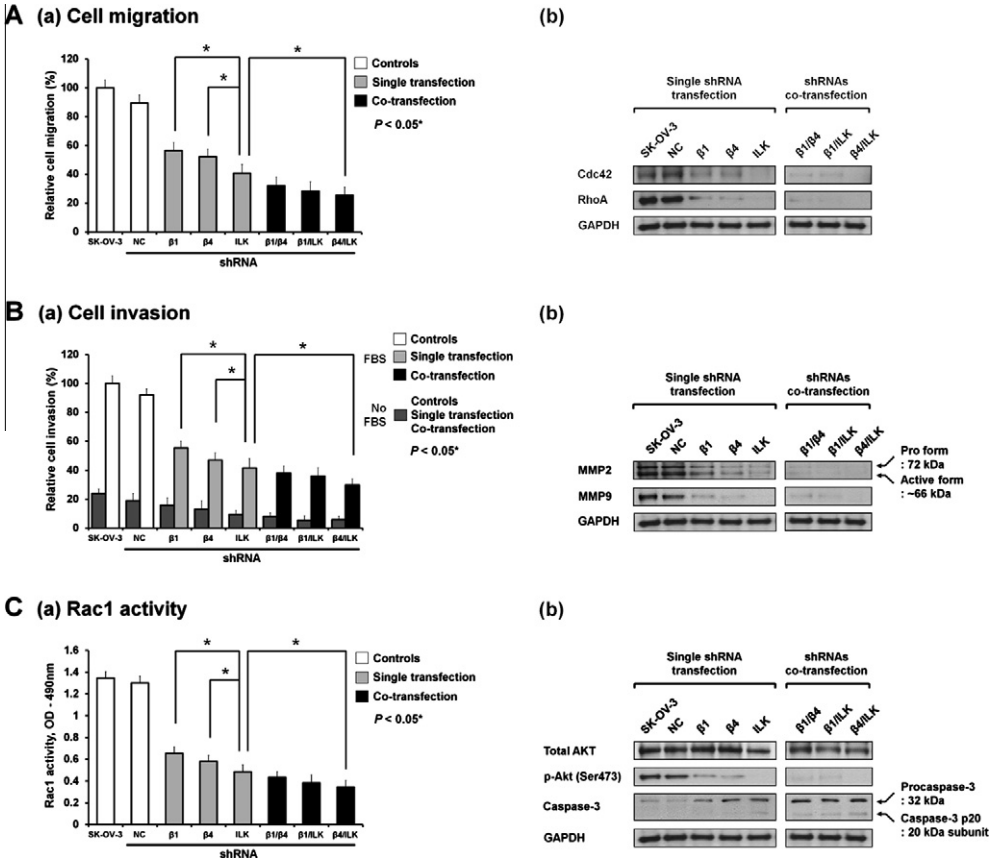

Fig. 3. Effect of shRNA-mediated depletion of β1 and β4 integrins and ILK, alone or in combination, on cell migration, invasion, and *in vitro* Rac1 activity. (A) Effects on cell migration. A-a: Cell migration assays. Cells were trypsinized and resuspended in culture medium at 48 h post-transfection. Cell migration assays were carried out 24 h after plating. A-b: Expression of Cdc42 and RhoA was examined 72 h after transfection. (B) Effects on cell invasion. B-a: Cell invasion assays. Cells were trypsinized and resuspended in culture medium at 48 h post-transfection. Cell invasion assays were carried out 48 h after plating. B-b: Expression of MMP-2 and MMP-9 was examined 96 h after transfection. (C) Effects on Rac1 activity. A-a: Rac1 activity assays performed 96 h after transfection. B-b: Expression of Akt, p-Ser473 Akt, and caspase-3 was examined 96 h after transfection. GAPDH served as a loading control. Columns, means from three independent experiments, each performed in triplicate; error bars, SD. $^{*}P < 0.05$, and $^{**}P < 0.01$, compared with ILK shRNA; *P*-values calculated using Student's *t*-test.

invasion-related molecules in SK-OV-3 cells. The shRNA treatment-induced reduction of expression of the cell migration-related molecules, Cdc42 and RhoA (Fig 3A-b), and the cell invasion-related molecules, MMP-2 and MMP-9 (Fig. 3B-b), showed a very similar pattern to the results for cell migration (Fig. 3A-a) and invasion assays (Fig. 3B-a). The most effective treatment was combination depletion of β4 integrin/ILK, and the most effective single depletion treatment was that of ILK. However, there were no differences between the effects of ILK depletion and the other combination treatments on cell migration- and invasion-related molecules.

3.7. Effect of ILK and β4 integrin depletion by shRNA on PI3K/Akt/Rac1 cascade in human ovarian cancer cell line SK-OV-3

To investigate the effect of shRNAs on Rac1 activity, we performed a Rac1 activity assay. The inhibition of Rac1 activity by shRNA showed a very similar pattern to the results for cell migration (Fig. 3A-a) and invasion assays (Fig. 3B-a). The most effective treatment consisted of combination depletion of β4 integrin/ILK, which decreased Rac1 activity by 73.5%, compared with negative control shRNA transfection. The most effective single depletion treatment was that of ILK, which decreased Rac1 activity by 62.7%, compared with negative control shRNA transfection (Fig. 3C-a; $P < 0.05$). In addition, of the combination depletions, only β4 integrin/ILK depletion reduced Rac1 activity statistically significantly more than ILK shRNA single depletion treatment ($P = 0.037$).

3.8. Effect of ILK and β4 integrin depletion by shRNA on Akt and caspase-3 activation in human ovarian cancer cell line SK-OV-3

Using Western blot analysis, the data showed that all shRNA treatments effectively decreased p-Ser473 Akt, which activates the PI3K/Akt pathway, compared with mock or negative control shRNA plasmid transfection, in SK-OV-3 cells, whereas total Akt levels were not affected by shRNA treatment (Fig. 3C-b). Meanwhile, inhibition of p-Ser473 Akt showed a very similar pattern to cell migration- and invasion-related molecules (Fig. 3A-b and B-b). In addition, down-regulation of ILK and β4 integrin enhanced apoptotic signaling through the activation of caspase-3. Therefore, the inhibition of ILK and β4 integrin leads to activation of caspase-3 and inhibition of p-Ser473 Akt. Consequently, it is possible to cause cell apoptosis by enhancing apoptotic signaling by blocking the PI3K/Akt/Rac1 cascade in ovarian cancer cells (Fig. 3C-b).

3.9. Effect of ILK and integrin β4 depletion by shRNA on cell proliferation in human ovarian cancer cell line SK-OV-3

We performed cell proliferation assays and observed a significant reduction in proliferation of shRNA-transfected cells. The reduction of proliferation by single shRNA treatment showed a very similar pattern to the results for cell migration (Fig. 3A-a) and invasion assays (Fig. 3B-a), whereas the reduction pattern of combination treatments differed slightly from the single treatments (Fig. 4A-a). The most effective treatment consisted of

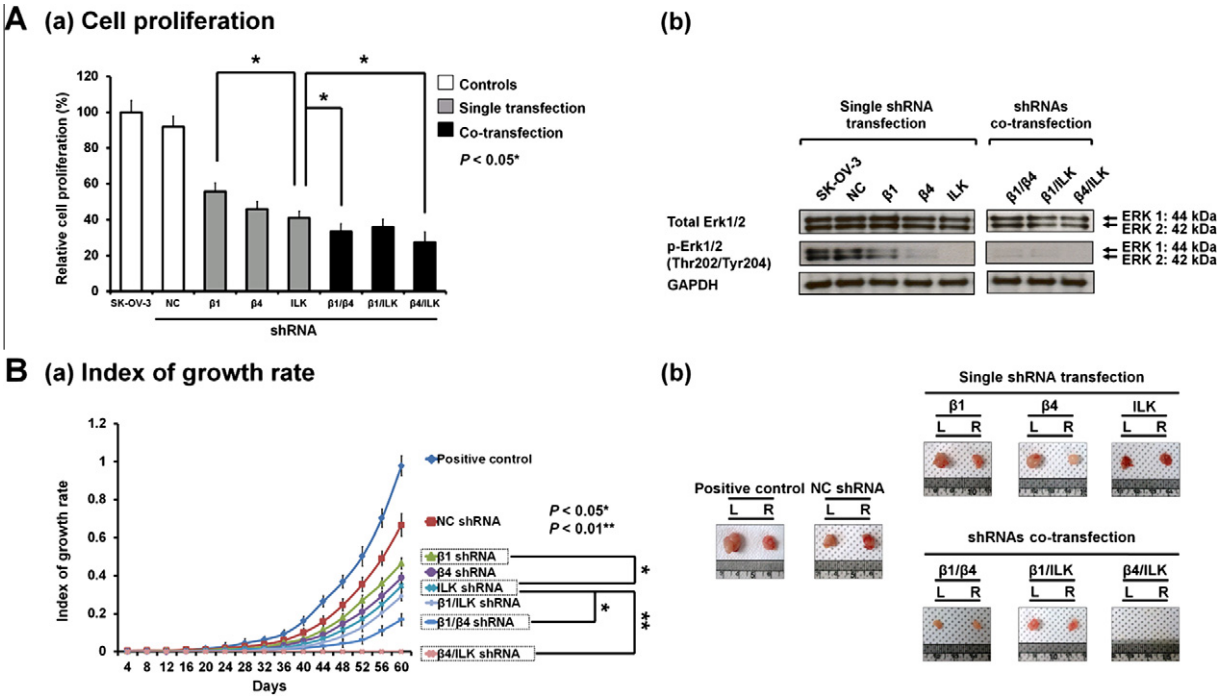

Fig. 4. Effect of shRNA-mediated depletion of β1 and β4 integrins and ILK, alone or in combination, on *in vitro* cell proliferation and *in vivo* growth of ovarian cancer xenografts in nude mice. (A) Effects on cell proliferation. A-a: Cell proliferation assays performed at 96 h after transfection. A-b: Expression of Erk1/2 and p-Thr202/Tyr204 Erk1/2 was examined 96 h after transfection. GAPDH served as a loading control. (B) Xenograft tumorigenicity assay. B-a: Index of the growth rate. Cells (1×10^6 per mouse) were injected subcutaneously in both sides of the dorsal flanks of female nude mice 48 h after transfection. Tumors were measured every 4 days for 60 days. B-b: Images of the tumors excised from the nude mice subcutaneously injected with control or shRNA-transfected cells. The mice were sacrificed 60 days after inoculation of the cells. Columns, means from three independent experiments, each performed in triplicate; error bars, SD. $^{*}P < 0.05$, and $^{**}P < 0.01$, compared with ILK shRNA; *P*-values calculated using Student's *t*-test.

combination depletion of β4 integrin/ILK, which decreased cell proliferation by 70.1%, compared with negative control shRNA transfection. The most effective single depletion treatment was that of ILK, which decreased cell proliferation by 55.3%, compared with negative control shRNA transfection (Fig. 4A-a; $P < 0.05$). Meanwhile, single depletion of β1 integrin ($P = 0.025$) and combination depletions of β1/β4 integrin ($P = 0.023$) and β4 integrin/ILK ($P = 0.037$) each reduced cell proliferation statistically significantly more than ILK shRNA single depletion, whereas other treatments did not.

3.10. Effect of ILK and integrin β4 depletion by shRNA on Erk1/2 activation in human ovarian cancer cell line SK-OV-3

By Western blot analysis, we showed that all shRNA treatments effectively decreased p-Thr202/Tyr204 Erk1/2 expression compared with mock or negative control shRNA plasmid transfection in SK-OV-3 cells, whereas total Erk1/2 expression was not affected by shRNA treatment (Fig. 4A-b). The inhibition pattern of p-Thr202/Tyr204 Erk1/2 followed a very similar pattern to the inhibition of cell proliferation (Fig. 4A-a). The most effective treatment consisted of combination depletion of β4 integrin/ILK, and the most effective single depletion treatment was that of ILK.

3.11. In vivo xenograft model of human ovarian cancer cells

By monitoring the tumor growth rate of each group of xenografts for 60 days, we concluded that down-regulation of β4 integrin and ILK strongly suppressed tumorigenesis in nude mice (Fig. 4B-a; Supplementary Table S4 and Fig. S3). Gross tumor size was also lower in those mice that received combination treatment compared with those that received shRNA control treatment (Fig. 4B-b). In addition, these results were consistent with the result of cell proliferation assays (Fig. 4B-a). Particularly, tumor growth rate after single depletion of β1 integrin ($P = 0.019$) and combination depletions of β1/β4 integrin ($P = 0.022$) and β4 integrin/ILK ($P = 0.0058$) caused statistically significantly augmented inhibition of cell proliferation, compared with ILK shRNA single depletion treatment (Fig. 4B-b).

4. Discussion

We investigated the potential of targeting integrins and ILK as combination therapy for highly aggressive ovarian cancer. Our data showed that targeting ILK alone and ILK in combination with β4 integrin effectively inhibited the PI3K/Akt/Rac1 cascade in invasive human ovarian cancer cell line SK-OV-3 by blocking the activation of Akt and Rac1 in *in vitro* and *in vivo* assays. In particular, our data discloses for the first time an unanticipated role and a synergistic effect for the combination of ILK with β4 integrin as an anticancer target in ovarian cancer, which elevates this integrin from candidate to culprit status in tumor progression. Therefore, implicit in these findings is the idea that combined targeting of ILK/β4 integrin in the oncogenic process adds therapeutic value to experimental approaches aimed at interfering with cancer growth and progression in solid tumors. Future studies must elucidate the factors responsible for tumor susceptibility to these inhibitors. Furthermore, a further elucidation of the mechanisms by β4 integrin expression will help in our understanding of the invasion and metastasis of ovarian cancer cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.09.114.

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