BioChip J. (2016) 10(3): 174-181
DOI 10.1007/s13206-016-0303-8

Original Article

Anti-angiogenic Mechanism of IPS-05002, a Novel Antagonist against Integrin α5β1, Determined by ProteoChip-based Antibody Array

Min-A Kim[1] **& In-Cheol Kang**[1,2,*]

Received: 04 February, 2016 / Accepted: 19 February, 2016 / Published online: 10 June, 2016

Abstract IPS-05002, a novel antagonist of integrin α5β1, was screened from a phytochemical compound library using ProteoChip-based integrin α5β1-fibronectin interaction assay method. The novel compound inhibited VEGF-stimulated human umbilical vein endothelial cell (HUVEC) proliferation, adhesion, and migration in a dose-dependent manner. It also suppressed tubular network formation. Differential expression profiling of cell cycle proteins in VEGF-induced HUVECs in the presence of IPS-05002 showed up-regulation of IKB-β, XRCC4, and down-regulation of Cdc6 compared with HUVECs induced by VEGF alone. In conclusion, these data suggest that IPS-05002 will be a potent inhibitor for VEGF-mediated angiogenesis.

Keywords: Integrin, Angiogenesis, Natural products, Small molecule antagonist, VEGF, Antibody microarray

Introduction

Angiogenesis is the formation of new blood vessels from endothelial cells. It is the major process of wound healing, organ regeneration like physiological and pathological situation. Then, tumor neovascularization can be a sign of cancer growth, metastasis, and migration. Also, it is associated with basic fibroblast growth factors (bFGFs) and vascular endothelial growth factors (VEGFs)[1-6]. Integrins are heterodimeric glycoproteins that mediate transmembrane signals between extracellular signal molecules and an intracellular cytoskeleton in endothelial cells[7-10]. Several integrins including α5β1 and αvβ3/β5 are over-expressed around tumor neovasculature during angiogenic switch, leading to a new anti-angiogenic approach[11-13]. Interestingly, mature vasculatures express few or no integrin α5 subunit except for liver sinusoid and lymph nodes whereas tumor neovasculatures express high level of integrin α5 subunit[14-17]. Integrin α5β1 can interact with different ligands such as endostatin, VEGFR-1, Angiopoietin-2 and Tie-2 and their interaction modulates integrin α5β1-mediated angiogenic signaling[18-20]. Integrin α5 subunit enhances survival signals in endothelial cells whereas it blocks apoptotic signals in regardless of attachment to extracellular matrix *in vitro* and *in vivo*[21]. Therefore, blocking integrin α5 subunit with a small peptide or an antibody results in anti-angiogenic effects and inhibited tumor growth by integrin-mediated death pathway[22,23]. Due to its biological role in angiogenesis, integrin α5β1 has been known to be a therapeutic target for tumor angiogenesis. Several antagonists of integrin α5β1 have been demonstrated as angiogenesis inhibitors[24-28]. Our previous report also demonstrated an anti-angiogenic function of A5-1, a novel peptide antagonist against integrin α5β1 from a hexapeptide library, PS-SPCL[29].

In this paper, we have screened IPS-05002, a small molecule antagonist against integrin α5β1 from a phytochemical library using ProteoChip-based integrin α5β1-fibronectin binding assay system. We assessed anti-angiogenic function of these antagonists in differ-

[1]Department of Biological Science, College of Natural Science
[2]BioChip Research Center, Hoseo University, Asan 31499, Republic of Korea
*Correspondence and requests for materials should be addressed to I.-C. Kang (✉ickang@office.hoseo.ac.kr)

ent angiogenesis assays *in vitro* and *ex vivo* and investigated its cellular mechanism using antibody-arrayed proteomics technology.

Results

Inhibitory Effect of IPS-05002 on Integrin α5β1-fibronectin Interaction

In previous study, integrin α5β1 was immobilized on a ProteoChip surface by an automatic spotting machine (CM 1000, Proteogen) and interacted with Cy5-labeled fibronectin[32]. According to construction method of integrin α5β1 microarray in our previous report, the integrin α5β1 microarray was prepared for discovering IPS-05002, a small molecule antagonist against the integrin from a phytochemical compound library. As shown in Figure 1, it was demonstrated that IPS-05002 inhibited the integrin α5β1-fibronectin interaction in a dose-dependent manner. The chip-based method led to a screening of the small inhibitor IPS-05002, which appeared to be the most effective in suppressing the integrin-fibronectin interaction from the compound library. The chemical structures of IPS-05002(3-(4-fluorobenzyl)-6-hydroxy-5-(2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indol-1-yl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one) is as novel as that of the integrin inhibitors (Figure 1B).

Anti-angiogenic Function of IPS-05002

To examine the biological activity of IPS-05002 against integrin α5β1, *in vitro* VEGF-induced HUVEC proliferation, adhesion, and migration assay were carried out. IPS-05002 markedly suppressed the proliferation and adhesion of HUVEC compared with GRGDSP-treated positive control group and VEGF-treated control group (Figure 2A and 2B). The inhibitory efficacy of IPS-05002 is much higher than that of GRGDSP peptide. Half-maximal inhibition of the proliferation on endothelial cells by IPS-05002 was determined at a concentration of 20.5 ± 0.56 μM, whereas GRGDSP peptide appeared to be an IC_{50} value of more than 1.7 mM (Figure 2A). IPS-05002 inhibited HUVEC adhesion onto Matrigel-coated surface in a dose-dependent manner (Figure 2B). Further attempts were made to examine the effects of IPS-05002 on HUVEC migration onto Matrigel-coated matrix (Figure 3A). IPS-05002 significantly perturbed VEGF-induced HUVEC migration compared with GRGDSP-treated control group in a dose and time-dependent fashion. Half-maximal suppression of HUVEC migration by IPS-05002 was observed to be 15.52 μM (Figure 3A). To assess the anti-migration efficacy of IPS-05002, *in vitro* capillary tube formation assay using HUVECs was employed. HUVECs seeded on Matrigel-coated plate were treated for 16 h with IPS-05002 or GRGDSP peptide in the presence of VEGF, and the formation of tubular structure using the HUVECs was observed by a phase-contrast microscopy. IPS-05002 completely suppressed VEGF-induced capillary tube formation at a concentration of 50 μM whereas GRGDSP peptide inhibited tubular network formation at a dose of 8.5 mM (Figure 3B). It is noted that the inhibitory effect of IPS-05002 appears to be much higher than that of GRGDSP peptide. Taken together, these data suggest that IPS-05002, a newly screened antagonistic small molecule compound against integrin α5β1, should be a potent angiogenesis inhibitor.

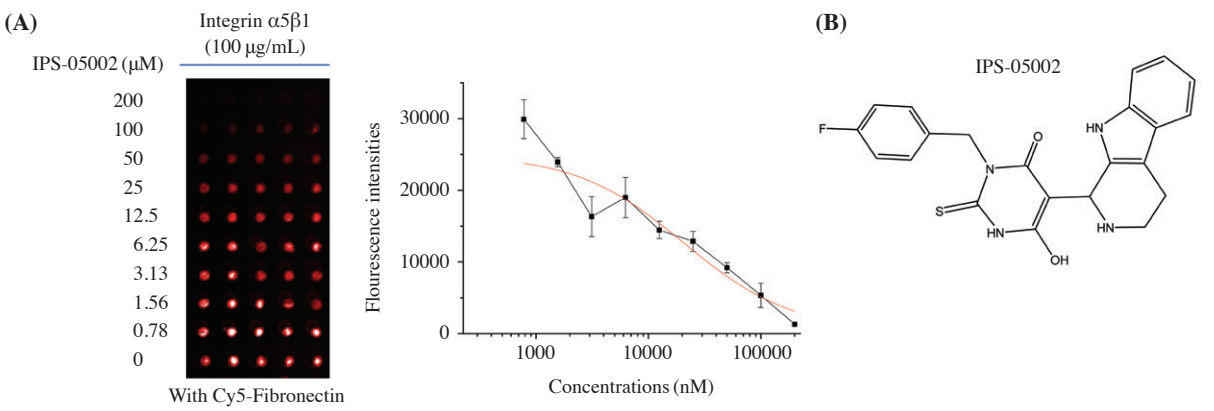


Figure 1. Antagonistic effect of IPS-05002 on the integrin α5β1-fibronectin interaction. (A) The integrin α5β1 microarray was incubated with different concentrations of IPS-05002 mixed with Cy5-labeled fibronectin. Scanned images of integrin α5β1-fibronectin interaction inhibited by IPS-05002 were observed in a dose-dependent manner. (B) Structure of IPS-05002 selected from the ProteoChip-based screening was shown in the right panel.

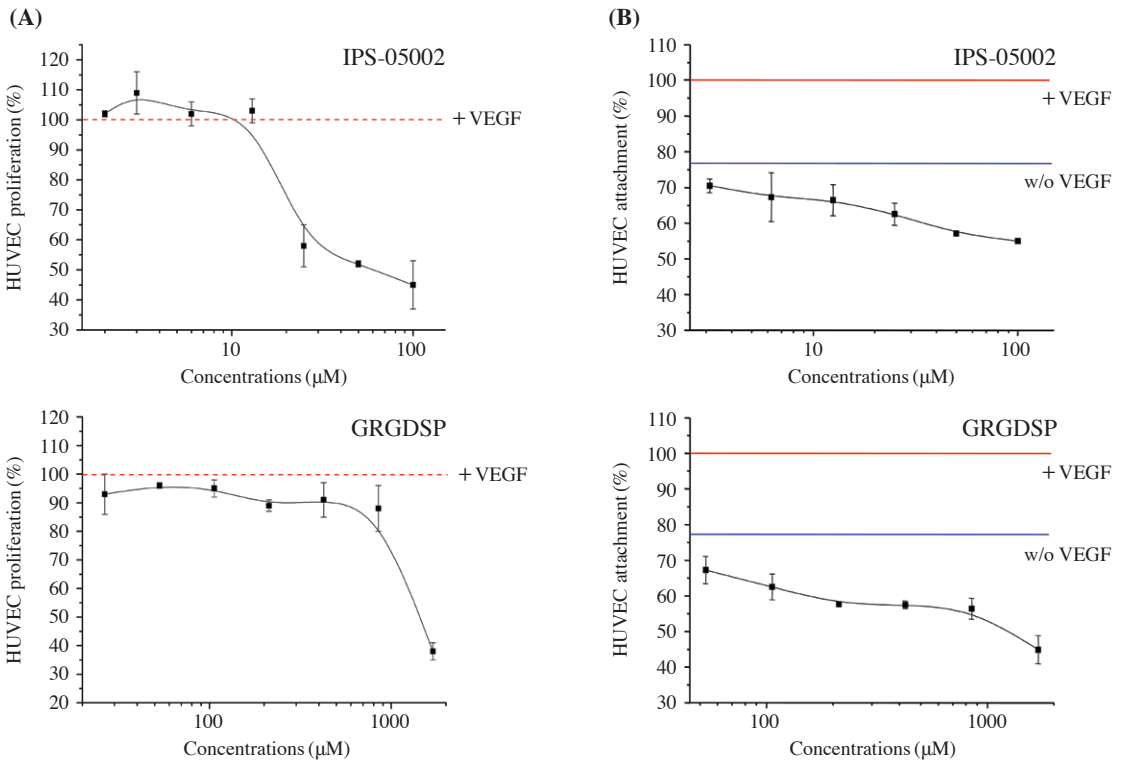
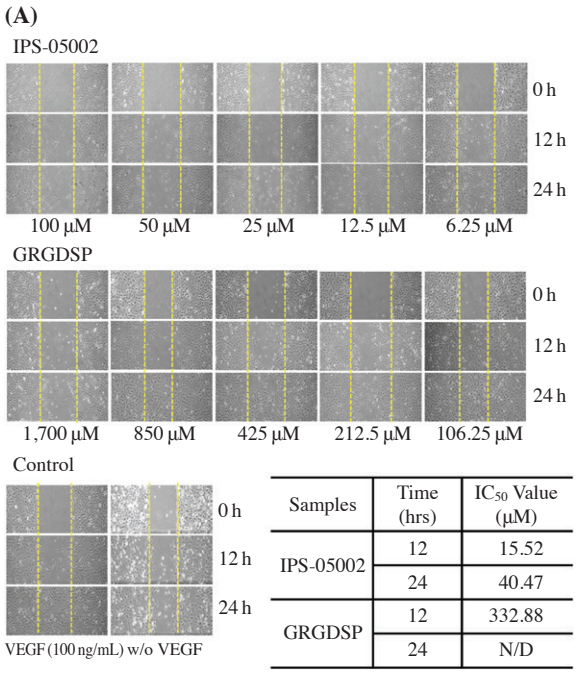


Figure 2. Inhibitory effects of IPS-5002 on HUVEC proliferation and adhesion. (A) HUVECs were incubated with different concentrations of IPS-05002 and RGD for 72 hr in the presence of VEGF (10 ng/mL). After incubation, unbound cells were removed with PBS and incubated for 2 hr with 0.1 M MTT solution. Absorbance was measured at 595 nm using an ELISA reader. (B) HUVECs were incubated with different concentrations of IPS-05002 and RGD for 90 min in the presence of VFGF (100 ng/mL) on Matrigel-coated 96-well ELISA plates. After incubation, unbound cells were removed with PBS, and attached cells were fixed with methanol and stained with crystal violet.

Proteomic Analysis of Anti-proliferative Effect of IPS-05002 in HUVECs

To investigate the anti-angiogenic mechanism of IPS-05002 in VEGF-stimulated HUVECs, we examined differential expression profiling of cell signaling proteins in HUVECs treated with IPS-05002 using IPS-Forwarded Phase Antibody Array containing selected 48 antibodies against cell signaling proteins. HUVEC lysates obtained from IPS-05002 and VEGF-treated HUVECs or VEGF-treated HUVECs were labeled with Cy3 or Cy5, respectively. Both lysates were mixed and spotted on the antibody microarray. The fluorescence intensities of the spots were detected using a fluorescence scanner, and the differential protein expression pattern between the two samples was then observed. To identify proteins that appeared changed expression levels in response to IPS-05002, the distribution of fluorescence intensities (Cy3 and Cy5) for all of the spots was normalized with the mean values of the Cy5 : Cy3 ratios of the protein spots. The average normalized median of ratios was used to represent the expression levels of each protein (Figure 4A). Based on the data from three independent experiments, IkB-β and XRCC4 were defined as up-regulated and Cdc6 was down-regulated in response of IPS-05002 in HUVECs (Figure 4B). The levels of all other proteins were unchanged according to the antibody microarray analysis. To validate the antibody microarray results, immunoblot assay was employed. IkB-β and XRCC4 were proteins up-regulated and Cdc6 was down-regulated in HUVECs treated with IPS-05002, as analyzed by Western blotting (Figure 4C). These proteins are presented as a basal level in HUVECs in the absence of VEGF treatment. There was no change in the expression level of IkB-β in the presence or absence of VEGF[33]. This finding confirms that the immunoblotting data are consistent with those obtained from the antibody microarray. IkB-β is NF-kB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) inhibitor protein. NF-kB is cell inflammation signaling transcription factor and plays a role in an angiogenesis signaling via ex-



Samples	Time (hrs)	IC_{50} Value (μM)
IPS-05002	12	15.52
	24	40.47
GRGDSP	12	332.88
	24	N/D

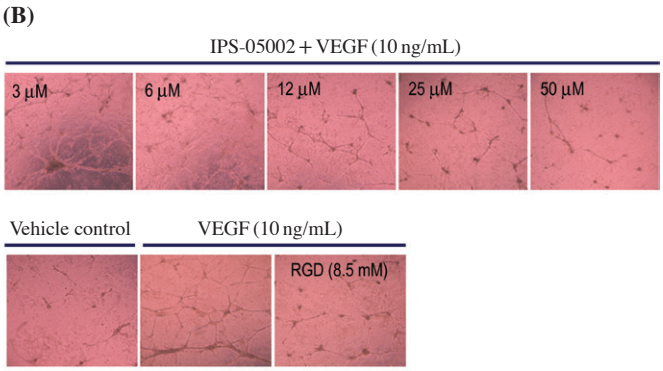


Figure 3. Inhibitory effects of IPS-05002 on HUVEC migration and capillary tube formation. (A) Dose-dependent inhibition of HUVECs migration by IPS-05002 and GRGDSP was examined. The width of wound area was captured using microscopy taken every 2 hours. (B) HUVECs were seeded on Matrigel in 24-well plates in serum-free media containing IPS-05002 in the presence of VFGF (10 ng/mL).

pression of various target gene[34,35]. XRCC4 is known to be DNA repair protein and Cdc6 is an important protein of DNA replication promotion through G1[36,37]. Taken together, these data strongly suggest that the up-regulation of IkB-β and XRCC4, and the down-regulation of Cdc6 in response to IPS-05002 in VEGF-stimulated HUVECs may result in the suppression of the angiogenic events of the cells.

Discussion

We have discovered IPS-05002, a small molecule antagonist against integrin α5β1 from a phytochemical library using a ProteoChip-based protein-protein interaction (PPI) assay system, which is a new PPI inhibitor screening technology based on competition of protein-protein interaction (Figure 1). A ProteoChip was used for a library screening of novel antagonistic peptides against integrin αvβ3 and integrin α5β1 that showed an anti-angiogenic activity[29,32]. In this study, we have demonstrated anti-angiogenic function of IPS-05002 using *in vitro* HUVEC-based assays and *ex vivo* CAM angiogenesis assay. HUVE cell proliferation, migration, adhesion, tubular network formation were also inhibited by IPS-05002 (Figures 1-3). These results suggest that the blocking of integrin α5β1 by IPS-05002 may result in suppression of HUVEC-based angiogenic events. We then investigated its anti-proliferative mechanism using an antibody microarray. In our previous reports, we demonstrated that proteomic approach using our proprietary antibody microarray technology, which was constructed on a ProteoChip was applicable for analysis of the expression patterns of endogenous cell signaling proteins in HUVECs and NIH3T3 cells[38-41]. Using the antibody array-based proteomic analysis, we investigated that the anti-proliferative mechanism of IPS-05002 recognizing integrin α5β1 was due to up-regulation of IkB-β and XRCC4 and down-regulation of Cdc6 in HUVECs (Figure 4). In this work, several lines of experimental evidence strongly support that IPS-05002, a novel small molecule antagonist of integrin α5β1, may be a potent inhibitor of angiogenesis.

Materials and Methods

Prefabrication of Integrin α5β1 Microarray

The ProteoChip integrin microarray was prefabricated

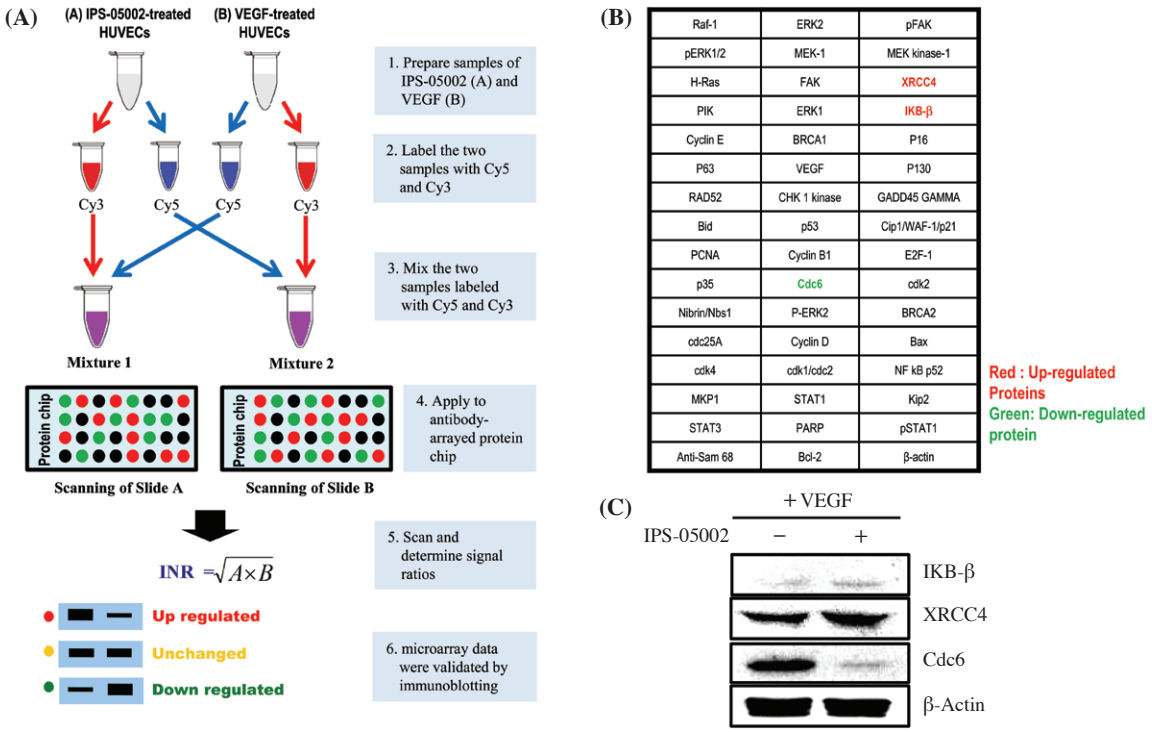


Figure 4. Proteomic analysis of cellular signaling proteins in IPS-05002-treated HUVECs using an antibody arrayed-protein chip. (A) Schematic representation of cell signaling protein profiling in the compound-treated HUVECs using antibody microarray chip. The cell lysate labeled with Cy5 or Cy3 were prepared and incubated on antibody microarray for 1 h at 37°C (B) The antibody map of the antibody array chip. (C) The protein expression data obtained from the antibody microarray was confirmed by immunoblot analysis. Western blots were developed using an ECL system (Santa Cruz Biotechnology) for the detection of signals.

by a robotic arrayer (CM-2000, Proteogen Inc, Korea) equipped with Stealth Micro-spotting Pins (SMP10, Tele-Chem, CA). ProLinker^{TM}-coated ProteoChip (Proteogen, Korea) was spotted on integrin α5β1 (100 μg/mL diluted with 30% glycerol in PBS). Integrin α5β1 (Millipore, USA) microarray was incubated at 4°C for 16 hrs and washed three times with PBST (0.05% Tween 20 in PBS). The integrin microarray was stored at 4°C until use.

Competitive Binding Assay Using Integrin α5β1 Microarray

Integrin α5β1 microarray was blocked with 3% BSA for 1 h and washed three times with PBST. Fibronectin (BD Bioscience, USA) was labeled with a fluorescent dye, Cy5 (Sigma, USA) according to the manufacturer's instruction[30]. Cy5-labeled fibronectin mixed with different concentrations of IPS-05002 was spotted on the integrin microarray at 37°C. After incubation for 1 h, integrin microarray chip was rinsed three times with PBST. Fluorescence intensities of the spots on the integrin chip were detected by a fluorescence laser scanner. We used GRGDSP peptide (Peptron, Korea) and Cy5-labeled or non-labeled fibronectin as positive control.

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were purchased from InnoPharmaScreen Inc. (Asan, Korea). HUVECs were cultured in a complete M199 medium (Wellgene, Korea) in a humidified atmosphere of 5% CO_2 at 37°C.

HUVEC Proliferation Assay *in Vitro*

HUVEC Cell proliferation was confirmed using a MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2 H-tetrazoliumbromide, Sigma, USA] assay protocol. Gelatin (Junsei, Japan)-coated 96-well tissue culture plates were cultured with HUVECs (2×10^3 cells/well) overnight. IPS-05002 was treated to cells in the presence of VFGF (Vexxon, Korea) in a dose-dependent manner. After incubation for 72 h, MTT solution (50 μg/well) was added to each well and incubated for 2 h at 37°C. After the supernatant was discarded, formazan crystals were dissolved in 100 μL of DMSO. The absorbance

of the plate was measured at 595 nm using an ELISA reader (Molecular Devices, USA). The presented data were measured in triplicate.

HUVEC Adhesion Assay

Ninety six-well plate (NUNC, USA) was coated with 40 μg/mL of Matrigel (BD science, USA) diluted in PBS for overnight at 4°C. Each well was washed twice with PBS and blocked for 1 hr at room temperature (RT) with 1% BSA in PBS. After washing twice with PBS, HUVECs (2×10^4 cells) mixed with IPS-05002 or RGD in the presence of VEGF were seeded on each well and incubated for 90 min at 37°C in 5% CO_2. After incubation, unbound cells were removed each well twice with PBS and bounded cells were stained with the staining solution (50% methanol, 10% acetic acid, 0.2% comassie blue in DW) for 10 min at RT. The absorbance of each well was measured at 595 nm using an ELISA reader (Molecular Devices, USA). VEGF-control cells were incubated in the presence of VEGF alone.

Wound-healing HUVEC Migration Assay

Six-well culture dish (SPL, USA) was added with HUVECs (5×10^5 cells). A wound area was generated with a sterilized 200 μL micropipette tip in confluent cultures of HUVECs on the dish. After rinsing of cellular debris with PBS, IPS-05002 in different concentrations was treated in the presence or absence of VFGF in the cells. The width of wound area was photographed every 2 h using an inverted microscopy (Olympus, Japan).

Capillary Tubular Network Formation Assay

Capillary tube formation of HUVECs was performed as described with modification[31]. Twenty four-well culture plate was coated with Matrigel at 37°C for 30 min. Each well was added to HUVECs (7.5×10^4 cells) in the presence of IPS-05002 (100, 50, 25, 12.5 and 6.25 μM) or GRGDSP peptide (1700, 850, 425, 212.5 and 106.25 μM) as a positive control. After incubation for 18 h, the capillary tubes were stained with Diff-Quick solution (Becton Dickinson, San Jose, CA) and observed under a phase-contrast microscope.

Protein Extraction and Labeling

When HUVECs reached confluence, they were serum starved by incubation in M199 containing 1% FBS for 8 hr. The cells were treated with or without IPS-05002 in the presence of VEGF. After 24 hr incubation, the cells were washed twice with PBS and harvested in 5 mM EDTA-PBS. The harvested cells were then maintained on ice for 10 min and centrifuged for 5 min at 1,300 rpm. The cell pellet was washed with PBS and recentrifuged. HUVECs lysates were extracted with Lysis-M™ (Roche, Germany) mammalian cell extraction buffer. Each protein was labeled with Cy3 and Cy5 (GE healthcare, UK) as manufacturer's manual. Free dyes were removed by a Spin column (S5059, Sigma, USA) and stored at 4°C.

Expression Profile Analysis of Cell Cycle Proteins Using Protein Chip-based Antibody Array

We conducted an antibody array using protein A (200 μg/mL)-coated ProteoChip. Specific antibodies were chosen against cell cycle-related proteins in HUVECs. Each antibody was arrayed on the chip by 10-fold dilution in 30% glycerol. After overnight at 4°C, the antibody chip was blocked with 3% BSA in PBS for 1 hr at room temperature. After rinsing with PBST (0.05% tween-20 in PBS), the chip was dried and stored at 4°C until use. Applying the labeled cell lysates diluted in the reaction buffer solution to the antibody array was carried out and incubated for 1 h at 37°C in the dark room. After reaction, the chip was rinsed with PBST and dried. Fluorescence intensity of the antibody microarray was detected by Gene-Pix 4100A microarray Scanner (Axon Instruments, CA) with 532 and 635 nm laser. Scanned images were analyzed with GenePix Pro (version 6.0) software package (Axon instruments) and data were analyzed with Excel (Microsoft, Redmond, WA) and Origin 6.1.

Acknowledgements This research was supported by the Academic Research fund of Hoseo University in 2013 (2013-0033).

References

1. Folkman, J. & Shing, Y. Angiogenesis. *J. Biol. Chem.* **267**, 10931-10934 (1991).
2. Folkman, J. Angiogenesis and apoptosis. *Semin. Cancer Biol.* **13**, 159-167 (2003).
3. Fan, T.P., Jaggar, R. & Bicknell, R. Controlling the vasculature: angiogenesis, anti-angiogenesis and vascular targeting of gene therapy. *Trends Pharmacol. Sci.* **16**, 57-66 (1995).
4. Naumov, G.N., Akslen, L.A. & Folkman, J. Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switc. *Cell Cycle*. **5**, 1779-1787 (2006).
5. Nguyen, M. Angiogenic factors as tumor markers. *Invest New Drugs* **15**, 29-37 (1997).

6. Rak, J., Yu, J.L., Klement, G. & Kerbel, R.S. Oncogenes and angiogenesis: signaling three-dimensional tumor growth. *J. Investig. Dermatol. Symp. Proc.* **5**, 24-33 (2000).
7. Avraamides, C.J., Garmy-Susini, B. & Varner, J.A. Integrins in angiogenesis and lymphangiogenesis. *Nat. Rev. Cancer* **8**, 604-617 (2008).
8. Raymond, K., Faraldo, M.M., Deugnier, M.A. & Glukhova, M.A. Integrins in mammary development. *Semin. Cell Dev. Biol.* **23**, 599-605 (2012).
9. Barczyk, M., Carracedo, S. & Gullberg, D. Integrins. *Cell Tissue Res.* 339, 269-280 (2010).
10. Campbell, I.D. & Humphries, M.J. Integrin structure, activation, and interactions. *Cold Spring Harb. Perspect. Biol.* **3**, a004994 (2011).
11. Avraamides, C.J., Garmy-Susini, B. & Varner, J.A. Integrins in angiogenesis and lymphangiogenesis. *Nat. Rev. Cancer* **8**, 604-617 (2008).
12. Carlson, T.R., Hu, H., Braren, R., Kim, Y.H. & Wang, R.A. Cell-autonomous requirement for beta1 integrin in endothelial cell adhesion, migration and survival during angiogenesis in mice. *Development* **135**, 2193-2202 (2008).
13. Schaffner, F., Ray, A.M. & Dontenwill, M. Integrin α5β1, the Fibronectin Receptor, as a Pertinent Therapeutic Target in Solid Tumors. *Cancers* **5**, 27-47 (2013).
14. Kim, S., Bell, K., Mousa, S.A. & Varner, J.A. Regulation of angiogenesis in vivo by ligation of integrin alpha5beta1 with the central cell-binding domain of fibronectin. *Am. J. Pathol.* **156**, 1345-1362 (2000).
15. Magnussen, A. *et al*. Rapid access of antibodies to alpha5beta1 integrin overexpressed on the luminal surface of tumor blood vessels. *Cancer Res.* **65**, 2712-2721 (2005).
16. Zhang, H., Li, C. & Baciu, P.C. Expression of integrins and MMPs during alkaline-burn-induced corneal angiogenesis. *Invest. Ophthalmol. Vis. Sci.* **43**, 955-962 (2002).
17. Bussolati, B., Deambrosis, I., Russo, S., Deregibus, M.C. & Camussi, G. Altered angiogenesis and survival in human tumor-derived endothelial cells. *FASEB J.* **17**, 1159-1161 (2003).
18. Sudhakar, A. *et al*. Human tumstatin and human endostatin exhibit distinct antiangiogenic activities mediated by αvβ3 and α5β1 integrins. *Proc Nat Acad Sci U S A* **100**, 4766-4771 (2003).
19. Orecchia, A. *et al*. Vascular endothelial growth factor receptor-1 is deposited in the extracellular matrix by endothelial cells and is a ligand for the α5β1 integrin. *J. Cell. Sci.* **116**, 3479-3489 (2003).
20. Felcht, M. *et al*. Angiopoietin-2 differentially regulates angiogenesis through TIE2 and integrin signaling. *J. Clin. Invest.* **122**, 1991-2005 (2012).
21. Kim, S., Bakre, M., Yin, H. & Varner, J.A. Inhibition of endothelial cell survival and angiogenesis by protein kinase A. *J. Clin. Invest.* **110**, 933-941 (2002).
22. Stupack, D., Puente, X.S., Boutsaboualoy, S., Storgard, C.M. & Cheresh, D.A. Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. *J. Cell Biol.* **155**, 459-470 (2001).
23. Bhaskar, V. *et al*. A function blocking anti-mouse integrin alpha5beta1 antibody inhibits angiogenesis and impedes tumor growth in vivo. *J. Transl. Med.* **5**, 1-11 (2007).
24. Goodman, S.L. & Picard, M. Integrins as therapeutic targets. *Trends Pharmacol. Sci.* **33**, 405-412 (2012).
25. Smallheer, J.M. *et al*. Synthesis and biological evaluation of nonpeptide integrin antagonists containing spirocyclic scaffolds. *Bioorg Med Chem Lett.* **14**, 383-387 (2004).
26. Bhaskar, V. *et al*. a chimeric integrin alpha5beta1 antibody, inhibits the growth of VX2 tumors in rabbits. *Invest. New Drugs* **26**, 7-12 (2008).
27. Livant, D.L. *et al*. The PHSRN sequence induces extracellular matrix invasion and accelerates wound healing in obese diabetic mice. *J. Clin. Invest.* **105**, 1537-1545 (2000).
28. Zeng, Z.Z. *et al*. alpha(5)beta(1) Integrin Ligand PHSRN Induces Invasion and alpha(5) mRNA in Endothelial Cells to Stimulate Angiogenesis. *Transl. Oncol.* **2**, 8-20 (2009).
29. Kim, E.Y., Bang, J.Y., Chang, S.I. & Kang, I.C. A novel integrin α5β1 antagonistic peptide, A5-1, screened by Protein Chip system as a potent angiogenesis inhibitor. *Biochem. Biophys. Res. Commun.* **377**, 1288-1293 (2008).
30. Sukhanov, S. & Delafontaine, P. Protein chip-based microarray profiling of oxidized low density lipoprotein-treated cells. *Proteomics* **5**, 1274-1280 (2005).
31. Koch, S. & Claesson-Welsh, L. Signal transduction by vascular endothelial growth factor receptors. *Cold Spring Harb. Perspect. Med.* **2**, a006502 (2012).
32. Lee, Y., Kang, D.K., Chang S.I., Han, M.H. & Kang, I.C. High-throughput screening of novel peptide inhibitors of an integrin receptor from the hexapeptide library by using a protein microarray chip. *J. Biomol. Screen.* **9**, 687-694 (2004).
33. Mechtcheriakova, D. *et al*. Specificity, diversity, and convergence in VEGF and TNF-a signaling events leading to tissue factor upregulation via EGR-1 in endothelial cells. *FASEB J.* **15**, 230-242 (2001).
34. Hayden, M.S. & Ghosh, S. Shared principles in NF-kappaB signaling. *Cell* **132**, 344-362 (2008).
35. Hayden, M.S. & Ghosh, S. Regulation of NF-κB by TNF family cytokines. *Semin. Immunol.* **26**, 253-266 (2014).
36. Herrero, A.B., San Miguel, J. & Gutierrez, N.C. Deregulation of DNA double-strand break repair in multiple myeloma: implications for genome stability. *PLoS One* **10**, e0121581 (2015).
37. Speck, C., Chen, Z., Li, H. & Stillman, B. ATPase-dependent cooperative binding of ORC and Cdc6 to origin DNA. *Nat. Struct. Mol. Biol.* **12**, 965-971 (2005).
38. Ahn, E.H. *et al*. Profiling of Differential Protein Expression in angiogenin-Induced HUVECs using Antibody-Arrayed ProteoChip. *Proteomics* **6**, 1104-1109

(2006).
39. Ajjappala, B.S. *et al*. Protein chip analysis of pluripotency-associated proteins in NIH3T3 fibroblast. *Proteomics* **9**, 3968-3978 (2009).
40. Kang, H., Park, B.R., Yoo, H.S., Kwon, K.R. & Kang, I.C. Anti-angiogenic function of a Korean Ginseng and Toad venom complex, Doksamsumsu-dan (DSSSD) analyzed by a forwarded phase antibody microarray. *BioChip Journal* **9**, 222-231 (2015).
41. Bang, J.Y. *et al*. Pharmacoproteomic Analysis of a Novel Cell-permeable Peptide Inhibitor of Tumor-induced Angiogenesis. *Mol. Cell. Proteomics* **10**, 10. 1074/mcp.M110.005264.1-11 (2011).