Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 301 (2003) 198–203
BBRC
www.elsevier.com/locate/ybbrc

Simvastatin stimulates VEGF release via p44/p42 MAP kinase in vascular smooth muscle cells

Motoki Takenaka,[a] Kouseki Hirade,[a] Kumiko Tanabe,[b] Shigeru Akamatsu,[b] Shuji Dohi,[b] Hiroyuki Matsuno,[a] and Osamu Kozawa[a,*]

[a] *Department of Pharmacology, Gifu University School of Medicine, Gifu 500-8705, Japan*
[b] *Department of Anesthesiology and Critical Care Medicine, Gifu University School of Medicine, Gifu 500-8705, Japan*

Received 18 December 2002

Abstract

It has been shown that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) modulate vascular smooth muscle cell functions. In the present study, we investigated the effect of simvastatin on vascular endothelial growth factor (VEGF) release, and the underlying mechanism, in a rat aortic smooth muscle cell line, A10 cells. Administration of simvastatin increased the VEGF level in rat plasma in vivo. In cultured cells, simvastatin significantly stimulated VEGF release in a dose-dependent manner. Simvastatin induced the phosphorylation of p44/p42 MAP kinase but not p38 MAP kinase or SAPK (stress-activated protein kinase)/JNK (*c-Jun N*-terminal kinase). PD98059 and U-0126, inhibitors of the upstream kinase that activates p44/p42 MAP kinase, significantly reduced the simvastatin-induced VEGF release in a dose-dependent manner. The phosphorylation of p44/p42 MAP kinase induced by simvastatin was reduced by PD98059 or U-0126. Moreover, a bolus injection of PD98059 truly suppressed the simvastatin-increased VEGF level in rat plasma in vivo. These results strongly suggest that p44/p42 MAP kinase plays a role at least partly in the simvastatin-stimulated VEGF release in vascular smooth muscle cells.

Keywords: Simvastatin; VEGF; MAP kinase; Vascular smooth muscle cells

It is well known that the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are useful therapeutic agents for patients with hypercholesterolemia, an essential risk factor of atherosclerosis [1]. In addition to the reduction of cholesterol biosynthesis through inhibiting the mevalonate pathway, accumulating evidence indicates that statins have several pleiotropic effects [1]. Vascular smooth muscle cells play a crucial role in the pathogenesis of hypertension and atherosclerosis [2]. As for vascular smooth muscle cells, it has been shown that atrovastatin, simvastatin, and lovastatin induce apoptosis of vascular smooth muscle cells [3]. In addition, it has recently been reported that simvastatin reduces plasminogen activator inhibitor-1 release by vascular smooth muscle cells [4]. However, the direct cellular effects of statins in vascular smooth muscle cells have not yet been precisely clarified.

Vascular endothelial growth factor (VEGF) has been characterized as a heparin-binding angiogenic growth factor displaying high specificity for endothelial cells, enhances vascular permeability, and promotes angiogenesis [5]. VEGF is expressed and secreted by numerous tumor and normal cells, including vascular smooth muscle cells [5]. In vascular smooth muscle cells, it has been shown that various growth factors and cytokines (such as fibroblast growth factor, platelet-derived growth factor, endothelin, transforming growth factor β, and interleukin-1) stimulate VEGF production [6–10]. As for the mechanism behind this VEGF production, it has been reported that endothelin-1 and -3 each increase VEGF synthesis through activation of protein kinase C in human umbilical vein smooth muscle cells [10]. We previously reported that p38 mitogen-activated protein (MAP) kinase plays an important role in the pathway

* Corresponding author. Fax: +81-58-267-2959.
E-mail address: okozawa@cc.gifu-u.ac.jp (O. Kozawa).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(02)03014-0

mediating the transforming growth factor β- or vitamin D_3-induced synthesis of VEGF in an aortic smooth muscle cell line, A10 cells [11,12]. However, the precise mechanism underlying VEGF production and release in vascular smooth muscle cells is not fully understood.

In the present study, we investigated whether simvastatin affects VEGF release in vivo and in cultured vascular smooth muscle cells in vitro, and if so, the mechanism behind this VEGF release. We herein show that simvastatin-stimulated VEGF release is regulated by p44/p42 MAP kinase in these cells.

Materials and methods

Materials. Simvastatin, PD98059, and U-0126 were purchased from Calbiochem-Novabiochem (La Jolla, CA). Phospho-specific p38 MAP kinase antibodies, p38 MAP kinase antibodies, phospho-specific stress-activated protein kinase (SAPK)/*c-Jun N*-terminal kinase (JNK) antibodies, SAPK/JNK antibodies, phospho-specific p44/p42 MAP kinase antibodies, and p44/p42 MAP kinase antibodies were obtained from New England BioLabs (Beverly, MA). An enhanced chemiluminescence (ECL) Western blotting detection system was obtained from Amersham Japan (Tokyo, Japan). Other materials and chemicals were obtained from commercial sources. Simvastatin, PD98059, and U-0126 were dissolved in dimethyl sulfoxide. The maximum concentration of dimethyl sulfoxide was 0.1%, which did not affect the assay for VEGF and Western blot analysis.

Effect of simvastatin on VEGF level in rat plasma in vivo. Male Sprague–Dawley rats (SLC, Sizuoka, Japan) were used. Simvastatin (2 or 20 mg/kg per day) was orally administrated for 3 days. When indicated, PD98059 (75 μM) or vehicle was injected as a bolus via the jugular vein before the administration of simvastatin. Catheter (Natume, Tokyo, Japan) was inserted into the right femoral artery for the monitoring of blood pressure and pulse rate via a pressure transducer (AP601G; Nihon Koden, Tokyo, Japan). Blood samples (0.3 ml) were collected into sodium citrate (3.15%, final concentration) via the jugular vein after 24 h from the final administration of simvastatin.

Cell culture. Aortic smooth muscle A10 cells, which have been derived from rat aortic smooth muscle cells [13], were obtained from American Type Culture Collection (Rockville, MD). The cells were seeded into 35-mm (1×10^5) or 90-mm (5×10^5) diameter dishes and cultured in DMEM containing 10% fetal calf serum (FCS) at 37 °C in a humidified atmosphere of 5% CO_2/95% air. The cells were seeded into 35-mm-diameter dishes or 90-mm-diameter dishes in DMEM containing 10% FCS. After 5 days, the medium was exchanged for serum-free DMEM. The cells were used for experiments after 48 h.

Assay for VEGF. The cultured cells were stimulated by simvastatin in 1 ml DMEM for the indicated periods. When indicated, the cells were pretreated with PD98059 or U-0126 for 60 min. The reaction was terminated by collecting the medium and VEGF in the medium was measured by a VEGF enzyme immunoassay kit.

Western blot analysis of MAP kinases. The cultured cells were stimulated by simvastatin for the indicated periods. The cells were washed twice with phosphate-buffered saline and then lysed, homogenized, and sonicated in a lysis buffer containing 62.5 mM Tris/Cl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 50 mM dithiothreitol, and 10% glycerol. The cytosolic fraction was collected as a supernatant after centrifugation at 125,000*g* for 10 min at 4 °C. SDS–polyacrylamide gel electrophoresis (PAGE) was performed by the method of Laemmli [14] in 10% polyacrylamide gels. Western blotting was performed as described previously [15] by using phospho-specific p44/p42 MAP kinase antibodies or p44/p42 MAP kinase antibodies, with peroxidase-labeled antibodies raised in goat against rabbit IgG being used as secondary antibodies. Peroxidase activity on the nitrocellulose sheet was visualized on X-ray film by use of an ECL Western blotting detection system. When indicated, the cells were pretreated with PD98059 or U-0126 for 60 min.

Determination. The absorbance of enzyme immunoassay samples was measured at 450 nm with EL 340 Bio Kinetic Reader (Bio-Tek Instruments, Winooski, VT).

Statistical analysis. The data were analyzed by ANOVA followed by Bonferroni method for multiple comparisons between pairs. All data are presented as means ± SD of triplicate determinations. Each experiment was repeated three times with similar results.

Results

Effect of simvastatin on VEGF level in rat plasma in vivo

Simvastatin (2 and 20 mg/kg/day) significantly increased the level of VEGF in rat plasma after its administration in a dose-dependent manner (Table 1).

Effect of simvastatin on VEGF release in A10 cells

Simvastatin significantly stimulated VEGF release time-dependently up to 36 h (Fig. 1). The stimulatory effect of simvastatin on VEGF release was dose-dependent over the range 0.1–20 μM (data not shown). The maximum effect of simvastatin was observed at 20 μM.

Effects of simvastatin on phosphorylation of p38 MAP kinase, SAPK/JNK, and p44/p42 MAP kinase in A10 cells

In order to clarify whether or not simvastatin activates the MAP kinase superfamily in A10 cells, we examined the effect of simvastatin on the phosphorylation of p38 MAP kinase, SAPK/JNK or p44/p42 MAP kinase. Simvastatin induced the significant phosphorylation of p44/p42 MAP kinase (Fig. 2). The simvastatin-induced phosphorylation of p44/p42 MAP kinase reached its peak at 60 min after the stimulation. On the other hand, p38 MAP kinase or SAPK/JNK was not affected by simvastatin (Fig. 2).

Table 1
Effect of simvastatin on the level of VEGF in vivo

Simvastatin	VEGF (pg/ml)
–	10.4 ± 1.0
+ (2 mg/kg/day)	34.6 ± 5.5*
+ (20 mg/kg/day)	77.2 ± 3.5*

Simvastatin (2 or 20 mg/kg per day) was orally administrated for 3 days. Blood samples were collected after 24 h from the final administration of simvastatin. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$, compared with the control value.

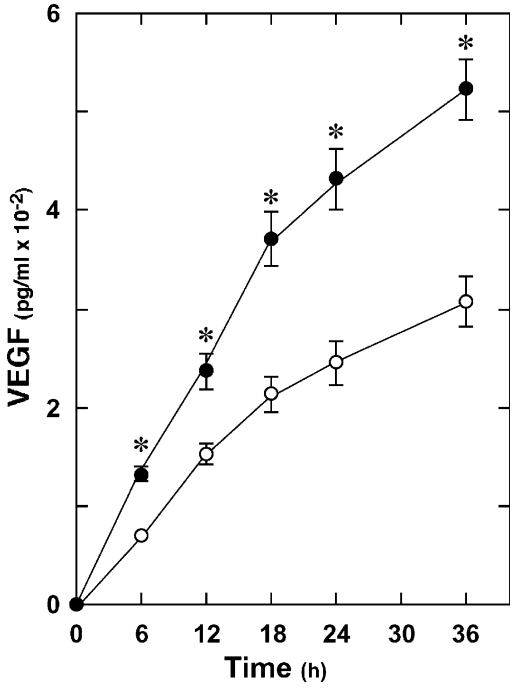

Fig. 1. Effect of simvastatin on VEGF release in A10 cells. The cultured cells were stimulated by 20 μM simvastatin for the indicated periods. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$, compared with the control value.

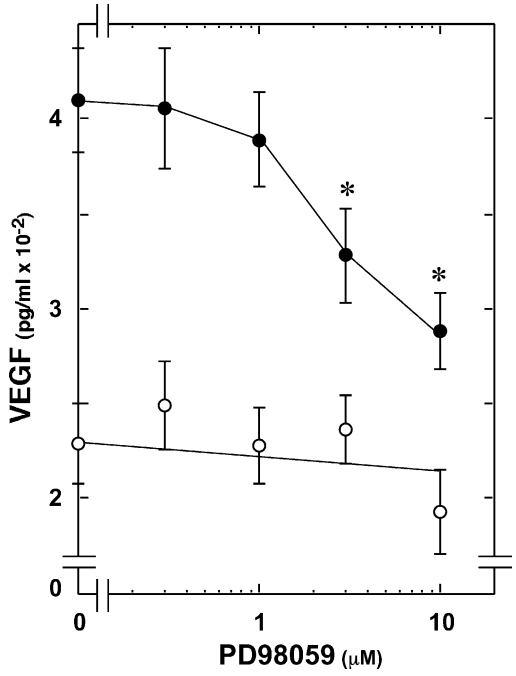

Fig. 3. Effect of PD98059 on the simvastatin-induced VEGF release in A10 cells. The cultured cells were pretreated with various doses of PD98059 for 60 min and then stimulated by 20 μM simvastatin for 24 h. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$, compared with the value of simvastatin alone.

Effect of PD98059 or U-0126 on the simvastatin-induced VEGF release in A10 cells

To clarify the involvement of p44/p42 MAP kinase in the simvastatin-induced VEGF release in A10 cells, we next examined the effect of PD98059, a specific inhibitor of upstream kinase that activates p44/p42 MAP kinase [16], on the VEGF release by simvastatin. The simvastatin-stimulated VEGF release was significantly reduced by PD98059, which alone had little effect on the level of VEGF (Fig. 3). In addition, U-0126, another inhibitor of upstream kinase that activates p44/p42 MAP kinase [17], which by itself did not affect the level of VEGF, suppressed the simvastatin-induced VEGF release (Table 2).

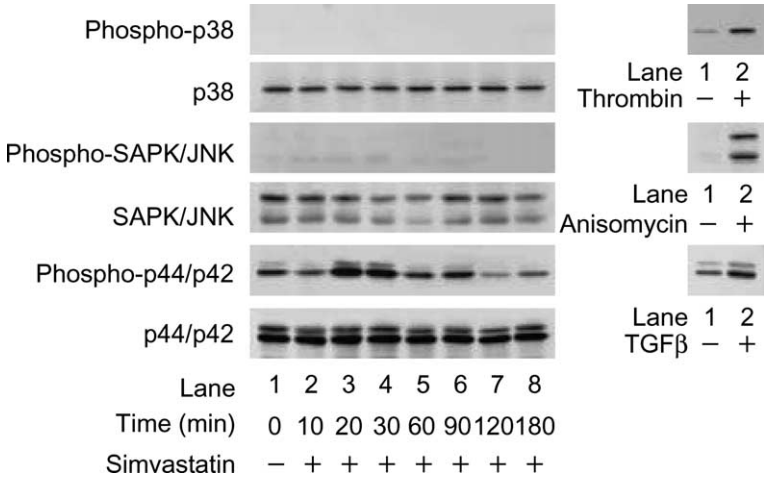

Fig. 2. Effects of simvastatin on the phosphorylation of p38 MAP kinase, SAPK/JNK, and p44/p42 MAP kinase in A10 cells. The cultured cells were incubated with 10 μM simvastatin for the indicated periods. The extracts of cells were subjected to SDS–PAGE against phospho-specific p38 MAP kinase antibodies, p38 MAP kinase antibodies, phospho-specific SAPK/JNK antibodies, SAPK/JNK antibodies, phospho-specific p44/p42 MAP kinase antibodies, and p44/p42 MAP kinase antibodies. Similar results were obtained with two additional and different cell preparations.

Table 2
Effect of U-0126 on the simvastatin-induced VEGF release in A10 cells

U-0126	Simvastatin	VEGF (pg/ml)
–	–	229 ± 21
–	+	410 ± 28
+	–	200 ± 15
+	+	287 ± 21*

The cultured cells were pretreated with 10 μM U-0126 for 60 min and then stimulated by 20 μM simvastatin for 24 h. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$, compared with the value of simvastatin alone.

Effect of PD98059 or U-0126 on the simvastatin-induced phosphorylation of p44/p42 MAP kinase in A10 cells

To further investigate the involvement of p44/p42 MAP kinase in the simvastatin-stimulated VEGF release in A10 cells, we examined the effect of PD98059 on the simvastatin-induced phosphorylation of p44/p42 MAP kinase. We found that the simvastatin-induced phosphorylation of p44/p42 MAP kinase was markedly reduced by PD98059 (Fig. 4A). In addition, U-0126 significantly attenuated the p44/p42 MAP kinase phosphorylation by simvastatin (Fig. 4B).

Effect of PD98059, on simvastatin-increased level of VEGF in rat plasma in vivo

In addition, we examined the effect of PD98059 on the simvastatin-increased level of VEGF in vivo.

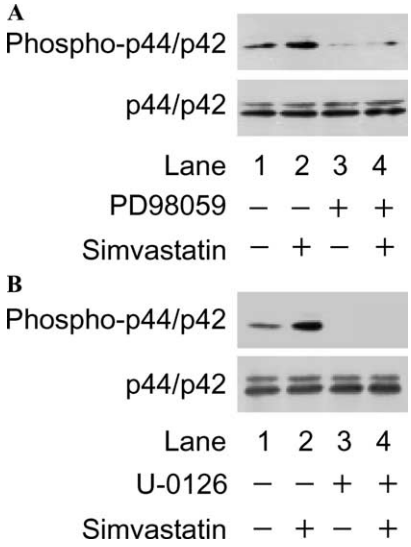

Fig. 4. Effects of PD98059 or U-0126 on the phosphorylation of p44/p42 MAP kinase induced by simvastatin in A10 cells. The cultured cells were pretreated with 10 μM PD98059 (A) or 10 μM U-0126 (B) for 60 min and then stimulated by 20 μM simvastatin for 3 h. The extracts of cells were subjected to SDS–PAGE against phospho-specific p44/p42 MAP kinase antibodies or p44/p42 MAP kinase antibodies. Similar results were obtained with two additional and different cell preparations.

Table 3
Effect of PD98059 on the simvastatin-stimulated VEGF level in vivo

PD98059	Simvastatin	VEGF (pg/ml)
–	–	10.4 ± 1.0
–	+	77.2 ± 3.5
+	–	10.9 ± 1.0
+	+	22.0 ± 2.1*

Simvastatin (20 mg/kg per day) was orally administered for 3 days. PD98059 (75 μM) or vehicle was injected as a bolus before the administration of simvastatin. Blood samples were collected after 24 h from the final administration of simvastatin. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$, compared with the value of simvastatin alone.

PD98059, which alone did not affect the basal level of VEGF, significantly inhibited the VEGF level increased by simvastatin (Table 3).

Discussion

In the present study, our initial finding was that an administration of simvastatin significantly increased the level of VEGF in rat plasma. It is well recognized that vascular smooth muscle cells are the main source of VEGF [18]. Thus, this finding led us to speculate that vascular smooth muscle cells may release VEGF into the blood in response to simvastatin. In accordance with our speculation, we then found that simvastatin-stimulated VEGF release from cultured aortic smooth muscle A10 cells. This is probably the first report showing that VEGF release from vascular smooth muscle cells can be induced by simvastatin, a statin.

We next investigated the mechanism underlying the simvastatin-induced VEGF release observed in A10 cells. The MAP kinase superfamily plays an important role in transducing extracellular signaling into a cellular response, the specificity of the cellular response to a given stimulus being determined by the activation of a particular MAP kinase pathway [19,20]. Three major MAP kinases, p44/p42 MAP kinase, SAPK/JNK, and p38 MAP kinase, are generally recognized as being the central elements used by mammalian cells to transduce such diverse messages [19,20]. VEGF release is reportedly increased through activation of MAP kinases in various cells. It has been reported that interleukin-1β induces VEGF gene expression through p38 MAP kinase and SAPK/JNK signaling in rat myocytes [21], and that VEGF expression is differently regulated by the p44/p42 MAP kinase and p38 MAP kinase pathways in *ras* transformed cells [22]. Thus, statins may affect VEGF release via activation of the MAP kinase superfamily in vascular smooth muscle cells. In the present study, we showed that simvastatin induced the phosphorylation of p44/p42 MAP kinase without affecting

the phosphorylation of p38 MAP kinase or SAPK/JNK among the MAP kinase superfamily in aortic smooth muscle A10 cells. It is well recognized that MAP kinases are activated by phosphorylation of tyrosine and threonine residues by dual-specificity MAP kinase kinase [23]. Therefore, our results suggest that simvastatin activates p44/p42 MAP kinase in vascular smooth muscle cells. To the best of our knowledge, our present study probably represents the first report showing the activation of p44/p42 MAP kinase by a statin in vascular smooth muscle cells.

We next examined whether p44/p42 MAP kinase is involved in the simvastatin-induced VEGF release in aortic smooth muscle A10 cells. First, PD98059, a well-known inhibitor of upstream kinase (MEK I) that activates p44/p42 MAP kinase [16], significantly reduced the simvastatin-stimulated release of VEGF. This result seems that p44/p42 MAP kinase is involved in simvastatin-stimulated VEGF release in A10 cells. Furthermore, we showed that the simvastatin-stimulated release of VEGF was suppressed by U0126, another inhibitor of upstream kinase that activates p44/p42 MAP kinase [17]. We found that the simvastatin-induced phosphorylation of p44/p42 MAP kinase was truly inhibited by PD98059 and U0126. Furthermore, we next investigated whether p44/p42 MAP kinase is involved in the pathway by which simvastatin stimulates the level of VEGF in vivo. PD98059 significantly inhibited the simvastatin-increased VEGF level in rat plasma. Therefore, it is probable that simvastatin increases VEGF level at least in part through the activation of p44/p42 MAP kinase. Taking these findings into account as a whole, it is most likely that simvastatin activates p44/p42 MAP kinase, resulting in regulating the release of VEGF in vascular smooth muscle cells. It has been reported that the activation of p44/p42 MAP kinase in vascular smooth muscle cells modulates vascular tone [24]. In addition, transient symptomatic hypotension reportedly develops in patients on simvastatin [25]. Thus, it is possible that simvastatin affects systemic blood pressure through the modulation of vascular smooth muscle tone by activation of the MAP kinase. Further investigations will be necessary to clarify the detailed effect of statins on vascular system.

VEGF is a highly specific mitogen for vascular endothelial cells [5]. The expression of VEGF is potentiated by hypoxia, by activated oncogenes, and by a variety of cytokines [5]. VEGF induces endothelial proliferation, promotes cell migration, and inhibits apoptosis [5]. In vivo, VEGF induces angiogenesis as well as permeabilization of blood vessels [5]. The angiogenic properties of VEGF have recently been exploited to induce in vivo angiogenesis for the treatment of diseases associated with an impaired blood supply [5], and VEGF is effective for the treatment of both limb and myocardial ischemia [26,27]. Statins are generally administered to patients with hypercholesterolemia, an essential risk factor of atherosclerosis. The present results showed that simvastatin-stimulated VEGF release in vivo and in vitro. Therefore, it is possible that statins may be useful for patients with atherosclerosis through VEGF release in addition to patients with hypercholesterolemia. Further investigations will be necessary to clarify the clinical justification for statins use.

In conclusion, these results strongly suggest that p44/p42 MAP kinase plays a role at least partly in the simvastatin-stimulated VEGF release in vascular smooth muscle cells.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (09671041, 12470015) from the Ministry of Education, Science, Sports, and Culture of Japan. We are very grateful to Yukari Okamoto for her skillful secretarial assistance.

References

[1] M. Takemoto, J.K. Liao, Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 1712–1719.
[2] R. Ross, The pathogenesis of atherosclerosis: a perspective for the 1990s, Nature 362 (1993) 801–809.
[3] C. Guijarro, L.M. Blanco-Colio, M. Ortego, C. Alonso, A. Ortiz, J.J. Plaza, C. Diaz, G. Hernandez, J. Egido, 3-Hydoxy-3-methylglutaryl coenzyme a reductase and isoprenylation inhibitors induce apoptosis of vascular smooth muscle cells in culture, Cir. Res. 83 (1998) 490–500.
[4] T. Bourcier, P. Libby, HMG CoA reductase inhibitors reduce plasminogen activator inhibitor-1 expression by human vascular smooth muscle cells and endothelial cells, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 556–562.
[5] G. Neufeld, T. Cohen, S. Gengrinovitch, Z. Poltorak, Vascular endothelial growth factor (VEGF) and its receptors, FASEB 13 (1999) 9–22.
[6] E. Brogi, T. Wu, A. Namiki, J.M. Isner, Indirect angiogenic cytokines upregulate VEGF and bFGF gene expression in vascular smooth muscle cells, whereas hypoxia upregulates VEGF expression only, Circulation 90 (1994) 649–652.
[7] J. Li, M.A. Perrella, J.-C. Tsai, S.-F. Yet, C.-M. Hsieh, M. Yoshizumi, C. Patterson, W.O. Endege, F. Zhou, M.-E. Lee, Induction of vascular endothelial growth factor gene expression by interleukin-1β in rat aortic smooth muscle cells, J. Biol. Chem. 270 (1995) 308–312.
[8] G.T. Stavri, Y. Hong, I.C. Zachary, G. Breier, P.A. Baskerville, S. Ylä-Herttuala, W. Risau, J.F. Martin, J.D. Erusalimsky, Hypoxia and platelet-derived growth factor-BB synergistically upregulate the expression of vascular endothelial growth factor in vascular smooth muscle cells, FEBS Lett. 358 (1996) 311–315.
[9] G.T. Stavri, I.C. Zachary, P.A. Baskerville, J.F. Martin, J.D. Erusalimsky, Basic fibroblast growth factor upregulates the expression of vascular endothelial growth factor in vascular smooth muscle cells: synergistic interaction with hypoxia, Circulation 92 (1995) 11–14.
[10] A. Pedram, M. Razandi, R.-M. Hu, E.R. Levin, Vasoactive peptides modulate vascular endothelial cell growth factor produc-

tion and endothelial cell proliferation and invasion, J. Biol. Chem. 272 (1997) 17097–17103.
[11] T. Yamamoto, O. Kozawa, K. Tanabe, S. Akamatsu, H. Matsuno, S. Dohi, T. Uematsu, Involvement of p38 MAP kinase in TGF-β-stimulated VEGF synthesis in aortic smooth muscle cells, J. Cell Biochem. 82 (2001) 591–598.
[12] T. Yamamoto, O. Kozawa, K. Tanabe, S. Akamatsu, H. Matsuno, S. Dohi, H. Hirose, T. Uematsu, 1,25-Dihydroxyvitamin D_3 stimulates vascular endothelial growth factor release in aortic smooth muscle cells: role of p38 mitogen-activated protein kinase, Arch. Biochem. Biophys. 398 (2002) 1–6.
[13] B.W. Kimes, B.L. Brandt, Characterization of two putative smooth muscle cell lines from rat thoracic aorta, Exp. Cell Res. 98 (1976) 349–366.
[14] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[15] K. Kato, H. Ito, K. Hasegawa, Y. Inaguma, O. Kozawa, T. Asano, Modulation of the stress-induced synthesis of hsp27 and α B-crystallin by cyclic AMP in C6 rat glioma cells, J. Neurochem. 66 (1996) 946–950.
[16] D.R. Alessi, A. Cuenda, P. Cohen, D.T. Dudley, A.R. Saltiel, PD98059 is a specific inhibitor of the activation of mitogen-activated protein kinasein vitro and in vivo, J. Biol. Chem. 270 (1995) 27489–27494.
[17] M.F. Favata, K.Y. Horiuchi, E.J. Manos, A.J. Daulerio, D.A. Stradley, W.S. Feeser, D.E. Van Dyk, W.J. Pitts, R.A. Earl, F. Hobbs, R.A. Copeland, R.L. Magolda, P.A. Scherle, J.M. Trzaskos, Identification of a novel inhibitor of mitogen-activated protein kinase, J. Biol. Chem. 273 (1998) 18623–18632.
[18] E. Tischer, R. Mitchell, T. Hartman, M. Silva, D. Gospodarowicz, J.C. Fiddes, J.A. Abraham, The human gene for vascular endothelial growth factor, J. Biol. Chem. 266 (1991) 11947–11954.
[19] C. Widmann, S. Gibson, M.B. Jarpe, G.L. Johnson, Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human, Physiol. Rev. 79 (1999) 143–180.
[20] L. Chang, M. Karin, Mammalian MAP kinase signalling cascades, Nature 410 (2001) 37–40.
[21] T. Tanaka, H. Kanai, K. Sekiguchi, Y. Aihara, T. Yokoyama, M. Arai, T. Kanda, R. Nagai, M. Kurabayashi, Induction of VEGF gene transcription by IL-1β is mediated through stress-activated MAP kinases and Sp1 sites in cardiac myocytes, J. Mol. Cell Cardiol. 32 (2000) 1955–1967.
[22] E. Okajima, U.P. Thorgeirsson, Different regulation of vascular endothelial growth factor expression by the ERK and p38 kinase pathways in v-*ras*, v-*raf*, and v-*myc* transformed cells, Biochem. Biophys. Res. Commun. 270 (2000) 108–111.
[23] J. Raingeaud, S. Gupta, J.S. Rogers, M. Dickens, J. Han, R.J. Ulevitch, R.J. Davis, Pro-inflammatory cytokines and environmrntal stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation of tyrosine and threonine, J. Biol. Chem. 270 (1995) 7420–7426.
[24] D.T. Ward, A.C. Alder, J. Ohanian, V. Ohanian, Noradrenaline-induced paxillin phosphorylation, ERK activation and MEK-regulated contraction in intact rat mesenteric arteries, J. Vasc. Res. 39 (2002) 1–11.
[25] J. French, H. White, Transient symptomatic hypotension in patients on simvastatin, Lancet 30 (1989) 807–808.
[26] I. Baumgartner, A. Pieczek, O. Manor, R. Blair, M. Kearney, K. Walsh, J.M. Isner, Constitutive expression of phVEGF$_{165}$ after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia, Circulation 97 (1998) 1114–1123.
[27] K.G. Lathi, P.R. Vale, D.W. Losordo, R.M. Cespedes, J.F. Symes, D.D. Esakof, M. Maysky, J.M. Isner, Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease: anesthetic management and results, Anesth. Analg. 92 (2001) 19–25.