Metastatic effect of LY-6K gene in breast cancer cells

SUH HEE CHOI*, HYUN KYUNG KONG*, SO YOUNG PARK and JONG HOON PARK

Department of Biological Science, Sookmyung Women's University, 52 Hyochangwonro, Yongsan-gu, Seoul 140-742, Korea

Received April 2, 2009; Accepted June 12, 2009

DOI: 10.3892/ijo_00000371

Abstract. The over-expression of urokinase receptor in breast cancer cells results in increased tumor invasion, growth and metastasis. As LY-6K belongs to the Ly6/uPAR superfamily, we assessed the association of LY-6K with metastasis in breast cancer cell lines and how the LY-6K gene mediates cancer cell metastasis. The migration ability of cells in which expression of LY-6K gene is up- or down-regulated was investigated by transfecting LY-6K naked DNA and treating LY-6K siRNA. Assays of wound healing, migration and invasion were performed. The assays demonstrated that cell migration significantly increased when LY-6K gene is over-expressed or LY-6K protein is abnormally expressed in breast cancer cells. In addition, LY-6K caused cancer cell metastasis and it is linked to the Ras/ERK signaling pathway. Our results suggest that LY-6K meaningfully participates in breast cancer cell metastasis by influencing cell migration and invasion through the Ras/ERK pathway.

Introduction

The Ly-6/urokinase receptor (uPAR) superfamily belongs to groups of lymphocyte antigens that have a C-terminal consensus sequence motif CCXXXXCN and several repeats of the Ly-6/uPAR domain that exhibit a distinct 8 or 10 disulfide bonding pattern between cysteine residues (1,2). This superfamily can be classified into two subfamilies based on the presence or absence of a glycosylphosphatidylinositol (GPI)-anchoring signal sequence (3). GPI-anchored Ly-6/uPAR receptor proteins include retinoic acid-induced gene E (RIG-E, or human Ly-6E), E48 antigen (human Ly-6D), prostate stem cell antigen (PSCA), CD59 or protectin, lynx1 and uPAR (4,5). The urokinase-type plasminogen activator receptor associates dynamically with integrins and initiates signaling events that alter cell adhesion, migration, proliferation and differentiation (6). Lymphocyte antigen 6 complex, locus K (LY-6K) as a novel gene is a typical family member with conserved Ly-6 features. The LY-6K gene is located on chromosome 8q24 and, like other Ly-6 antigens, has 10 cysteine residues in a consensus motif; the gene theoretically harbors the sequence that determines GPI anchoring. Recently, the LY-6K gene was proposed as a potential target antigen for the diagnosis and therapy of head and neck squamous cell carcinoma (HNSCC), although expression of LY-6K in HNSCC is lower (7). Moreover, the LY-6K gene expression is prolific in human breast cancer tissue, and so may represent a novel breast cancer marker (8).

Invasion and metastasis require proteolytic degradation of surrounding matrices. The plasminogen activator/plasmin system and matrix metalloproteinases (MMPs) are involved in this process of various breast cancers (9). The generation of pericellular plasmin by uPA in tumor cells may directly contribute to plasmin-mediated proteolysis or indirectly lead to activation of other proteinases that are capable of extracellular matrix breakdown. Both plasminogen and uPA bind to specific cell-surface receptors, and can help migrating cells to degrade tissue barriers during normal and pathological processes (10). uPAR is primarily responsible for the initiation of the plasminogen activator/plasmin cascade, playing a decisive role in the activation of plasminogen into plasmin. uPA/uPAR are over-expressed in breast cancer and its high levels correlate with poor prognosis in breast cancer patients (11-13). In addition, the activation of Ras and its downstream signal-controlled kinase-activating protein kinase (extracellular signal-regulated kinase/mitogen activated protein kinase; ERK/MARK) pathway play an important role in integrin ligation (14). Furthermore, integins complexed with uPAR may activate the Ras/ERK pathway to control invasion in cancer cells. Recently, it was demonstrated that uPA/PAR and integrin enhance adhesive and invasive capabilities through the Ras/ERK signaling pathway (2).

Both LY-6K and uPAR are included in the LY-6/uPAR superfamily. Focusing on this relationship, we investigated whether the over-expression of LY-6K in non-invasive cells would have an effect on cell migration in breast cancer cells by uPAR-mediated metastasis. In addition, we sought to confirm whether the invasive signal was an integrin-related Ras/ERK pathway.

Materials and methods

DNA manipulation. The bacterial artificial chromosome clone RPII-907C16 harboring the LY-6K gene was purchased from the BACPAC Resources Center (http://bacpac.chori.org). For

Correspondence to: Dr Jong Hoon Park, Department of Biological Science, Sookmyung Women's University, 52 Hyochangwonro, Yongsan-gu, Seoul 140-742, Korea
E-mail: parkjh@sookmyung.ac.kr

*Contributed equally

Key words: LY-6K, breast cancer, metastasis, Ras/ERK pathway

transfection, the LY-6K full coding sequence was inserted into pEGFP-N1 (Clontech, Mountain View, CA). The LY-6K gene was amplified using primers (5'-GGAATTCAGATGA GGCTCCAAAGACCCCGA-3' and 5'-TGCGGTCGACTCA AGACAGGCTGAGGCCGG-3'). This polymerase chain reaction (PCR) product containing the complete open reading frame of the LY-6K gene with *Eco*RI and *Sal*I linkers was purified and inserted into the *Eco*RI/*Sal*I restriction enzyme sites of pEGFP-N1. For RNA interference (RNAi) of LY-6K, each 100 pmol of LY-6K small interfering RNA (siRNA; siLY-6K) and negative control siRNA purchased from Bioneer (Daejeon, Korea) were used.

Cell culture and transfection. MCF7 and MCF7-ADR cells (Roswell Park Cancer Institute, Buffalo) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS). MCF7 cell is a human breast adenocarcinoma cell line and MCF7-ADR is a multidrug resistant (MDR) human breast cancer MCF7 subline that possesses adriamycin resistance (15). HCC1937 BL cells, in the peripheral blood of breast cancer patients (American Type Culture Collection, Manassas, VA) were cultured in RPMI-1640 supplemented with 10% FBS. The cells were also treated with 50 μM PD98059 (Calbiochem, San Diego, CA) and 5 μM GW5074 (Santa Cruz Biotechnology, Santa Cruz, CA). The day before transfection, MCF7 and MCF7-ADR cells were plated at a density of 1-2x10^6 cells in 100-mm diameter culture plates. Transient transfection of LY-6K and LY-6K siRNA was performed on cells grown to 50-80% confluency using RNAiMax (Invitrogen, Carlsbad, CA) and Fugene HD (Roche, Basel, Switzerland) according to the manufacturer's recommendations.

Semi-quantitative reverse transcription-PCR (RT-PCR). Total RNAs were extracted using TRizol reagent (Invitrogen) from several breast cancer cell lines as well as normal HCC1937BL cells. The 18S rRNA gene (5'-GTAACCCGTTGAACCCC ATT-3' and 5'-CCACCAATCGGTAGTAGCG-3') was used as a positive control. Residues 106-556 of the LY-6K coding sequence was amplified using the primers 5'-CTGCGAAGG TTCCAGAAGG-3' and TAGGTGGCCCCTCTAATTG-3'. For LY-6K PCR, the initial denaturing phase of 5 min at 95˚C was followed by a 30-cycle amplification phase including denaturation at 95˚C for 1 min, annealing at 60˚C for 1 min and elongation at 72˚C for 1 min. Amplification was terminated at 72˚C for 5 min. The PCR conditions for 18S rRNA were the same as those for LY-6K.

Immunoblot analysis. MCF7 cells were lysed in 25 mM Tris-HCl (pH 7.5) containing 150 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 2% glycerol, 1% NP-40, and protease inhibitor cocktail (lysis buffer). Cell lysates were clarified by centrifugation at 14,000 x g and 4˚C for 20 min, and the supernatants were incubated with the antibody of interest. LY6K antibody is purchased from Novus Biological (Littleton, CO). ERK, p-ERK, MEK, p-MEK, Raf-1, p-Raf-1, MMP2 and MMP9 antibodies were purchased from Cell Signaling Technology (Danvers, MA). Horseradish peroxidase-conjugated goat anti-rabbit antibodies and goat anti-mouse antibodies (both from Chemicon) that were both diluted 1:5000 served as secondary antibodies. The analyzed protein levels were normalized by detecting the expression level of ß-actin (Santa Cruz Biotechnology) and immunoblots were visualized by enhanced chemiluminescence.

Migration assays

Monolayer wound healing assay. MCF7 cells were seeded in wells of a 6-well plate (2x10^5 cells/well) and cultured in DMEM with 10% FBS. After 24 h incubation, cells were transfected with EGFP-N1-LY-6K using Fugene HD transfection reagent (Roche). After 48 h incubation, plates displaying confluent growth of MCF7 were scraped by a yellow tip (16). Following this, wound healing was observed at 15, 18 and 24 h.

Migration assay. The insert of a 24-well transwell apparatus (Corning, Corning, NY) was incubated at 37˚C for 1-2 h to adjust to room temperature. LY-6K transfected MCF7 cells and siRNA transfected MCF7-ADR cells (4x10^5 cells/ml) were prepared in serum-free medium. FBS containing medium (600 μl) was added to the lower chamber and 100 μl of prepared cell suspension was added to the insert. After 24 h, cells remaining in the insert (non-invading cells) were gently retrieved using a cotton tipped swab and allowed to air-dry for 20-30 min. Crystal violet solution (500 μl) was added to each well of the apparatus. After 20 min, invasive cells that traversed the membrane separating the insert from the lower chamber were stained by dipping the lower surface of the membrane into the stain. The stained membranes were washed several times using water and allowed to air-dry. Cells adhering to the membrane were quantified by dissolving the stained cells in 10% acetic acid and transferring the mixture to a 96-well plate for colorimetric determination of the optical density at 570 nm. The optical density readings were compared using a standard curve.

Invasion assay. Matrigel (BD, Franklin Lakes, NJ) was thawed at 4˚C overnight and diluted (1-5 mg/ml) in serum-free cold DMEM. A 100 μl volume of the diluted matrigel was added to the upper chamber of a transwell apparatus and incubated at 37˚C for 4-5 h to allow the gel to swell. Transfected cells were harvested from tissue culture flasks and washed three times in medium containing 1% FBS. LY-6K transfected MCF7 cells and siRNA transfected MCF7-ADR cells (4x10^5 cells/ml) were prepared in serum-free medium. Medium containing FBS (600 μl) was added to the lower chamber of the Transwell apparatus and 100 μl of prepared cell suspension was added to each insert. After 24 h, non-invading cells were retrieved and invasive cells quantified as described above.

Confocal microscopy. MCF7-ADR cells (2x10^5 cells/ml) were cultured on microscope cover glass contained in the wells of a 6-well plate. Cells that grew on the surface of the glass were fixed in 4% paraformaldehyde for 15 min, washed, and permeabilized with 0.2% Triton in phosphate buffered saline for 15 min. Each slide were incubated with LY-6K antibody diluted 1:500 and 4-6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, MO) diluted 1:2000 for 10 min in the dark at room temperature. The cells were stained with

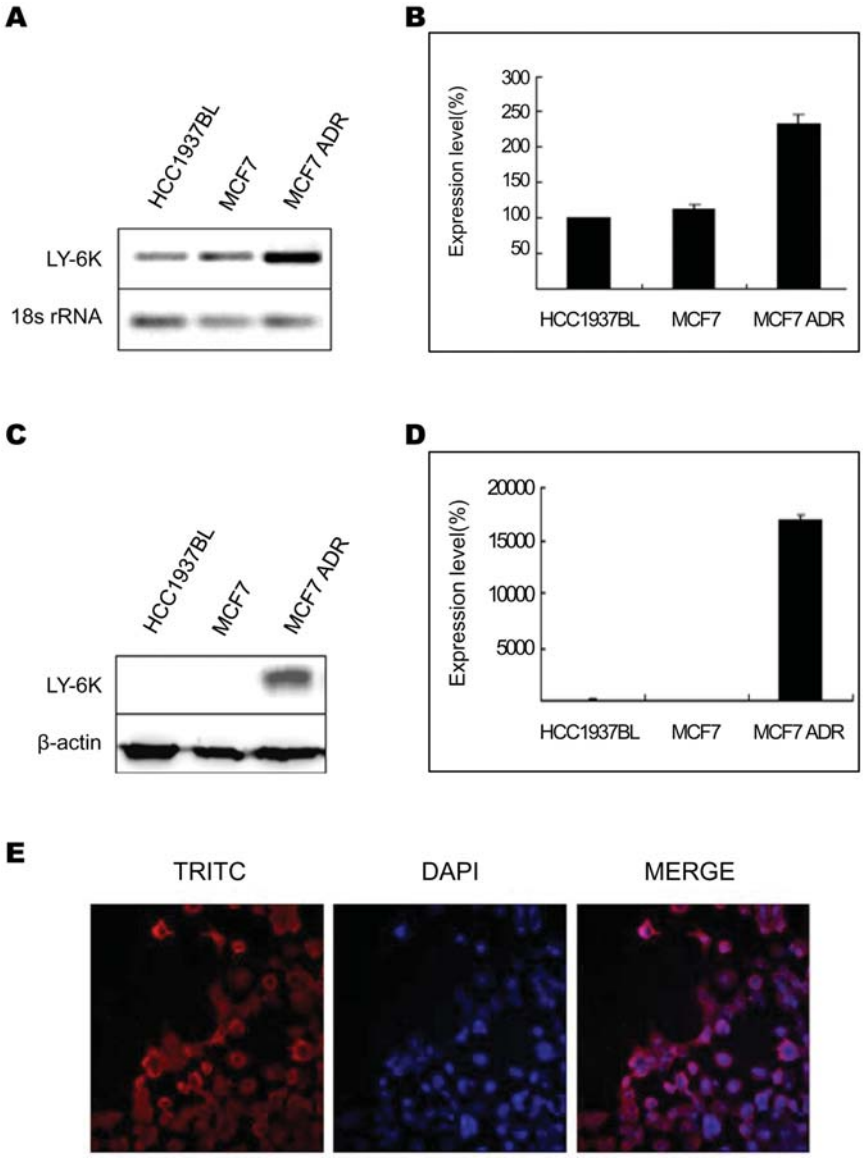

Figure 1. LY-6K gene expression in breast cancer cell lines. (A) LY-6K mRNA was assayed in MCF7, MCF7-ADR and HCC1937 cells by RT-PCR. The expression level of LY-6K mRNA was normalized to the signal detected with the 18s RNA gene. (B) The amplified PCR product was quantified using the Multigauge program and expressed relative to 18s RNA. (C) LY-6K protein detection in MCF7, MCF7-ADR and HCC1937 cells by Western blotting. The normalization of expression used ß-actin. (D) Band quantity applied to the Multigauge program. (E) Detection of LY-6K expression in MCF7-ADR cells using anti-rabbit-TRITC and DAPI staining. Subcellular localization of endogenous LY-6K protein in MCF7-ADR cells is evident.

anti-rabbit-tetramethylrhodamine isothiocyanate (TRITC; Sigma-Aldrich) to amplify the LY-6K signal for 15 min in the dark at room temperature. Each cover glass that was then retrieved was positioned on a microscopic slide glass and immunofluorescence imaging was conducted using a confocal laser scanning microscope (Olympus, Tokyo, Japan).

Results

Expression of LY-6K in breast cancer cells. In a previous study, we confirmed that LY-6K is a novel breast cancer marker. To explore the potential of LY-6K for breast cancer diagnosis and treatment, expression of LY-6K mRNA was ascertained using several breast cancer cell lines. Among these cell lines, MCF7 and MCF7-ADR displayed obviously different expression patterns (17). LY-6K mRNA and protein expression levels were presently assessed as well as in HCC1937BL cells as a negative control. Although LY-6K mRNA existed in all cells (Fig. 1A and B), protein expression was markedly elevated in MCF7-ADR cells (Fig. 1C and D). Immunofluorescence analysis to detect the subcellular localization of endogenous LY-6K in MCF7-ADR cells determined that LY-6K was mostly located in the cytoplasm of tumor cells with scant levels detected in the nucleus (Fig. 1E). However, LY-6K expression was not evident in HCC1937BL and MCF7 cells. Thus, MCF7 and MCF7-ADR breast cancer cells differently expressed LY-6K at the levels of both transcription and translation, suggesting that the ubiquity of the LY-6K gene in breast cancer cells is not reflected in LY-6K protein production.

Effect of LY-6K in metastasis. Over-expression of uPAR in breast cancer cells results in increased tumor invasion, growth and metastasis (11). As the novel breast cancer marker

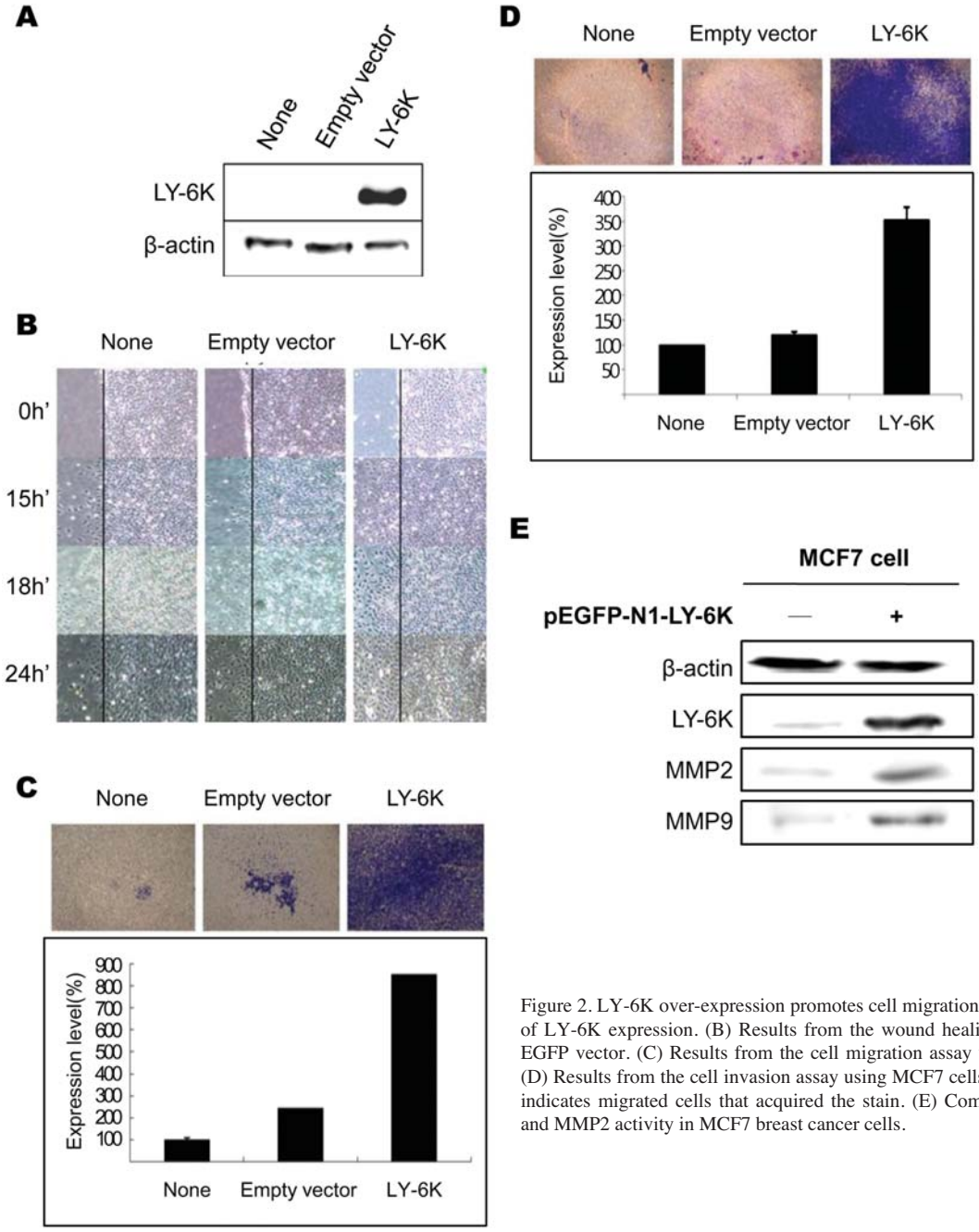

Figure 2. LY-6K over-expression promotes cell migration. (A) Confirmation of LY-6K expression. (B) Results from the wound healing assay. Control, EGFP vector. (C) Results from the cell migration assay using MCF7 cells. (D) Results from the cell invasion assay using MCF7 cells. The purple color indicates migrated cells that acquired the stain. (E) Comparison of MMP9 and MMP2 activity in MCF7 breast cancer cells.

LY-6K is also a member of the LY-6/uPAR superfamily, we determined whether LY-6K was related to cell metastasis. To assess this, three migration assays were performed. MCF7 cells were chosen as the control for the metastasis assay, because LY-6K protein was not detected in normal MCF7 cells (Fig. 1C). Following transfection of LY-6K naked DNA into MCF7 cells, LY-6K protein was expressed (Fig. 2A). LY-6K expression influenced the mobility of MCF7 cells (Fig. 2B). To ascertain the influence of LY-6K on cell migration and invasion, the transwell apparatus was exploited to detect cell migration through the membrane barrier between Transwell inserts and the receptacles. Cells over-expressing LY-6K displayed the most pronounced migration tendency, as evident by a more intense cell staining of the membrane face opposite the insert chamber in which the cells were originally added. Non-transfected and empty-vector-transfected cells showed far less evidence of migration. LY-6K transfected cells displayed an approximately 8-fold greater migration (Fig. 2C) and about a 4-fold greater invasion capacity (Fig. 2D) than non-transfected cells. CD147, which is an inducer of extracellular MMP, promotes invasion of cancer cells by stimulating the production of MMPs. Their production critically influences tumor progression including growth and invasion (18). LY-6K transfected MCF7 cells significantly expressed both MMP2 and MMP9 (Fig. 2E).

Influence of LY-6K siRNA on MCF7-ADR cell migration. To verify the role of LY-6K on cell migration, RNAi was utilized to specifically suppress the expression of endogenous LY-6K in MCF7-ADR cells. Confirmation was provided by RT-PCR and Western blotting (Fig. 3A). Transwell cell migration and invasion assays established that MCF7-ADR cells treated

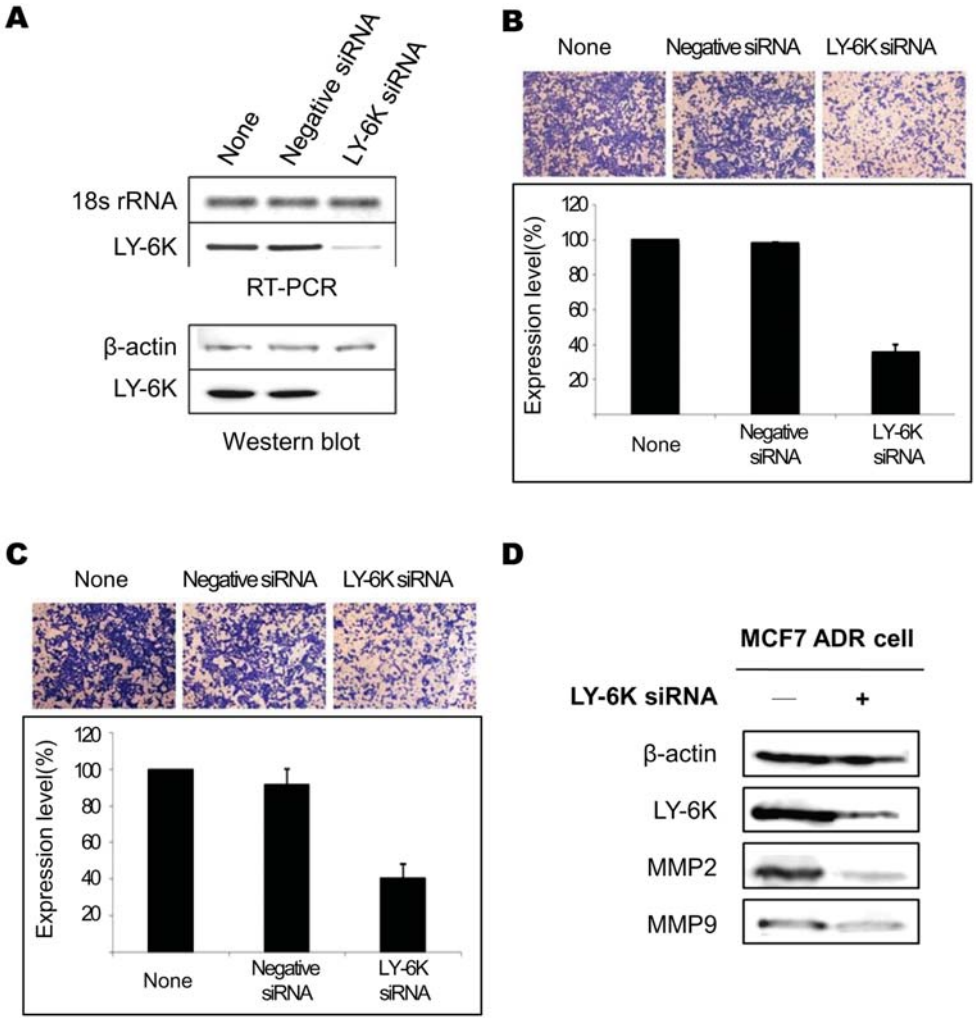

Figure 3. siRNA knockdown of LY-6K inhibits cell migration. (A) mRNA and protein expression levels of LY-6K in MCF7-ADR cells treated with LY-6K siRNA. LY-6K siRNA and negative control siRNA were transfected into MCF7-ADR cells. (B) Results of cell migration assay using MCF7-ADR cells. (C) Results of cell invasion assay using MCF7-ADR cells. The purple color indicates migrated cells. (D) Comparison of MMP9 and MMP2 activity in MCF7 breast cancer cells.

with LY-6K siRNA migrated less than negative control siRNA treated and non-transfected MCF7-ADR cells (Fig. 3B and C). Expression of both MMP2 and MMP9 was also significantly decreased in LY-6K siRNA treated MCF7-ADR cells (Fig. 3D).

Effects of LY-6K over-expression on Ras/ERK pathway-mediated migration. Activation of Ras and its downstream signal controlled by the ERK/MARK pathway is an important contributor to integrin ligation (12). When in a complex with uPAR, integrins can activate the ERK pathway to control invasion in cancer cells. The recent demonstration that uPA/PAR and integrin enhances cell adhesion and invasion via the ERK signaling pathway (13), prompted us to consider that the over-expression of LY-6K might affect the ERK pathway. To assess this speculation, we examined MCF7 and MCF7-ADR cells, which differentially express LY-6K. Expression of ERK was identical in both cell lines (Fig. 4A). However, phosphorylated (activated) ERK was readily evident in MCF7-ADR cells but not in MCF7 cells, and LY-6K protein was detected only in MCF7-ADR cells. We speculated that LY-6K may be influential in the migration-relevant ERK pathway. In uPAR-rich cells uPA-bound uPAR frequently interacts with α5ß1 integrin, causing its activation. This leads to integrin-dependent recruitment of FAK/EGFR complex, resulting in activation of Ras/ERK signaling, which in turn induces a mitogenically high ERK/p38 signaling ratio. Down-regulation of uPAR or blockage of α5ß1 function results in integrin inactivation, disassembly of the complex, inactivation of its intracellular signaling components and reduced ERK activation. Based on this information, we examined Raf-1, MEK and ERK activity. In MCF7 cells, which do not express LY-6K, endogenous LY-6K was expressed following transfection. As shown in Fig. 4B, expression of LY-6K was coincident with the activation of Raf-1, MEK and ERK. When endogenous LY-6K expression in MCF7-ADR cells was abrogated, phosphorylation of ERK was reduced (Fig. 4C). As shown in Fig. 4D, MCF7 cells treated with the MEK inhibitor PD98059 or the Raf-1 inhibitor GW5074 24 h after LY-6K transfection displayed reduced ERK pathway activity. This result was consistent with the suggestion that the pathway associated with migration caused by LY-6K is similar to the ERK pathway-mediated integrin activation. Several distinct ERK pathways are important in the regulation of

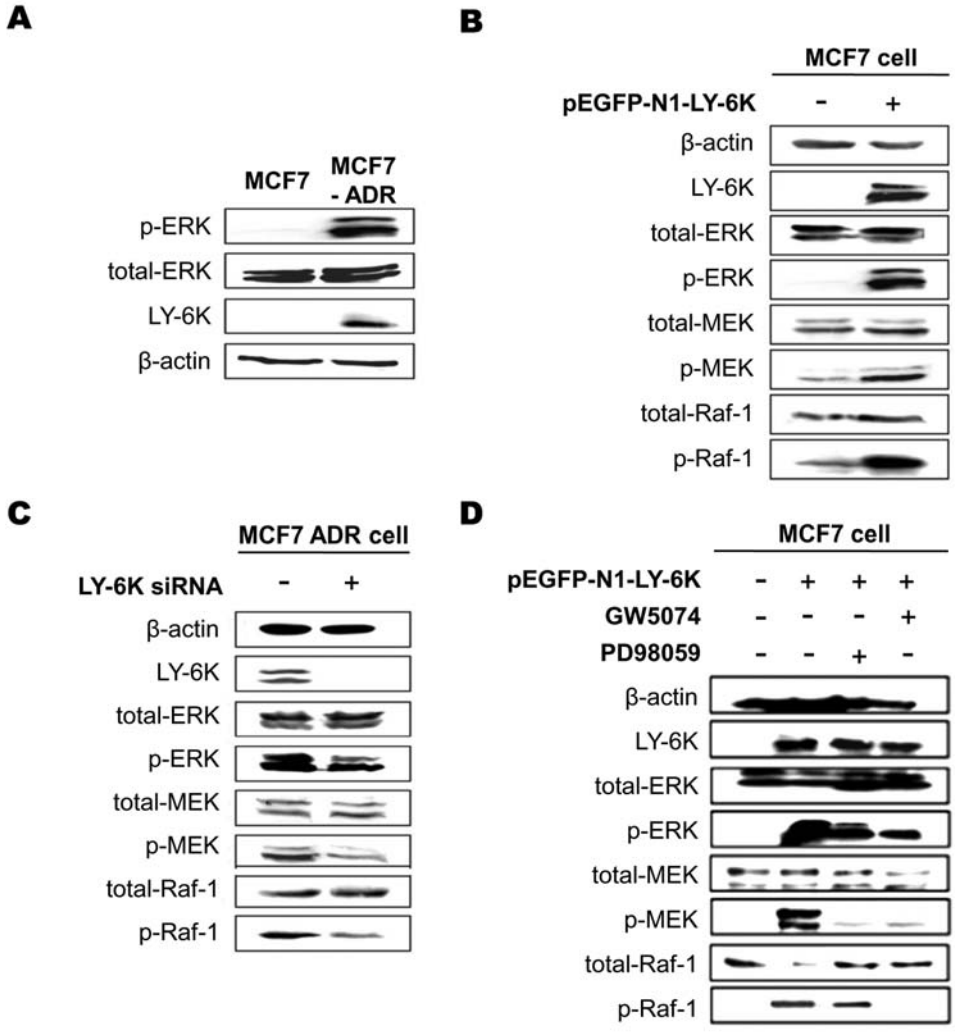

Figure 4. Relationship of ERK pathway and LY-6K. (A) Comparison of ERK expression in MCF7 and MCF7-ADR cells reveals that expression depends on LY-6K. (B) Exuberant and simultaneous expression of LY-6K and phospho-ERK in MCF7 cells. (C) Reduction of phospho-ERK upon treatment of MCF7-ADR cells with LY-6K siRNA. (D) Confirmation of pathway using the inhibitor.

cell proliferation, differentiation, development, inflammation, survival and migration (19-21). To examine the cellular effect of over-expression of the LY-6K gene, a proliferation assay was conducted. The tetrazolium hydroxide-based XTT assay is a colorimetric and short-term suspension culture assay that measures the metabolic activity of living cells, similar to the 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay. pEGFP-N1-LY-6K was transfected into HEK293 normal kidney cells and MCF7 breast cancer cells. LY-6K-transfected cells transfected with control DNA or vector (pEGFP-N1) did not differ (data not shown), indicating that cell proliferation was not affected by LY-6K.

Discussion

LY-6K is a potential target antigen in HNSCC diagnosis and therapy (7). Moreover, the LY-6K gene is also expressed in human breast cancer tissue, making LY-6K exploitable as a novel breast cancer marker (8). Our study provides experimental evidence to support a new role of LY-6K in breast cancer cells. LY-6K was not induced uniformly in all breast cancer cell lines examined. Ly-6K mRNA was detected in both MCF7 and MCF7-ADR cells, but the expressed protein was not detected in MCF7 cells.

Because LY-6K belongs to the Ly6/uPAR superfamily, which is related to metastasis, we first sought to determine whether LY-6K is related to cell metastasis. The three migration assay methods we used conclusively demonstrated that the migration of LY-6K-transfected MCF7 cells is enhanced and that these cells display enhanced invasiveness than their non-transfected counterparts. Furthermore, the inhibition of endogenous LY-6K expression by siRNA in MCF7-ADR cells reduced cell migration in spite of their own metastatic character. Therefore, we can conclude that the LY-6K gene affects breast cancer cell metastasis, similarly to uPAR.

As LY-6K is related to breast cancer cell metastasis, we confirmed the pathway by which this gene mediates cancer cell metastasis. The activation of Ras and its downstream signal-controlled kinase-activate protein kinase ERK/MARK pathway is an important contributor to integrin ligation. The integrin-uPAR complex can activate the Ras pathway to control invasion in cancer cells. The uPA/PAR and integrin-mediated enhancement of adhesive and invasive capabilities through the Ras/ERK signaling pathway has been described

(12). We focused on the influence of LY-6K on the ERK pathway. In MCF7 cells, expression of endogenous LY-6K increased the expression of the activated ERK pathway. Moreover, in MCF7-ADR cells that exuberantly expressed LY-6K, this expression as well as the activation of Raf-1, MEK and ERK were all reduced by transient tranfection with LY-6K siRNA. The best explanation is that the cell migration pathway of LY-6K is similar to the integrin-relevant Ras/ERK pathway. LY-6K, however, does not affect cell proliferation. In normal cells (HEK293) and breast cancer cells (MCF7), over-expression of the LY-6K gene does not alter cell proliferation. Unfortunately, we do not yet know how the LY6K gene is expressed and regulated in breast cancer cells, but studies on LY-6K promoter region-related p53 and NF-κB will hopefully overcome this obstacle. In conclusion, we suggest that as a novel breast cancer marker, the LY-6K gene plays an important role in breast cancer metastasis. Functionally, LY-6K gene expression may affect breast cancer cells independent from p53-mediated cell cycle regulation, and may influence metastasis as a member of Ly6/uPAR superfamily via the Ras/ERK signaling pathway. We are planning further studies on the effect of LY-6K gene expression *in vivo*, to clarify the potential for LY-6K as a diagnostic biomarker in breast cancer.

Acknowledgements

This work was supported by the Sookmyung Women's University Research Grants 2007, and the SRC/ERC program of MOST/KOSEF (Research Center for Women's Disease). We would like to thank Dr Robert Clarke at Georgetown University for giving the MCF7 cell lines.

References

1. Casey JR, Petranka JG, Kottra J, Fleenor DE and Rosse WF: The structure of the urokinase-type plasminogen activator receptor gene. Blood 84: 1151-1156, 1994.
2. Ploug M and Ellis V: Structure-function relationships in the receptor for urokinase-type plasminogen activator. Comparison to other members of the Ly-6 family and snake venom alpha-neurotoxins. FEBS Lett 349: 163-168, 1994.
3. Adermann K, Wattler F, Wattler S, *et al*: Structural and phylogenetic characterization of human SLURP-1, the first secreted mammalian member of the Ly-6/uPAR protein superfamily. Protein Sci 8: 810-819, 1999.
4. Shan X, Bourdeau A, Rhoton A, *et al*: Characterization and mapping to human chromosome 8q24.3 of Ly-6-related gene 9804 encoding an apparent homologue of mouse TSA-1. J Immunol 160: 197-208, 1998.
5. Brakenhoff RH, Gerretsen M, Knippels EM, *et al*: The human E48 antigen, highly homologous to the murine Ly-6 antigen ThB, is a GPI-anchored molecule apparently involved in keratinocyte cell-cell adhesion. J Cell Biol 129: 1677-1689, 1995.
6. Blasi F and Carmeliet P: uPAR: a versatile signalling orchestrator. Nat Rev Mol Cell Biol 3: 932-943, 2002.
7. De Nooij-van Dalen AG, van Dongen GA, Smeets SJ, *et al*: Characterization of the human Ly-6 antigens, the newly annotated member Ly-6K included, as molecular markers for head-and-neck squamous cell carcinoma. Int J Cancer 103: 768-774, 2003.
8. Lee JW, Lee YS, Yoo KH, *et al*: LY-6K gene: a novel molecular marker for human breast cancer. Oncol Rep 16: 1211-1214, 2006.
9. Andreasen PA, Kjoller L, Christensen L and Duffy MJ: The urokinase-type plasminogen activator system in cancer metastasis: a review. Int J Cancer 72: 1-22, 1997.
10. Grondahl-Hansen J, Hilsenbeck SG, Christensen IJ, Clark GM, Osborne CK and Brunner N: Prognostic significance of PAI-1 and uPA in cytosolic extracts obtained from node-positive breast cancer patients. Breast Cancer Res Treat 43: 153-163, 1997.
11. Xing RH and Rabbani SA: Overexpression of urokinase receptor in breast cancer cells results in increased tumor invasion, growth and metastasis. Int J Cancer 67: 423-429, 1996.
12. Lengyel E, Wang H, Gum R, Simon C, Wang Y and Boyd D: Elevated urokinase-type plasminogen activator receptor expression in a colon cancer cell line is due to a constitutively activated extracellular signal-regulated kinase-1-dependent signaling cascade. Oncogene 14: 2563-2573, 1997.
13. Sawai H, Okada Y, Funahashi H, *et al*: Interleukin-1alpha enhances the aggressive behavior of pancreatic cancer cells by regulating the alpha6beta1-integrin and urokinase plasminogen activator receptor expression. BMC Cell Biol 7: 8, 2006.
14. Webster GA and Perkins ND: Transcriptional cross talk between NF-kappaB and p53. Mol Cell Biol 19: 3485-3495, 1999.
15. Mehta K, Devarajan E, Chen J, Multani A and Pathak S: Multidrug-resistant MCF-7 cells: an identity crisis? J Natl Cancer Inst 94: 1652-1654, 2002.
16. Rodriguez LG, Wu X and Guan JL: Wound-healing assay. Methods Mol Biol 294: 23-29, 2005.
17. Brunner N, Boysen B, Jirus S, *et al*: MCF7/LCC9: an anti-estrogen-resistant MCF-7 variant in which acquired resistance to the steroidal antiestrogen ICI 182,780 confers an early cross-resistance to the nonsteroidal antiestrogen tamoxifen. Cancer Res 57: 3486-3493, 1997.
18. Yan L, Zucker S and Toole BP: Roles of the multifunctional glycoprotein, emmprin (basigin; CD147), in tumour progression. Thromb Haemost 93: 199-204, 2005.
19. Nguyen DH, Catling AD, Webb DJ, *et al*: Myosin light chain kinase functions downstream of Ras/ERK to promote migration of urokinase-type plasminogen activator-stimulated cells in an integrin-selective manner. J Cell Biol 146: 149-164, 1999.
20. Jo M, Thomas KS, Somlyo AV, Somlyo AP and Gonias SL: Cooperativity between the Ras-ERK and Rho-Rho kinase pathways in urokinase-type plasminogen activator-stimulated cell migration. J Biol Chem 277: 12479-12485, 2002.
21. Xie L, Overbeek PA and Reneker LW: Ras signaling is essential for lens cell proliferation and lens growth during development. Dev Biol 298: 403-414, 2006.