EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 38, No. 2, 153-161, April 2006

βig-h3 triggers signaling pathways mediating adhesion and migration of vascular smooth muscle cells through αvβ5 integrin

Byung-Heon Lee[1]*, Jong-Sup Bae[1]*, Rang-Woon Park[1], Jung-Eun Kim[2], Jae-Yong Park[3] and In-San Kim[1,4]

[1]Department of Biochemistry and
Cell and Matrix Biology Research Institute
[2]Department of Molecular Medicine
[3]Department of Internal Medicine
School of Medicine, Kyungpook National University
Daegu 700-422, Korea
[4]Corresponding author: Tel, 82-53-420-4821;
Fax, 82-53-422-1466; E-mail, iskim@mail.knu.ac.kr
*These authors contributed equally to this work.

Accepted 20 March 2006

Abbreviations: FAK, focal adhesion kinase; PI3K, phosphatidylinositide 3-kinase; TGF-β, transforming growth factor-β; VSMCs, vascular smooth muscle cells

Abstract

Adhesion and migration of vascular smooth muscle cells (VSMCs) play an important role in the pathogenesis of atherosclerosis. These processes involve the interaction of VSMCs with extracellular matrix proteins. Here, we investigated integrin isoforms and signaling pathways mediating the adhesion and migration of VSMCs on βig-h3, a transforming growth factor (TGF)-β-inducible extracellular matrix protein that is elevated in atherosclerotic plaques. Adhesion assays showed that the αvβ5 integrin is a functional receptor for the adhesion of aortic VSMCs to βig-h3. An YH18 motif containing amino acids between 563 and 580 of βig-h3 was an essential motif for the adhesion and growth of VSMCs. Interaction between the YH18 motif and the αvβ5 integrin was responsible for the migration of VSMCs on βig-h3. Inhibitors of phosphatidylinositide 3-kinase, extracellular signal-regulated kinase (ERK), and Src kinase reduced the adhesion and migration of VSMCs on βig-h3. βig-h3 triggered phosphorylation and activation of AKT, ERK, focal adhesion kinase, and paxillin mediating the adhesion and migration of VSMCs. Taken together, these results suggest that βig-h3 and αvβ5 integrin play a role in the adhesion and migration of VSMCs during the pathogenesis of atherosclerosis.

Keywords: atherosclerosis; βIG-H3 protein; integrin αvβ5; muscle, smooth, vascular; transforming growth factor β

Introduction

Atherosclerosis involves multiple processes including endothelial dysfunction, inflammation, vascular proliferation, and alteration in extracellular matrix. Recent studies have emphasized the role of inflammation in mediating all stages of atherosclerosis (Libby *et al*., 2002). In addition to inflammation, migration and proliferation of vascular smooth muscle cells (VSMCs) are the key processes of neointima formation in atherosclerosis and vascular injury. VSMCs may contribute to the development of atherosclerosis through the production of inflammatory cytokines such as monocyte chemoattractant protein-1, and through the synthesis of matrix proteins (Newby and Zaltsman, 2000; Dzau *et al*., 2002; Libby, 2002). VSMCs may also be important in maintaining the stability of the plaque through the formation of a firm fibrous cap (Newby and Zaltsman, 2000; Dzau *et al*., 2002; Libby, 2002). These processes involve the interaction of VSMCs with matrix and changes in intracellular signaling pathways that regulate cell migration.

Integrins are cell surface heterodimeric proteins composed of α and β subunits and responsible for cell adhesion to matrix. Different integrins mediate binding of different cell types to specific matrix proteins such as collagen (e.g., α1β1, α2β1) or fibronectin (e.g., αvβ3, α5β1) (Hynes, 2002). Among them, β1 and β3 integrins have been involved in the adhesion and migration of VSMCs on fibronectin, laminin, and collagen (Clyman *et al*., 1990; Clyman *et al*., 1992). In addition to adhesion, integrins initiate intracellular signaling pathways by recruiting and activating protein kinases such as focal adhesion kinase (FAK). These signaling pathways are involved in the regulation of cell migration, proliferation, and survival (Giancotti and Ruoslahti, 1999).

βig-h3 is a 68-kDa matrix protein whose expression is highly induced by transforming growth factor-β (TGF-β) in several cell types including keratinocytes, fibroblasts, mammary epithelial cells, and melanoma

cells (Skonier *et al*., 1994). βig-h3 plays a role in cell adhesion, migration, and wound healing (LeBaron *et al*., 1995; Kim *et al*., 2000; Bae *et al*., 2002; Park *et al*., 2004). In addition, βig-h3 is involved in certain human diseases. Mutations of βig-h3, for example, have been identified and are implicated in corneal dystrophies (Munier *et al*., 1997; Endo *et al*., 1999). βig-h3 is down-regulated in melorheostosis and involved in osteogenesis (Kim *et al*., 2000). Moreover, βig-h3 is elevated in the aorta of diabetic rats and its production is induced by high glucose in VSMCs (Ha *et al*., 2003), suggesting a role for βig-h3 in the development of diabetic angiopathy. Of interest is that βig-h3 is elevated in macrophages and VSMCs in atherosclerotic and restenotic human arteries (O'Brien *et al*., 1996). These findings suggest a role for βig-h3 in the pathogenesis of atherothrombosis and restenosis. Here we investigated integrin isoforms and intracellular signaling pathways that are responsible for the adhesion and migration of VSMCs on βig-h3.

Materials and Methods

Recombinant proteins, reagents, antibodies, and cells

Recombinant proteins including βig-h3, D-I, D-II, D-III, D-IV, D-IV-LAAR, YH18, YH18-Con, and ΔH1H2(6) were prepared as previously described (Kim *et al*., 2000; 2002). Phosphatidylinositide 3-kinase (PI3K) inhibitor LY294002, ERK kinase inhibitor PD98059, protein tyrosine kinase inhibitor genistein, and Src family kinase inhibitor PP2 were purchased from Calbiochem (San Diego, CA). Rabbit polyclonal anti-human βig-h3 antibody was prepared by immunizing rabbits with human recombinant βig-h3 protein. Rabbit polyclonal anti-FAK and mouse monoclonal anti-phospho-ERK were from Santa Cruz Biotechnologies (Santa Cruz, CA). Rabbit polyclonal anti-AKT and anti-phospho-AKT were from Cell Signaling Technology (Beverly, MA). Anti-ERK, anti-phospho-FAK, anti-paxillin, and anti-phospho-paxillin were from Transduction Laboratories (Lexington, KY). Aortic VSMCs (Clonetics, CC-2571) were grown in DMEM supplemented with 10% FBS, sodium pyruvate, penicillin G and streptomycin. All experiments were performed using cells between the third and the fifth passage.

Cell adhesion assays

Ninety-six-well culture plates (BD Bioscicences, Mountain View, CA) were coated with the indicated proteins at 4°C overnight. Then, the plates were rinsed three times in phosphate-buffered saline (PBS), and uncoated surfaces were blocked with 2% heat-inactivated BSA for 1 h at 37°C. Aortic VSMCs (2×10^4 cells) were plated to each well and incubated for 1 h, and then unattached cells were removed by rinsing twice with PBS. Attached cells were incubated with 50 mM citrate buffer, pH 5.0, containing 3.75 mM *p*-nitrophenyl- *N*-acetyl-D-glucosaminide (hexosaminidase substrate) and 0.25% Triton X-100 for 1 h at 37°C. The reaction was stopped, and color was developed by the addition of 50 mM glycine buffer, pH 10.4, containing 5 mM EDTA. Absorbance was measured at 405 nm in a microplate reader (Titertek Instrument, Inc., Humtsville, AL).

Cell migration assays

Cell migration assays were performed as previously described (Kim *et al*., 2000; 2002). For cell migration, the under surface of the membrane of trans-well plates (8 μm pore size, Costar, Cambridge, MA) was coated with proteins at 4°C overnight and blocked with 2% BSA. Then aortic VSMCs (10^5 cells per well in 200 μl medium) were seeded in the upper compartment of filters. After 6 h of migration, cells in the upper chamber of the filter were removed, and nonmigrating cells on the top of the filters were removed with a cotton swab. Migrating cells on the lower side of the filter were fixed with 8% glutaraldehyde and then stained with 0.25% crystal violet in 20% methanol. Each experiment was repeated in duplicate, and counting was done in nine randomly selected microscopic high power fields ($\times$ 200).

Cell proliferation assays

Cell proliferation was assayed using twenty-four well culture plates coated with the indicated proteins. Aortic VSMCs (5×10^4 cells) that were synchronized by serum starvation for 24 h were plated onto each well. After incubation for 24 h, cell proliferation was measured by counting cell numbers using a hematocytometer.

Inhibition of adhesion and migration

Various chemical kinase inhibitors or function blocking antibodies against integrins were examined for their ability to prevent cells from adhering or migrating on the prepared substrata. Cells (2×10^5 cells/ml) were pre-incubated individually with the indicated reagents at 37°C for 30 min. The pre-incubated cells were transferred onto plates or trans-wells coated with proteins and then incubated further for 1 h at 37°C. Attached or migrated cells were then quantified as described above.

Immunoblotting

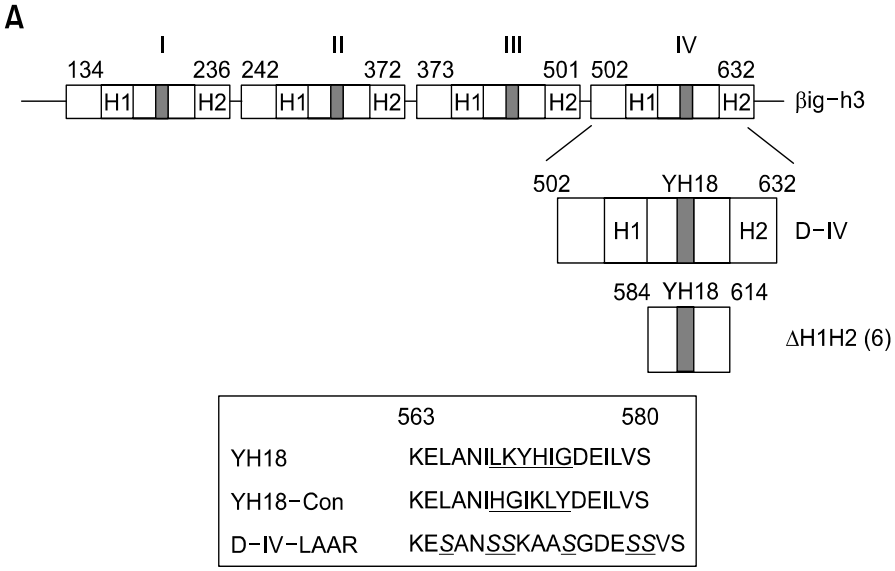
Cells lysates were prepared and subjected to SDS-PAGE, transfer, and blotting with the indicated antibodies as previously described (Lee and Ruoslahti, 2005). The intensity of a protein band was quantified by densitometry of immunoblots using Fuji Film MacBAS Software (Fuji Photo Film, Tokyo, Japan).

Results

The αvβ5 integrin is a functional receptor for aortic VSMCs adhesion to βig-h3

Figure 1A shows diagrams of βig-h3 and its domains. βig-h3 consists of four Fas-1 domains, which are designated as D-I, D-II, D-III, and D-IV domain (Kim *et al*., 2002; Nam *et al*., 2003). Each domain has two conserved Fas-1 regions known as H1 and H2. ΔH1H2(6) is a fragment of D-IV domain that lacks both H1 and H2 region and also the carboxy-terminal EPDIM motif. D-IV-LAAR is a mutant D-IV domain, where all conserved amino acids had been substituted. The YH18 motif contains 18 amino acids including tyrosine and histidine at the positions between 563 and 580, while the YH18-Con contains a scrambled sequence.

Previous studies have shown that the αvβ3 and αvβ5 integrins mediate VSMC migration on vitronectin (Clyman *et al*., 1992; Kappert *et al*., 2001; Stawowy *et al*., 2004). In this regard, we investigated the effects of function-blocking antibodies against αvβ3 and αvβ5 integrins on the adhesion of aortic VSMCs to βig-h3. A function-blocking antibody



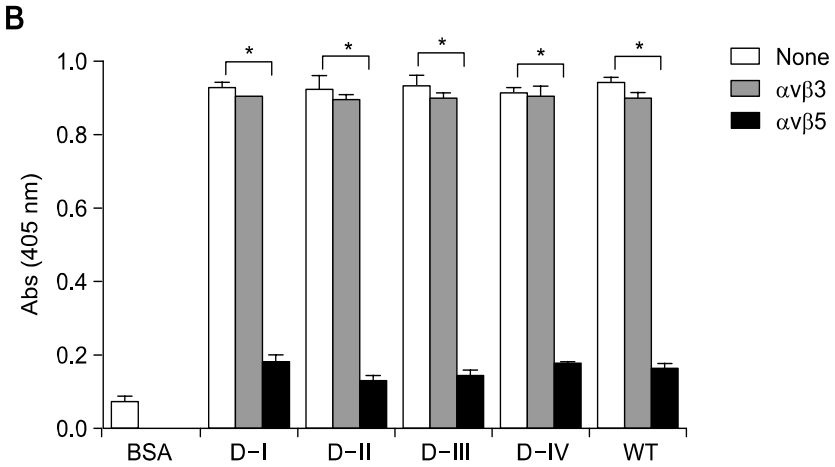


Figure 1. The αvβ5 integrin is a functional receptor of aortic VSMCs for the adhesion on βig-h3. (A) Diagrams showing βig-h3 and its domains. The underlined letters represent scrambled amino acid sequences. Italicized underlined letters represent mutated amino acids. (B) Cells were pre-incubated with or without function-blocking monoclonal antibodies against the αvβ3 or αvβ5 integrin and then plated onto the culture dishes coated with wild-type (WT) βig-h3, D-I, D-II, D-III, and D-IV domain for 1 h. The number of cells attached to the substrates was then measured. Data represent mean ± SD of three separate experiments. *$P < 0.05$ by Student's *t*-test.

against the integrin αvβ5 inhibited the adhesion of aortic VSMCs to wild-type βig-h3 as well as D-I, D-II, D-III, and D-IV domains of βig-h3 (Figure 1B). In contrast, a function-blocking antibody against the αvβ3 integrin did not inhibit the adhesion of aortic VSMCs to βig-h3 and individual domains (Figure 1B). These findings indicate that the αvβ5 integrin is the functional receptor on aortic VSMCs for adhesion to βig-h3.

The YH18 motif of βig-h3 mediates the adhesion and growth of aortic VSMCs

We have previously reported that the YH18 motif of βig-h3 mediates the adhesion of fibroblasts and endothelial cells through interaction with the αvβ5 and αvβ3 integrins, respectively (Kim *et al*., 2002; Nam *et al*., 2003). In this regard, we investigated a protein motif(s) responsible for the adhesion of aortic VSMCs to βig-h3. The adhesion of VSMCs to βig-h3, D-IV domain, and ΔH1H2(6) were as efficient as the cell adhesion to vitronectin (Figure 2A). In contrast, VSMCs adhered to a D-IV-LAAR mutant at a lesser degree (Figure 2A). The adhesion of aortic VSMCs to βig-h3 and D-IV as well as vitronectin was blocked by the YH18 motif, but not by the YH18-Con (Figure 2B). These findings indicate that the YH18 motif within βig-h3 D-IV domain is an essential element for the adhesion of aortic VSMCs to βig-h3.

To determine the effect of βig-h3 on the growth of aortic VSMCs, cells were synchronized by serum starvation and then plated onto dishes coated with the indicated proteins. βig-h3 as well as D-IV domain and ΔH1H2(6) supported the growth of aortic VSMCs, while the D-IV-LAAR mutant or BSA supported little the cell growth (Figure 2C). These results indicate that βig-h3 and its domains containing the YH18 motif play a role in supporting the growth of aortic VSMCs.

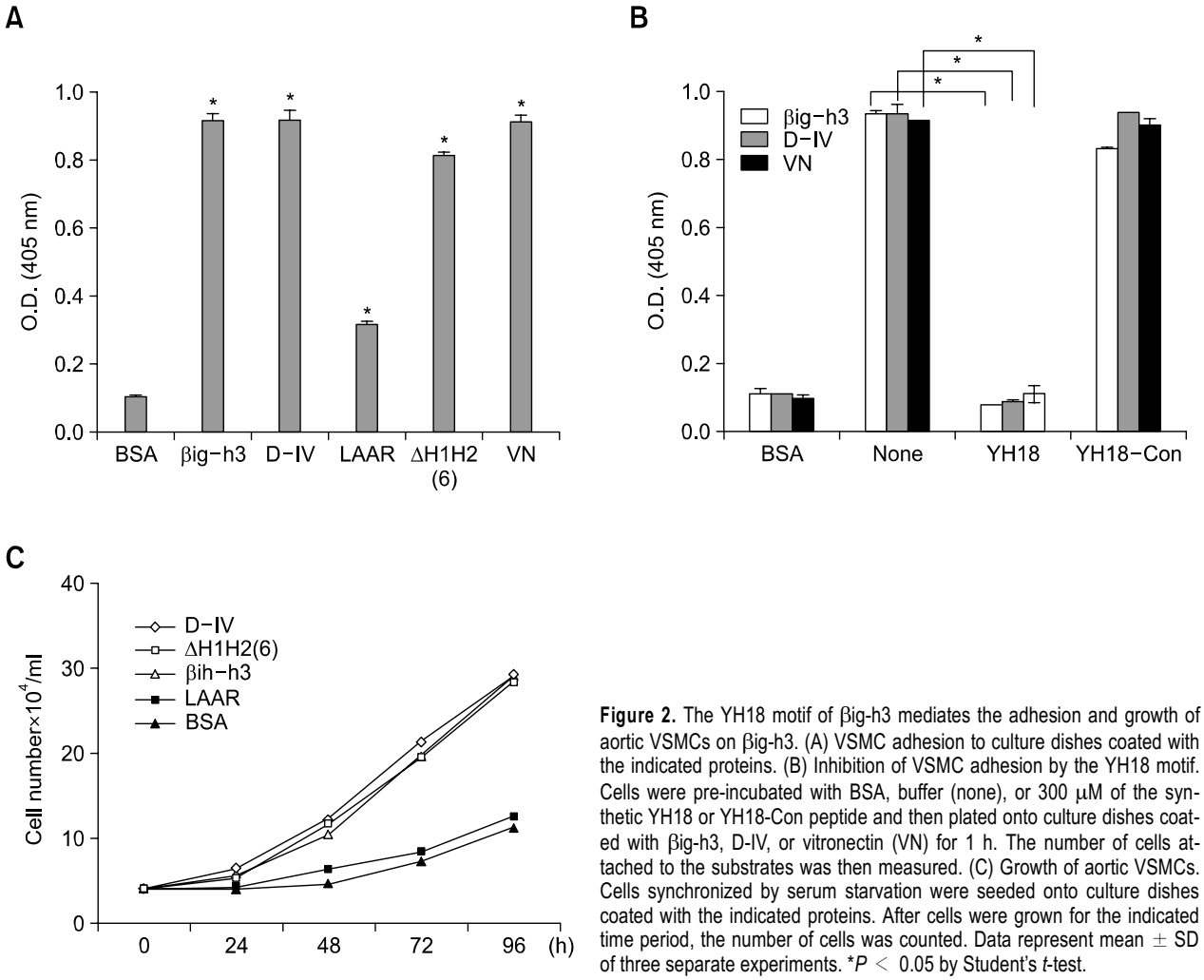


Figure 2. The YH18 motif of βig-h3 mediates the adhesion and growth of aortic VSMCs on βig-h3. (A) VSMC adhesion to culture dishes coated with the indicated proteins. (B) Inhibition of VSMC adhesion by the YH18 motif. Cells were pre-incubated with BSA, buffer (none), or 300 μM of the synthetic YH18 or YH18-Con peptide and then plated onto culture dishes coated with βig-h3, D-IV, or vitronectin (VN) for 1 h. The number of cells attached to the substrates was then measured. (C) Growth of aortic VSMCs. Cells synchronized by serum starvation were seeded onto culture dishes coated with the indicated proteins. After cells were grown for the indicated time period, the number of cells was counted. Data represent mean ± SD of three separate experiments. *$P < 0.05$ by Student's *t*-test.

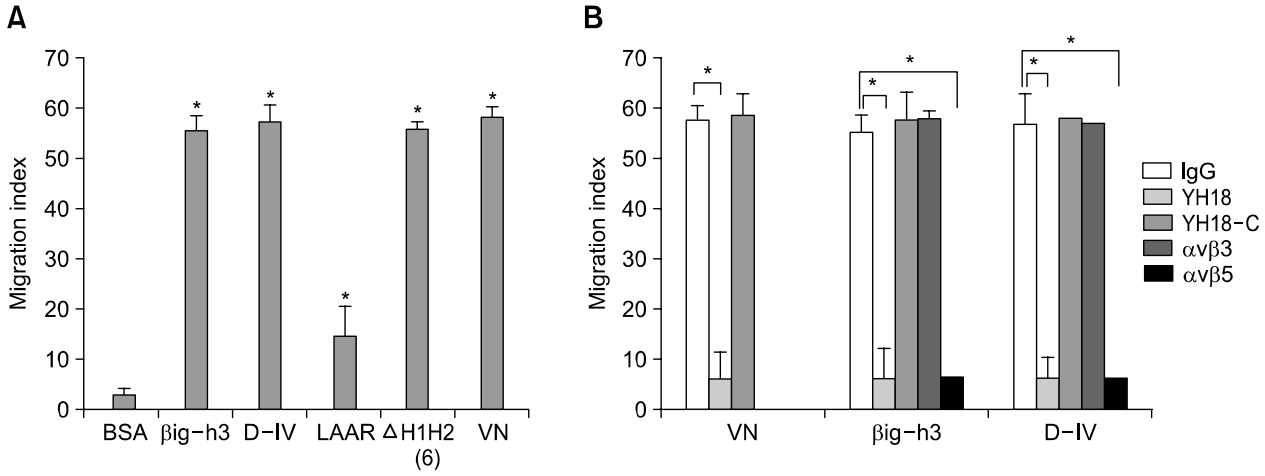


Figure 3. βig-h3 supports migration of aortic VSMCs through the interaction between the YH18 motif and the αvβ5 integrin. (A) Cells were plated onto the culture dishes coated with the indicated proteins, and then cell migration was measured. (B) Cells were pre-incubated with the indicated peptides (300 μM) or antibodies (5 mg/ml) before plating onto culture dishes coated with the indicated proteins, and then cell migration was measured. Data represent mean ± SD of three separate experiments. *$P < 0.05$ by Student's *t*-test.

βig-h3 supports migration of aortic VSMCs through interaction between the YH18 motif and the αvβ5 integrin

Migration of aortic VSMCs was facilitated when cells were plated on βig-h3, D-IV domain, ΔH1H2(6), or vitronectin, while little migration was observed on D-IV-LAAR mutant or BSA (Figure 3A). The migration of aortic VSMCs on βig-h3 or D-IV domain was completely blocked by the YH18 motif or the function blocking antibody against the αvβ5 integrin, but not by the YH18-Con or the antibody against the αvβ3 integrin (Figure 3B). These results indicate that the migration of aortic VSMCs on βig-h3 is dependent on the interaction between the YH18 motif of βig-h3 and the αvβ5 integrin.

Adhesion and migration of aortic VSMCs on βig-h3 are suppressed by inhibitors for PI3K, ERK, protein tyrosine kinase, and Src kinase

To investigate protein kinases involved in the αvβ5 integrin-mediated adhesion and migration of aortic VSMCs on βig-h3, cells were incubated with several types of specific protein kinase inhibitors. The adhesion of aortic VSMCs on βig-h3 was suppressed in a dose-dependent manner by LY294002, PD98059, genistein, and PP2, which are specific inhibitors for PI3K, ERK, protein tyrosine kinase, and Src family kinase, respectively (Figure 4A). In addition to adhesion, the migration of aortic VSMCs on βig-h3 was also suppressed by these kinase inhibitors in a dose-dependent manner (Figure 4B).

Adhesion and migration of aortic VSMCs on βig-h3 involves phosphorylation of AKT, ERK, FAK, and paxillin

To investigate the downstream signaling pathways involved in the adhesion and migration of aortic VSMCs on βig-h3, the phosphorylation and activation of intracellular kinases were determined using phospho-specific antibodies against the kinases. Phosphorylation and thus activation of AKT, a downstream kinase of PI3K, were increased by the adhesion of aortic VSMCs to βig-h3 D-IV domain compared to poly-lysine, which mediates non-integrin dependent adhesion (Figure 5A). Phosphorylation and activation of ERK, FAK, and paxillin were also increased by the adhesion of aortic VSMCs to βig-h3 D-IV domain compared to poly-lysine (Figure 5B-D). In addition to adhesion, phosphorylation of AKT, FAK, and paxillin were increased when cells migrate on βig-h3 D-IV domain compared to non-migrating cells or migrating cells on non-coated culture plates (Figure 5A, C and D). Phosphorylation of ERK, however, was not increased by the migration of aortic VSMCs on βig-h3 D-IV domain (Figure 5B).

Discussion

In this study, we showed that βig-h3 mediates adhesion and migration as well as growth of aortic VSMCs through the interaction between the YH-18 motif and the αvβ5 integrin. We also showed that βig-h3 triggers phosphorylation and thus activation of intracellular signaling pathways including AKT,

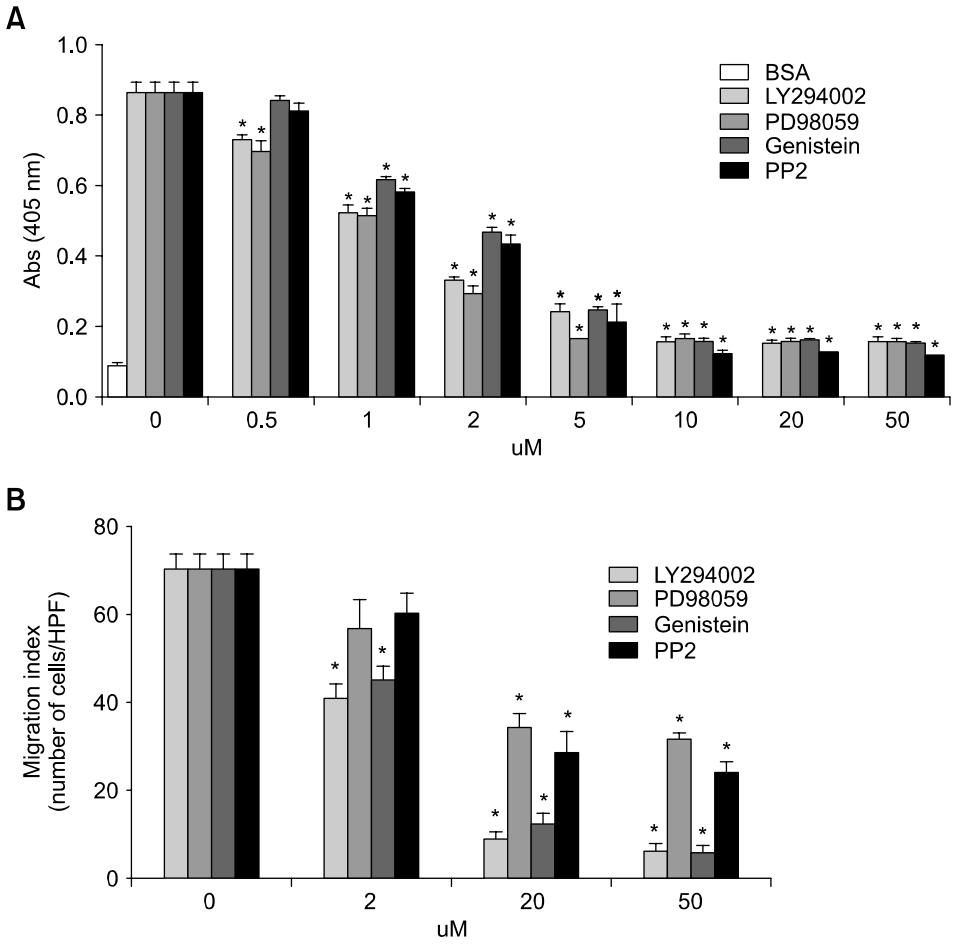


Figure 4. Adhesion and migration of aortic VSMCs on βig-h3 are inhibited by inhibitors for PI3K, ERK, and Src kinase. Cells were pre-incubated with PI3K, ERK, and Src kinase inhibitors at the indicated concentrations, and then plated onto culture dishes coated with βig-h3. After incubation for 1 h, cell adhesion (A) and migration (B) on the βig-h3-coated dishes were measured. Data represent mean ± SD of three separate experiments. *$P < 0.05$ by Student's *t*-test.

ERK, FAK and paxillin mediating adhesion and migration of VSMCs. These findings suggest that βig-h3 and the αvβ5 integrin play an important role in the adhesion, migration and proliferation of VSMCs that occur during neointima formation in atherosclerosis.

Alterations in extracellular matrix components have been described in atherosclerotic plaques and shown to influence cellular processes such as adhesion, migration and proliferation. For example, βig-h3 is elevated in human atherosclerotic and restenotic arteries, more abundantly in the intima than the media, and detected in the plaque macrophages as well as smooth muscle cells and endothelial cells (O'Brien *et al*., 1996). We also observed that βig-h3 is upregulated in human atherosclerotic plaques (data not shown). TGF-β isoforms, the potent inducers of βig-h3, and their receptors are increased in atherosclerotic lesions (Bobik *et al*., 1999). In the same context, we recently found that the expression of βig-h3 in cultured human monocyte-derived macrophages can be induced by tumor necrosis factor-α or IL-1β, inflammatory cytokines frequently increased in atherosclerotic microenvironment (data not published). Periostin, a matrix protein that is highly homologous to βig-h3, is also elevated in rat arteries after vascular injury and localized to smooth muscle cells of the neointima and adventitia and promotes migration of VSMCs (Lindner *et al*., 2005). Fas-1 domains of βig-h3 and related proteins such as periostin, stabilin-1, and stabilin-2 have been shown to interact with integrins and modulate cell adhesion and migration (Gillan *et al*., 2002; Kim *et al*., 2002; Politz *et al*., 2002). These Fas-1 domain-containing proteins thus seem to play a common role in cell adhesion and migration. In addition to Fas-1 domain-containing family of proteins, some matrix proteins including collagen, fibronectin, thrombospondin-1, vitronectin, tenascin, and osteopontin have been shown to be elevated in response to vascular injury or in atherosclerotic plaues and promote migration of VSMCs (Giachelli *et al*., 1993; Liaw *et al*., 1994; Batchelor *et al*., 1998; Willis *et al*., 2004). In fact, osteopontin transgenic mice develop atherosclerosis (Chiba *et al*., 2002). Taken together, VSMC

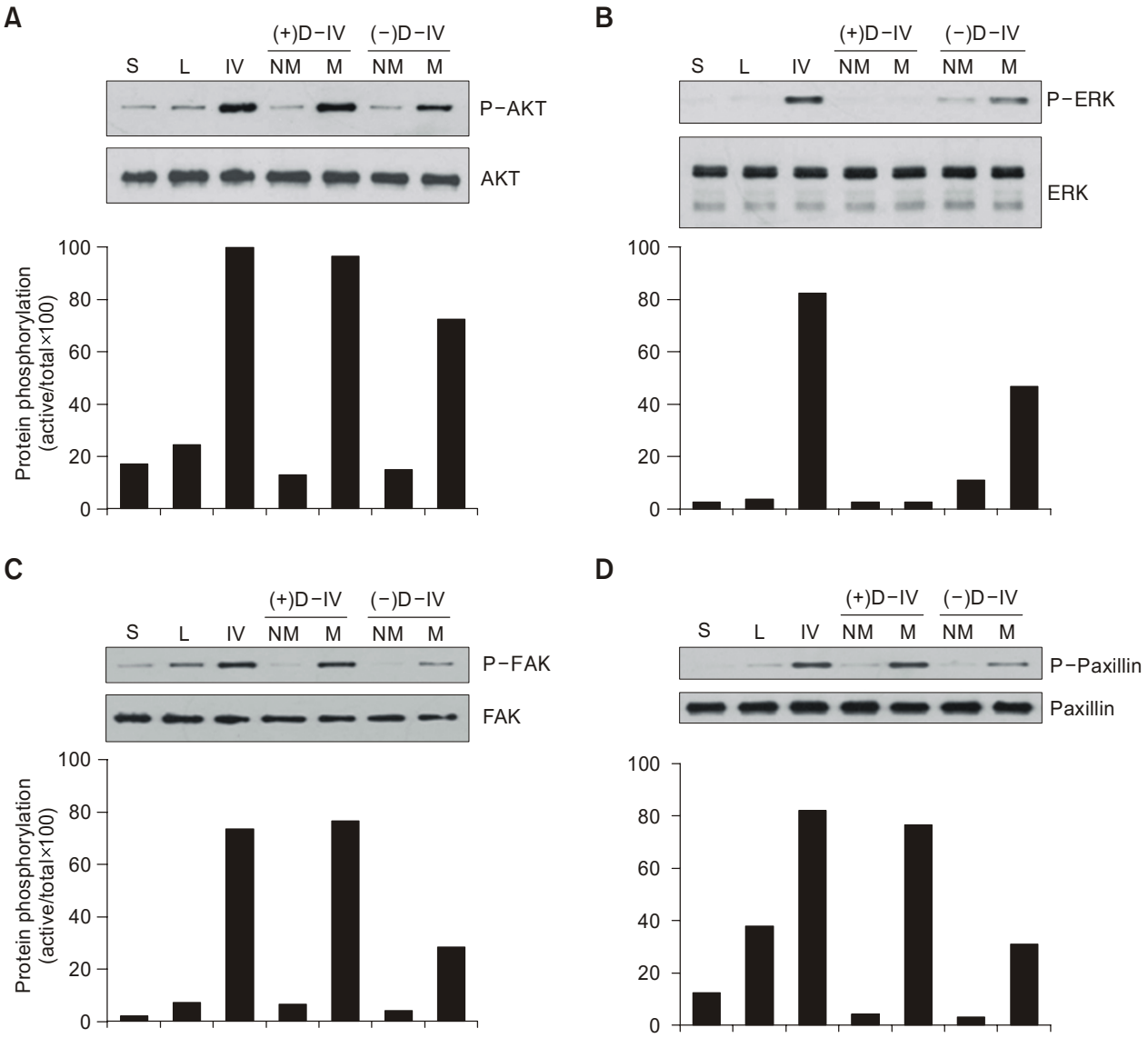


Figure 5. Phosphorylations of AKT, ERK, FAK, and paxillin are involved in the adhesion and migration of aortic VSMCs on βig-h3. For adhesion assays, cells were grown in suspension (S), or plated onto culture dishes coated with poly-L-lysin (L) or D-IV domain (IV). For migration, cells were grown in trans-well chamber coated with or without D-IV, and then migrating (M) and non-migrating (NM) cells were assayed, respectively. After incubation, cells were harvested, lysed, and then subjected to Western blotting using antibodies specific for phosphorylated or total form of AKT (A), ERK (B), FAK (C), and paxillin (D). Bars represent relative levels of phosphorylated protein normalized by total protein levels.

interaction with matrix proteins that are elevated in neointima as components of the atherosclerotic plaque may contribute to the pathogenesis of atherosclerosis and restenosis after injury.

Matrix proteins interact with distinct isoforms of integrins and promote cell adhesion and migration depending on cell types. For example, vitronectin has been shown to mediate VSMC adhesion mainly through the αvβ5 integrin but not through the αvβ3 integrin (Clyman *et al*., 1992; Kappert *et al*., 2001; Stawowy *et al*., 2004). Both the αvβ3 and αvβ5 integrins are elevated during neointima formation in injured aorta (Kappert *et al*., 2001). We showed here that similar to vitronectin, βig-h3 mediates VSMC adhesion and migration through the αvβ5 integrin but not the αvβ3 integrin. Migration of fibroblasts on βig-h3 is also facilitated through the αvβ5 integrin (Kim *et al*., 2002). On the other hand, βig-h3 promotes migration of keratinocytes and renal tubular epithelial cells through the α3β1 integrin (Bae *et al*., 2002; Park *et al*., 2004). Furthermore, we recently reported that βig-h3 promotes the migration

of vascular endothelial cells through the αvβ3 integrin, and a synthetic peptide containing the YH-18 motif of βig-h3 has anti-angiogenic activity by inhibiting the interaction between the αvβ3 integrin and βig-h3 (Nam *et al*., 2003). Given a key role of the αvβ5 integrin in VSMC migration on both βig-h3 and vitronectin that are elevated in atherosclerotic lesions, a function-blocking antibody against the αvβ5 integrin or a synthetic peptide containing the YH-18 motif could be a promising therapeutic approach against atherosclerosis.

Some tyrosine kinases and small GTP-binding proteins have been known to play a role in VSMC migration that is induced by growth factors and extracellular matrix (Abedi and Zachary, 1995; Willis *et al*., 2004). Platelet-derived growth factor, a potent inducer of VSMC migration, stimulates the phosphorylation of PI3K, ERK, FAK, and paxillin (Abedi *et al*., 1995; Cospedal *et al*., 1999). Hepatocyte growth factor induces VSMC migration by phosphorylating PI3K, Akt, ERK, FAK, and Pyk2 (Taher *et al*., 2002; Ma *et al*., 2003). We showed here that βig-h3 triggers phosphorylation and activation of Akt, FAK and paxillin but not that of ERK for mediating migration of VSMC through the interaction with the αvβ5 integrin. Phosphorylation of ERK, however, is still required in the adhesion of VSMCs to βig-h3. Given the importance of adhesion in cell migration, it would explain the decrease of cell migration by a ERK kinase inhibitor as shown in migration assays. Similar to βig-h3, vitronectin also triggers the activation of FAK and Akt but not of ERK (Stawowy *et al*., 2004). Taken together, although VSMC migration on matrix shares some signaling pathways in common, distinct signaling pathways are still involved in the migration depending on the nature of growth factors and extracellular matrix proteins.

Acknowledgement

This work was supported by BioMedical Research Institute grant, Kyungpook National University Hospital (2004) (to B-H.L.) and by a program of National Research Laboratory (M10104000036-01J0000-01610) (to I-S.K.).

References

Abedi H, Dawes KE, Zachary I. Differential effects of platelet-derived growth factor BB on p125 focal adhesion kinase and paxillin tyrosine phosphorylation and on cell migration in rabbit aortic vascular smooth muscle cells and Swiss 3T3 fibroblasts. J Biol Chem 1995;270:11367-76

bedi H, Zachary I. Signalling mechanisms in the regulation of vascular cell migration. Cardiovasc Res 1995;30:544-56

Bae JS, Lee SH, Kim JE, Choi JY, Park RW, Yong Park J, Park HS, Sohn YS, Lee DS, Bae Lee E, Kim IS. Betaig-h3 supports keratinocyte adhesion, migration, and proliferation through alpha3beta1 integrin. Biochem Biophys Res Commun 2002; 294:940-8

Batchelor WB, Robinson R, Strauss BH. The extracellular matrix in balloon arterial injury: a novel target for restenosis prevention. Prog Cardiovasc Dis 1998;41:35-49

Bobik A, Agrotis A, Kanellakis P, Dilley R, Krushinsky A, Smirnov V, Tararak E, Condron M, Kostolias G. Distinct patterns of transforming growth factor-beta isoform and receptor expression in human atherosclerotic lesions. Colocalization implicates TGF-beta in fibrofatty lesion development. Circulation 1999; 99:2883-91

Chiba S, Okamoto H, Kon S, Kimura C, Murakami M, Inobe M, Matsui Y, Sugawara T, Shimizu T, Uede T, Kitabatake A. Development of atherosclerosis in osteopontin transgenic mice. Heart Vessels 2002;16:111-7

Clyman RI, McDonald KA, Kramer RH. Integrin receptors on aortic smooth muscle cells mediate adhesion to fibronectin, laminin, and collagen. Circ Res 1990;67:175-86

Clyman RI, Mauray F, Kramer RH. Beta 1 and beta 3 integrins have different roles in the adhesion and migration of vascular smooth muscle cells on extracellular matrix. Exp Cell Res 1992;200:272-84

Cospedal R, Abedi H, Zachary I. Platelet-derived growth factor-BB (PDGF-BB) regulation of migration and focal adhesion kinase phosphorylation in rabbit aortic vascular smooth muscle cells: roles of phosphatidylinositol 3-kinase and mitogen-activated protein kinases. Cardiovasc Res 1999;41: 708-21

Dzau VJ, Braun-Dullaeus RC, Sedding DG. Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. Nat Med 2002;8:1249-56

Endo S, Nguyen TH, Fujiki K, Hotta Y, Nakayasu K, Yamaguchi T, Ishida N, Kanai A. Leu518Pro mutation of the beta ig-h3 gene causes lattice corneal dystrophy type I. Am J Ophthalmol 1999;128:104-6

Giachelli CM, Bae N, Almeida M, Denhardt DT, Alpers CE, Schwartz SM. Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. J Clin Invest 1993;92:1686-96

Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999; 285:1028-32

Gillan L, Matei D, Fishman DA, Gerbin CS, Karlan BY, Chang DD. Periostin secreted by epithelial ovarian carcinoma is a ligand for alpha (V) beta (3) and alpha (V) beta (5) integrins and promotes cell motility. Cancer Res 2002;62:5358-64

Ha SW, Bae JS, Yeo HJ, Lee SH, Choi JY, Sohn YK, Kim JG, Kim IS, Kim BW. TGF-beta-induced protein beta ig-h3 is upregulated by high glucose in vascular smooth muscle cells. J Cell Biochem 2003;88:774-82

Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002;110:673-87

Kappert K, Blaschke F, Meehan WP, Kawano H, Grill M, Fleck E, Hsueh WA, Law RE, Graf K. Integrins alphavbeta3 and alphavbeta5 mediate VSMC migration and are elevated during

neointima formation in the rat aorta. Basic Res Cardiol 2001;96:42-9

Kim JE, Kim EH, Han EH, Park RW, Park IH, Jun SH, Kim JC, Young MF, Kim IS. A TGF-beta-inducible cell adhesion molecule, betaig-h3, is downregulated in melorheostosis and involved in osteogenesis. J Cell Biochem 2000;77:169-78

Kim JE, Kim SJ, Lee BH, Park RW, Kim KS, Kim IS. Identification of motifs for cell adhesion within the repeated domains of transforming growth factor-beta-induced gene, betaig-h3. J Biol Chem 2000;275:30907-15

Kim JE, Jeong HW, Nam JO, Lee BH, Choi JY, Park RW, Park JY, Kim IS. Identification of motifs in the fasciclin domains of the transforming growth factor-beta-induced matrix protein betaig-h3 that interact with the alphavbeta5 integrin. J Biol Chem 2002;277:46159-65

LeBaron RG, Bezverkov KI, Zimber MP, Pavelec R, Skonier J, Purchio AF. Beta IG-H3, a novel secretory protein inducible by transforming growth factor-beta, is present in normal skin and promotes the adhesion and spreading of dermal fibroblasts *in vitro*. J Invest Dermatol 1995;104:844-9

Lee BH, Ruoslahti E. alpha (5) beta (1) integrin stimulates Bcl-2 expression and cell survival through Akt, focal adhesion kinase, and Ca(2+)/calmodulin-dependent protein kinase IV. J Cell Biochem 2005;95:1214-23

Liaw L, Almeida M, Hart CE, Schwartz SM, Giachelli CM. Osteopontin promotes vascular cell adhesion and spreading and is chemotactic for smooth muscle cells *in vitro*. Circ Res 1994;74:214-24

Libby P. Inflammation in atherosclerosis. Nature 2002;420: 868-74

Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135-43

Lindner V, Wang Q, Conley BA, Friesel RE, Vary CP. Vascular injury induces expression of periostin: implications for vascular cell differentiation and migration. Arterioscler Thromb Vasc Biol 2005;25:77-83

Ma H, Calderon TM, Kessel T, Ashton AW, Berman JW. Mechanisms of hepatocyte growth factor-mediated vascular smooth muscle cell migration. Circ Res 2003;93:1066-73

Munier FL, Korvatska E, Djemai A, Le Paslier D, Zografos L, Pescia G, Schorderet DF. Kerato-epithelin mutations in four 5q31-linked corneal dystrophies. Nat Genet 1997;15:247-51

Nam JO, Kim JE, Jeong HW, Lee SJ, Lee BH, Choi JY, Park RW, Park JY, Kim IS. Identification of the alphavbeta3 integrin-interacting motif of betaig-h3 and its anti-angiogenic effect. J Biol Chem 2003;278:25902-9

Newby AC, Zaltsman AB. Molecular mechanisms in intimal hyperplasia. J Pathol 2000;190:300-9

O'Brien ER, Bennett KL, Garvin MR, Zderic TW, Hinohara T, Simpson JB, Kimura T, Nobuyoshi M, Mizgala H, Purchio A, Schwartz SM. Beta ig-h3, a transforming growth factor-beta-inducible gene, is overexpressed in atherosclerotic and restenotic human vascular lesions. Arterioscler Thromb Vasc Biol 1996;16:576-84

Park SW, Bae JS, Kim KS, Park SH, Lee BH, Choi JY, Park JY, Ha SW, Kim YL, Kwon TH, Kim IS, Park RW. Beta ig-h3 promotes renal proximal tubular epithelial cell adhesion, migration and proliferation through the interactionwith alpha3beta1 integrin. Exp Mol Med 2004;36:211-9

Politz O, Gratchev A, McCourt PA, Schledzewski K, Guillot P, Johansson S, Svineng G, Franke P, Kannicht C, Kzhyshkowska J, Longati P, Velten FW, Goerdt S. Stabilin-1 and -2 constitute a novel family of fasciclin-like hyaluronan receptor homologues. Biochem J 2002;362:155-64

Skonier J, Bennett K, Rothwell V, Kosowski S, Plowman G, Wallace P, Edelhoff S, Disteche C, Neubauer M, Marquardt H, Rodgers J, Purchio AF. Beta ig-h3: a transforming growth factor-beta-responsive gene encoding a secreted protein that inhibits cell attachment *in vitro* and suppresses the growth of CHO cells in nude mice. DNA Cell Biol 1994;13:571-84

Stawowy P, Kallisch H, Veinot JP, Kilimnik A, Prichett W, Goetze S, Seidah NG, Chretien M, Fleck E, Graf K. Endoproteolytic activation of alpha (v) integrin by proprotein convertase PC5 is required for vascular smooth muscle cell adhesion to vitronectin and integrin-dependent signaling. Circulation 2004;109:770-6

Taher TE, Derksen PW, de Boer OJ, Spaargaren M, Teeling P, van der Wal AC, Pals ST. Hepatocyte growth factor triggers signaling cascades mediating vascular smooth muscle cell migration. Biochem Biophys Res Commun 2002;298:80-6

Willis AI, Pierre-Paul D, Sumpio BE, Gahtan V. Vascular smooth muscle cell migration: current research and clinical implications. Vasc Endovascular Surg 2004;38:11-23