Korean J Biol Sci 8: 27-32, 2004

Fibronectin-Dependent Cell Adhesion is Required for Shear-Dependent ERK Activation

Heonyong Park*, Jaeyoung Shin, Jung Weon Lee[1] and Hanjoong Jo[2]

Department of Molecular Biology and Institute of Nanosensor and Biotechnology, Dankook University, Seoul 140-714, Korea;
[1]Cancer Research Institute, Seoul National University, Seoul 110-799, Korea;
[2]Division of Cardiology, Emory University School of Medicine, Atlanta, GA 30322, USA

Key Words:

Shear stress
Extracellular matrix
Extracellular signal
-activated regulated kinase
Fibronectin
Endothelial cells

Endothelial cells are subjected to hemodynamic shear stress, the dragging force generated by blood flow. Shear stress regulates endothelial cell shape, structure, and function, including gene expression. Since endothelial cells must be anchored to their extracellular matrices (ECM) for their survival and growth, we hypothesized that ECMs are crucial for shear-dependent activation of extracellular signal-activated regulated kinase (ERK) that is important for cell proliferation. Shear stress-dependent activation of ERK was observed in cells plated on two different matrices, fibronectin and vitronectin (the two most physiologically relevant ECM in endothelial cells). We then treated bovine aortic endothelial cells (BAECs) with Arg-Gly-Asp (RGD) peptides that block the functional activation of integrin binding to fibronectin and vitronectin, and a nonfunctional peptide as a control. Treatment of cells with the RGD peptides, but not the control peptide, significantly inhibited ERK activity in a concentration-dependent manner. This supports the idea that integrin adhesion to the ligands, fibronectin and vitronectin, mediates shear stress-dependent activation of ERK. Subsequently, whereas antagonists of vitronectin (LM 609, an antibody for integrin $\alpha_v\beta_3$ and XT 199, an antagonist specific for integrin $\alpha_v\beta_3$) did not have any effect on shear-dependent activation of ERK, antagonists of fibronectin (a neutralizing antibody for integrin $\alpha_5\beta_1$ or $\alpha_4\beta_1$ and SM256) had an inhibitory effect. These results clearly demonstrate that mechanoactivation of ERK requires anchoring of endothelial cells to fibronectin through integrins.

Hemodynamic shear stress regulates physiological functions of endothelial cells lining the interface between the inner vessel and blood. Shear stress is recognized by the endothelial cells, and regulaes vascular tone, vessel wall remodeling, cell adhesion, coagulation, and fibrinolysis (Davies, 1995). Shear stress also plays an important role in the anti-atherogenic process, because the focal atherosclerotic lesions occur in areas of unstable shear stress (Zarins et al., 1983; Ku et al., 1985). Shear stress has been shown to regulate a variety of cellular events, such as activation of heterotrimeric G-protein (Gudi et al., 1996), activation of K^+ channel (Olsen et al., 1988), production of inositol phosphate (Nollert et al., 1990), regulation of gene expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, platelet-derived growth factor-B, basic fibroblast growth factor, transforming growth factor β-1, tissue plasminogen activator, endothelial nitric oxide synthase and endothelin (Davies, 1995).

The extracellular matrices (ECMs) are synthesized and secreted by cells, support endothelium of the blood vessels and attach to integrins of the cell membrane. Accordingly, ECMs play key roles in physiological functions regulated by integrins. Integrins are expressed in most mammalian cells and different cells produce some different types of integrins (Hynes, 1992). Functionally, integrins have been found to mediate a series of signal transduction responsible for cell survival, growth, and differentiation and also play a crucial role in recognizing environmental stimuli. Some integrins are critical for cellular responses to physical stimuli via mitogen-activated protein kinases (MAP kinases). For example, integrin β3 subunit has been shown to mediate shear-dependent vasodilation (Muller et al., 1997). Therefore, it is highly possible that ECMs reglulate shear-dependent activation of MAP kinases.

*To whom correspondence should be addressed.
Tel: 82-2-709-2990, Fax: 82-2-793-0176
E-mail: heonyong@dankook.ac.kr

Mitogen-activated protein kinase family members are activated by various external stimuli such as growth factors (nerve and epidermal growth factors), ligands acting on G-protein-coupled receptors (α2-adrenergic agonists and lysophosphatidic acid) and physical stresses (ultraviolet radiation and hyperosmolarity) (Karin, 1994; Cobb et al., 1995). We have shown that shear stress differentially modulates activities of extracellular signal-activated regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in bovine aortic endothelial cells (BAECs) (Tseng et al., 1995; Pearce et al., 1996; Jo et al., 1997). MAP kinases control a variety of important cell signal transductions for cell proliferation, cell cycle progress and cell death. Previously we investigated that caveolin-1, a principal protein for plasma membrane microdomains called caveolae, regulates the shear-mediated ERK activation (Park et al., 1998; Park et al., 2000). Interestingly, both caveolin-1 and integrin regulate ERK activation. Caveolin-1 has structural properties to interact with diversity of proteins (Kim et al., 2003). Therefore, it is highly possible for the two proteins to be responsible for the shear-mediated activation of ERK.

Multiple fibronectin-binding integrins including integrin $\alpha_4\beta_1$ and RGD-directed integrins regulate ERK activity in response to stretch (MacKenna et al., 1998). Accordingly, determination of the detailed upstream signaling pathways for the shear-dependent activation of ERK provides an insight into cellular sensing systems for a physical force, shear stress. This further helps to understand the mechanisms by which shear stress works as an anti-atherogenic factor. Here, we took a variety of approaches to identify the extracellular matrix responsible for the shear-stimulated MAP kinase signaling and we also addressed whether RGD & SM256-dependent integrins are involved in shear-stimulated ERK pathway.

Materials and Methods

Cell culture and cell adhesion assay

BAEC obtained from descending thoracic aortas were maintained in a growth medium (DMEM (1 g/liter glucose, Life technologies, Inc.) containing 20% fetal bovine serum (FBS, Atlanta Biologicals) without antibiotics) at 37°C and 5% CO_2 (Ohno et al., 1995). Cells used in this study were between passages 3 and 10. For shear stress exposure, one million cells were seeded in glass slides (75×38 mm, Fisher), grown overnight and starved in a starvation medium (DMEM containing 0.5% fetal bovine serum). Next day the cells were exposed to shear stress. When RDG peptides or various integrin antagonists were used, peptides were added to starvation medium 2 h before the shear stress exposure. For cell adhesion assay, glass slides were coated with 1.4 $\mu g/cm^2$ various matrix proteins (vitronectin (VN), fibronectin (FN)) or poly-L-lysine (40 μg/plate) for 18 h. Confluent BAEC on T75 tissue culture flask (bought from Costar) were incubated for 1 h in 4 mM ethylenediamine tetra-acetic acid (EDTA) at 37°C and detached from plates by gentle pipetting. Then the cells were harvested by centrifugation at 1,000 rpm and washed with DMEM. Three million cells were plated on a glass slide and grown for 3 h before exposing to shear stress. For inhibition, integrin antagonists or antibodies were added to growth medium and then cells were subjected to shear stress.

Shear stress studies

The glass slides containing a confluent monolayer of BAEC were assembled into a parallel plate shear chamber forming a flow channel (220 μm height×25 mm width× 62 mm length) between the monolayer and a polycarbonate plate as previously described (Karin, 1994; Park et al., 2000). Non-pulsatile, laminar shear stress was controlled by changing the flow rate of the starvation medium delivered to the cells using the constant head flow-loop or a syringe pump (KD Scientific) as previously described (Davies, 1995; Jo et al., 1997).

Preparation of cell lysates

Following exposure to shear stress, cells were washed in ice-cold phosphate buffered saline (PBS), scraped in 0.25 ml of lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM vanadate, 1 mM dithiothreiotol, 1.0 % Triton X-100, and 0.1 mM phenylmethylsulfonyl fluoride), solubilized for 15 min to prepare Triton-soluble lysate as previously described (Jo et al., 1997). Entire solubilization procedures were performed at 4°C and the protein content of soluble cell lysates was measured by using a Bio-Rad DC assay kit (Bio-Rad).

Determination of ERK activation by western blot analysis

Soluble lysates (10 μg each) were resolved by 10% SDS-PAGE, transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore), and probed with antibodies (Ab) specific to phosphorylated forms of ERK1/2 (pERK1/2) (New England Biolabs) to determine the activation status of the MAP kinase as previously described (Jo et al., 1997). As a control, total amount of ERK1/2 was determined by Western blot analysis using an ERK1 Ab which also cross-reacts with ERK2 (UBI) (Jo et al., 1997). Goat anti-rabbit IgG conjugated to alkaline phosphatase was used as a secondary Ab and the membrane was developed by a chemiluminescent detection method (Jo et al., 1997).

Abbreviations

ECM, extracellular matrix; MAP kinases, mitogen-activated protein kinases; ERK, extracellular signal-

activated regulated kinase; BAECs, bovine aortic endothelial cells; VN, vitronectin; FN, fibronectin.

Results

Extracellular Matrices (ECMs) are required for shear-dependent activation of ERK

As shown in Fig. 1, ERK was highly activated in response to shear stress in BAEC grown in 20% FBS on ECM-coated glass slides, whereas shear-dependent activation of ERK was minimal in BAEC grown on poly-L-lysine (PLL) coated glass slides. It is of interest to note that cells were not spread out in the case of PLL coated slides. Therefore BAECs attached to the PLL coated slides were round and did not form their specific polygonal shape, indicating that PLL may work as an inhibitor for adhesion of BAECs to the glass slides. Actually, BAECs are adherent to uncoated glass slides and also highly responsive to shear stress only when they are grown in the presence of FBS. From the previous observation, the results in Fig. 1 have to be carefully interpreted. First of all, five- to six-fold stimulation of ERK activity induced by shear stress was observed in the cells on the glass slides coated with vitronectin (VN) and fibronectin (FN), when compared with cells attached to the PLL coated slide. This fold stimulation is comparable to that of ERK activity in cells attached to the uncoated glass slides in the presence of serum. While it is not clear how BAECs are adherent to the uncoated slides, the adherence of endothelial cell to matrices are essential for shear-dependent activation of ERK.

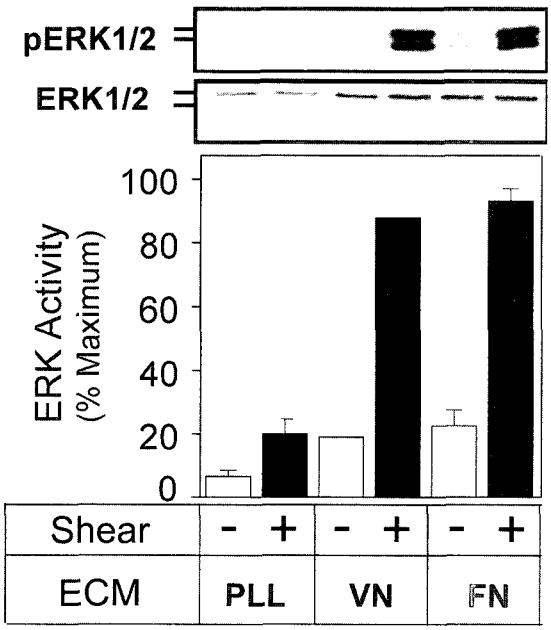

Fig. 1. Cellular adhesion onto extracellular matrices is required for shear-dependent activation of ERK. BAEC were seeded and grown on ECM-coated glass plates in the presence of 20% FBS for 3 h until they were attached. Cells were then exposed to shear stress (10 dyn/cm^2 for 5 min) and subsequently lysed on ice. Activity of ERK was determined by Western blot analysis with a phospho ERK (pERK) antibody and a total ERK antibody. Quantification of pERK bands performed by densitometry and is represented in the bottom panel. The bar graph shows the mean±S.E. (n=3).

In order to further dissect the results of Fig. 1, two separate questions can be addressed. One question is how BAECs adhere to the uncoated glass slides in the presence of serum and become responsible to the shear stress. The other is which matrix plays an important role in the shear-dependent activation of ERK. More queries on the latter are still posed, for example, role of two different ECMs and/or the effect of serum. Next experiments were designed to understand the above questions.

RGD peptides block shear-dependent activation of ERK

Three different RGD peptides were used to inhibit binding of integrins to ECM. Previously GRGESP was shown to have no inhibitory effect on cell attachment to ECM. GRGDSP inhibits cell adhesion to both fibronectin and vitronectin, whereas GRGDdSP inhibits cell adhesion to fibronectin (Pierschbacher et al., 1984). Thus we used the three peptides to conveniently determine integrins connecting to the shear stress-induced ERK signaling pathway. As shown in Fig. 2, GRGDdSP and GRGDSP inhibited the shear-stimulated ERK activity, but GRGESP that was used as a control had no effect. The inhibitory effect of GRGDdSP and GRGDSP indicates that integrins interacting with RGD sequence control the shear-stimulated ERK activity. More importantly, the inhibitory effect of the RGD peptides implicates that FN is an essential mediator for shear-dependent activation of ERK, because GRGDdSP has no effect on cell attachment to VN (Pierschbacher et al., 1984). In addition, effective dose of two different peptides in cell attachment to FN was known to be different. Effective dose for GRGDSP was found to be lower than that of GRGdDSP. In our experiment, GRGDSP inhibited the shear-stimulated ERK activity at a lower concentration than that of GRGDdSP, corresponding to previous dose-effect (Pierschbacher et al., 1984; Pierschbacher et al., 1987). In conclusion, ECM-integrin binding is necessary for shear-stimulated signaling pathways.

XT-199, an integrin $\alpha_v\beta_3$ (a vitronectin receptor) antagonist, has no effect on shear-dependent activation of ERK

To more clearly identify an ECM involved in shear-stimulated ERK signaling pathway, BAEC was pretreated with XT-199, an antagonist specific for integrin $\alpha_v\beta_3$ (a vitronectin receptor) known as a relevant vascular integrin

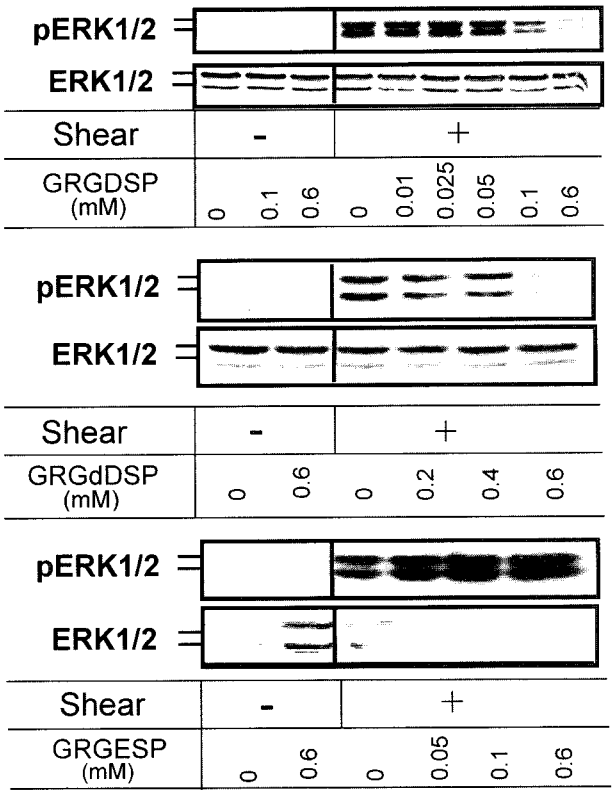

Fig. 2. RGD peptides inhibit mechanoactivation of ERK. BAEC grown on uncoated glass slides were treated with increasing amounts of GRGESP (control peptide), GRGDSP (inhibits adhesion to fibronectin and vitronectin), and GRGDdSP (inhibits adhesion to fibronectin) in 0.5% FBS-DMEM for 2 h. Cells were then exposed to shear stress (10 dyn/cm^2 for 5 min) or static control. ERK activity was measured by Western blot analysis as described in Materials and Methods. Shown in the bottom panel is quantitation of pERK bands ($\bar{x}$ ±S.E., n=3).

(Hantgan et al., 1998), before the shear stress and then ERK activity was measured. No inhibitory effect of XT-199 in the cells grown on the uncoated glass slides was shown (Fig. 3). No effect of XT-199 may result from a high and parallel effect of the ERK activity that was stimulated by integrins other than integrin $\alpha_v\beta_3$ in the presence of serum. Therefore to increase more specificity and remove the serum effect, VN coated glass slides were used for cell adhesion in the absence of serum. While BAEC were seeded and attached on VN-coated slides for 3 h, co-incubation with XT-199 had no effect on shear-dependent activation of ERK, more clearly suggesting that integrin $\alpha_v\beta_3$ is not involved in shear-dependent activation of ERK in our experimental condition. However, this data conflicts with the previous report (Li et al., 1997) showing that LM 609, an antibody against integrin $\alpha_v\beta_3$, blocks shear-dependent activation of ERK and JNK. To confirm whether LM 609 has an inhibitory effect, we also treated cells with LM609 (10 μg/ml) in our shear stress-exposing conditions and then monitored ERK activity. But we did not observe any inhibitory effect (Fig. 4). It is concluded that integrin $\alpha_v\beta_3$ is not involved

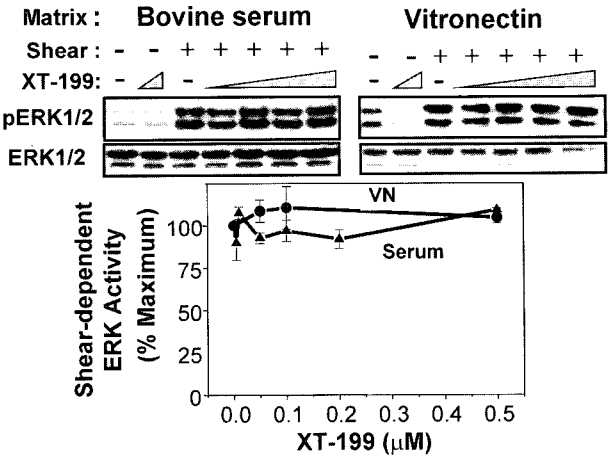

Fig. 3. Pretreatment of XT-199 has no effect on shear-dependent activation of ERK. BAEC grown in 20% FBS on uncoated glass slides or without serum on VN-coated slides were treated with increasing amounts of XT-199. In both cases, XT-199 was maximally used at 0.5 μg/ml. ERK activities were determined by Western blot analysis using pERK antibodies. The bottom line graph was plotted after quantification ($\bar{x}$ ±S.E, n=3).

in the shear-dependent activation of ERK in our experimental conditions.

SM 256, a non-specific integrin antagonist, inhibits shear-dependent activation of ERK in BAEC grown on FN-coated slides

We then tested whether SM256, a non-specific integrin antagonist (Mousa et al., 1999), has an effect on shear-dependent activation of ERK. Whereas pretreatment of cells grown on the uncoated slides with SM256 had no effect on ERK activity stimulated by shear stress, ERK activity in the cells on FN-coated slides was inhibited by pretreatment with SM 256 (Fig. 5). The ECM-dependent inhibitory effect of SM256 suggests that shear stress-dependent ERK signaling pathway is mediated by a

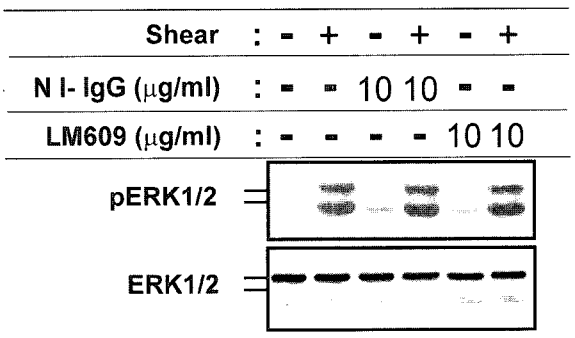

Fig. 4. Pretreatment of LM609 has no effect on shear-dependent activation of ERK. BAEC grown on uncoated glass slides in the presence of serum were treated with 10 μg/ml of LM609 or the same amount of non-immune serum. After 1 h pretreatment with the above antibodies, cells were exposed to shear stress as described in Methods and Materials. ERK activities were determined by Western blot analysis using pERK antibodies.

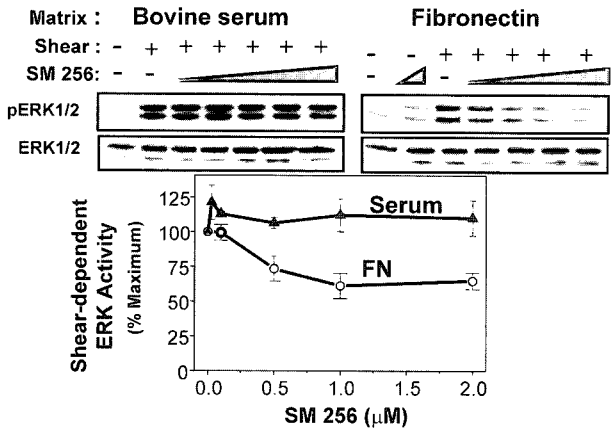

Fig. 5. Pretreatment of SM 256 inhibits shear-dependent activation of ERK in cells adherent to FN-coated slides. BAEC grown with 20% FBS on slides or without FBS on the FN-coated slides were treated with increasing amounts of SM256 (0-2 μM). The inhibitory effect of SM 256 was shown only in cells attached to FN-coated slides. ERK activity was determined by Western blot analysis using antibodies specific for phosphorylated form of ERK. Quantification was performed by densitometry and plotted in the line graph showing mean±S.E, n=3.

combination of integrins. In addition, the inhibitory effect of SM 256 in cells that were adherent to FN-coated slides implicates that integrin $\alpha_5\beta_1$, an FN-dependent and SM 256-antagonized integrin, is an upstream regulator for ERK activity in response to shear stress. These data are consistent with the previous report demonstrating that integrin $\alpha_5\beta_1$ was a signaling molecule for shear-induced cell adhesion and apoptosis (Urbich et al., 2000).

Integrins $\alpha_5\beta_1$ or $\alpha_4\beta_1$ inhibit shear-dependent activation of ERK

To investigate whether a specific integrin mediates ERK activity in response to shear stress, BAECs were pretreated with antibodies for different types of integrins and exposed to shear stress. Interestingly, shear-dependent activation of ERK was blocked by both neutralizing

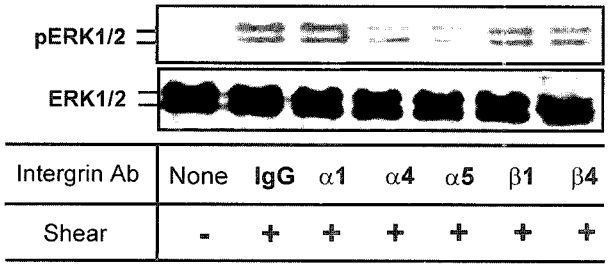

Fig. 6. Pretreatment of integrins α_4 and α_5-specific antibodies has an inhibitory effect on shear-dependent activation of ERK. BAEC grown on slides or attached to FN-coated slides were treated with non-immune IgG or various antibodies. The inhibitory effect was shown only in cells pretreated with antibodies for integrin α_5 and α_4. ERK activity was determined by Western blot analysis using antibodies specific for phosphorylated form of ERK.

antibodies against integrins α_5 and α_4, fibronectin-binding receptors (Fig. 6). The current results strongly support that fibronectin is a relevant ECM for shear sensing regimens in endothelium and its partner molecule.

Discussion

Here, ERK activity stimulated by shear stress was shown to be mediated by an ECM, fibronectin, implicating that integrins play an important role in shear-activation of ERK. The inhibitory effect of both GRGDSP and GRGDdSP suggests that fibronectin-binding integrins play an important role in the shear-dependent ERK pathway. Integrins interact with molecules on neighboring cells or ECM as well as form assemblies with other receptors in the same cell. In addition, integrins associate with signaling molecules in focal adhesion sites. Receptors associated with integrins are a variety of molecules influencing various important cell signal transductions, for instance, PDGF, GPI-linked receptors including uPAR, and others (Hynes, 1992; Porter et al., 1998; Wary et al., 1998; Wei et al., 1999). A scaffolding protein, caveolin-1 was also found to bind some integrins as well. Interestingly, we previously found that caveolin-1 regulates shear-dependent activation of ERK (Park et al., 1998; Park et al., 2000), further suggesting that integrins, the caveolin-1 partner molecules, somehow participate in shear-dependent activation of ERK.

XT-199, an antagonist specific for integrin $\alpha_v\beta_3$ has no effect on shear-dependent activation of ERK suggesting that integrin $\alpha_v\beta_3$ is not a regulator. This observation is correspondent to the finding that an inhibitory $\alpha_v\beta_3$ antibody, LM 609, had no effect on shear-activated ERK in cells grown on plain glass slides or attached to either FN or VN-coated plates (data not shown). This result is contradictory to the previous report (Li et al., 1997). However, since the activity of ERK was affected by subtle difference in growing condition (for instance, serum or ECMs), as shown in Fig. 5, this conflict may be caused by those subtle changes. On the other hand, SM 256 is an antagonist highly specific for integrin $\alpha_v\beta_3$ at micromolar concentration, but this reagent is also used as an antagonist for other integrin isoforms, such as $\alpha_{IIb}\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$. Therefore, SM 256 is a non-specific antagonist at higher concentrations when compared with XT-199 (Hantgan et al., 1998; Mousa et al., 1999). Since SM 256 had an inhibitory effect in cells grown on the fibronectin-coated slide, but not on serum- and vitronectin-coated slides (data not shown), the fibronectin-interacting integrins play an important role in shear-dependent activation of ERK. More specifically, two neutralizing antibodies against integrin α_5 and α_4 blocked the shear-dependent activation of ERK. Consequently, integrins that bind to fibronectin are involved in ERK signaling pathway in response to shear stress. These results further maintain that endothelial cell adhesion(s) to ECM is

potential mechanosensor(s) in blood vessel. In the future, it will be explored to determine the role of vitronectin and also to investigate co-localization and dynamics of the two molecules (integrins and caveolin-1) responsible for shear-dependent ERK signaling.

Acknowledgements

The present work was supported by a Korean Research Foundation grant (KRF-2002-003-C00107)

References

Cobb MH and Goldsmith EJ (1995) How MAP kinases are regulated. *J Biol Chem* 270: 14843-14846.

Davies PF (1995) Flow-mediated endothelial mechanotransduction. *Physiol Rev* 75: 519-560.

Gudi S, Clark CB, and Frangos JA (1996) Fluid flow rapidly activates G-proteins in human endothelial cells: involvement of G-proteins in mechanochemical signal transduction. *Circ Res* 79: 834-839.

Hantgan RR and Mousa SA (1998) Inhibition of platelet-mediated clot retraction by integrin antagonists. *Thrombosis Res* 89: 271-279.

Hynes RO (1992) Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69:11-25.

Jo H, Sipos K, Go YM, Law R, Rong J, and McDonald JM (1997) Differential effect of shear stress on extracellular signal-regulated kinase and N-terminal Jun kinase in endothelial cells. *J Biol Chem* 272: 1395-1401.

Karin M (1994) Signal transduction from the cell surface to the nucleus through the phosphorylation of transcription factors. *Curr Opin Cell Biol* 6: 415-424.

Kim J, Shin J, and Park H (2003) Structural characterization for N-terminal domain of caveolin-1. *Korean J Biol Sci* 7: 207-211.

Ku DN, Giddens DP, Zarins CK, and Glagov S (1985) Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low and oscillating shear stress. *Atherosclerosis* 5: 293-301.

Li S, Kim M, Hu YL, Jalali S, Schlaepfer DD, Hunter T, Chien S, and Shyy JY (1997) Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. *J Biol Chem* 272: 30455-30462.

MacKenna DA, Dolfi F, Vuori K, and Ruoslahti E (1998) Extracellular signal-regulated kinase and c-Jun NH2-terminal kinase activation by mechanical stretch is integrin-dependent and matrix-specific in rat cardiac fibroblasts. *J Clin Invest* 101: 301-310.

Mousa SA, Lorelli W, Mohamed S, Batt DG, Jadhav PK, and Reilly TM (1999) Alphavbeta3 integrin binding affinity and specificity of SM256 in various species. *J Cardiovasc Pharmacol* 33: 641-646.

Muller JM, Chilian WM, and Davis MJ (1997) Integrin signaling transduces shear stress-dependent vasodilation of coronary arterioles. *Circ Res* 80: 320-326.

Nollert MU, Eskin SG, and McIntire LV (1990) Shear stress increases inositol triphosphate level in human endothelial cells. *Biochem Biophys Res Comm* 170: 281-287.

Ohno M, Cooke JP, Dzau VJ, and Gibbons GH (1995) Fluid shear stress induces endothelial transforming growth factor beta-1 transcription and production. Modulation by potassium channel blockade. *J Clin Invest* 95: 1363-1369.

Olsen SP, Clapham DE, and Davies PE (1988) Hemodynamic shear stress activates a K^+ current in vascular endothelial cells. *Nature* 331: 168-170.

Park H, Go YM, St John PL, Maland MC, Lisanti MP, Abrahamson DR, and Jo H (1998) Plasma membrane cholesterol is a key molecule in shear stress-dependent activation of extracellular signal-regulated kinase. *J Biol Chem* 273: 32304-32311.

Park H, Go YM, Darji R, Choi JW, Lisanti MP, Maland MC, and Jo H (2000) Caveolin-1 regulates shear stress-dependent activation of extracellular signal-regulated kinase. *Am J Physiol Heart Circ Physiol* 278: H1285-H1293.

Pearce MJ, McIntyre TM, Prescott SM, Zimmerman GA, and Whatley RE (1996) Shear stress activates cytosolic phospholipase A2 (cPLA2) and MAP kinase in human endothelial cells. *Biochem Biophys Res Commun* 218: 500-504.

Pierschbacher MD and Ruoslahti E (1984) Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature* 309: 30-33.

Pierschbacher MD and Ruoslahti E (1987) Influence of stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity in cell adhesion. *J Biol Chem* 262: 17294-17298.

Porter JC and Hogg N (1998) Integrins take partners: cross-talk between integrins and other membrane receptors. *Trends Cell Biol* 8: 390-396.

Tseng H, Peterson TE, and Berk BC (1995) MAP kinase activation by flow in endothelial cells. *Circ Res* 77: 869-878.

Urbich C, Walter DH, Zeiher AM, and Dimmeler S (2000) Laminar shear stress upregulates integrin expression role in endothelial cell adhesion and apoptosis. *Circ Res* 87: 683-689.

Wary KK, Mariotti A, Zurzolo C, and Giancotti FG (1998) A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. *Cell* 94: 625-634.

Wei Y, Yang X, Liu Q, Wilkins JA, and Chapman HA (1999) A role for caveolin and the urokinase receptor in integrin-mediated adhesion and signaling. *J Cell Biol* 144: 1285-1294.

Zarins CK, Giddens DP, Bharadvaj BK, Sottiurai VS, Mabon RF, and Glagov S (1983) Carotid bifurcation atherosclerosis. Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress. *Circ Res* 53: 502-514.

[Received August 24, 2003; accepted October 8, 2003]