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Stress-Activated Protein Kinase/c-Jun *N*-terminal Kinase (JNK) Plays a Part in Endothelin-1-Induced Vascular Endothelial Growth Factor Synthesis in Osteoblasts

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Abstract We previously reported that endothelin-1 (ET-1) activates both p44/p42 mitogen-activated protein (MAP) kinase and p38 MAP kinase in osteoblast-like MC3T3-E1 cells, and that not p44/p42 MAP kinase but p38 MAP kinase participates in the ET-1-induced vascular endothelial growth factor (VEGF) synthesis. In the present study, we investigated the involvement of stress-activated protein kinase/c-Jun *N*-terminal kinase (JNK) in ET-1-induced VEGF synthesis in these cells. ET-1 significantly induced the phosphorylation of JNK in a dose-dependent manner in the range between 0.1 and 100 nM. SP600125, an inhibitor of JNK, markedly reduced the ET-1-induced VEGF synthesis. A combination of SP600125 and SB203580 additively reduced the ET-1-stimulated VEGF synthesis. SP600125 suppressed the ET-1-induced phosphorylation of JNK, while having no effect on the phosphorylation of p38 MAP kinase elicited by ET-1. SB203580, an inhibitor of p38 MAP kinase, hardly affected the ET-1-induced phosphorylation of JNK. These results strongly suggest that JNK plays a role in ET-1-induced VEGF synthesis in addition to p38 MAP kinase in osteoblasts. J. Cell. Biochem. 87: 417–423, 2002. © 2002 Wiley-Liss, Inc.

Key words: ET-1; VEGF; JNK

Endothelin (ET)-1 is a potent vasoconstrictive agent, which is produced and secreted by endothelial cells [Masaki, 1993; Pollock et al., 1995]. It is currently recognized that ET has a wide variety of effects on ubiquitous tissues including skeletal tissues through binding to specific receptors [Pollock et al., 1995]. Bone metabolism is regulated by two functional cells, osteoblasts and osteoclasts [Nijweide et al., 1986], the former responsible for bone formation and the latter for bone resorption. Accumulating evidence indicates that osteoblasts are responsible for bone resorptive agents such as parathyroid hormone and 1,25-$(OH)_2$ vitamin D_3 [Nijweide et al., 1986] through the upregulation of RANKL expression [Yasuda et al., 1998], suggesting that osteoblasts play pivotal roles in the regulation of bone remodeling. During bone remodeling, capillary endothelial cells provide the microvasculature, and osteoblasts and osteoprogenitor cells, which locally proliferate and differentiate into osteoblasts, migrate into the resorption lacuna. Thus, it is currently recognized that the activities of osteoblasts, osteoclasts, and capillary endothelial cells are closely coordinated via humoral factors as well as by direct cell-to-cell contact, and these functional cells cooperatively regulate bone metabolism [Erlebacher et al., 1995]. Both ET_A and ET_B receptor have been shown to exist on osteoblasts [Takuwa et al., 1990; Sakurai et al., 1992; Semler et al., 1995]. It has been reported that ET-1 stimulates bone resorption and decreases alkaline phosphatase activity in vitro, induces collagen and non-collagen protein synthesis in neonatal mouse calvaria, and stimulates the proliferation of osteoblasts [Takuwa et al., 1989, 1990; Tatrai et al., 1992; Stern et al., 1995].

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Regarding the intracellular signaling system of ET in bone cells, it has been reported that ET-1 stimulates Ca^{2+} mobilization, phosphoinositide hydrolysis by phospholipase (PL) C, and phosphatidylcholine hydrolysis by PLD in osteoblasts, including mouse osteoblast-like MC3T3-E1 cells [Takuwa et al., 1989, 1990; Tatrai et al., 1992a,b; Suzuki et al., 1994]. In addition, we have shown that ET-1 activates both p44/p42 mitogen-activated protein (MAP) kinase and p38 MAP kinase in MC3T3-E1 cells [Kawamura et al., 1999a,b]. As for the MAP kinase superfamily, it has been reported that stress-activated protein kinase/c-Jun *N*-terminal kinase (JNK), which belongs to the MAP kinase superfamily [Widmann et al., 1999], is activated by ET-1 in rat neonatal ventricular myocytes, porcine vascular smooth muscle cells, and immortalized rat Schwann cells, [Nemoto et al., 1998; Berti-Mattera et al., 2000; Chevalier et al., 2000]. However, the involvement of JNK in ET-1 signaling in osteoblasts has not yet been clarified.

Vascular endothelial growth factor (VEGF) is known as a specific mitogenic factor of vascular endothelium [Ferrara and Davis-Smyth, 1997]. In bone metabolism, it has recently been reported that inactivation of VEGF causes complete suppression of blood vessel invasion concomitant with impaired trabecular bone formation and expansion of hypertrophic chondrocyte zone in mouse tibial epiphyseal growth plate [Gerber et al., 1999]. Evidence is accumulating that VEGF is produced and secreted by osteoblasts in response to various humoral factors [Harada et al., 1994; Goad et al., 1996; Wang et al., 1996; Ferrara and Davis-Smyth, 1997; Schalaeppi et al., 1997]. We have shown that ET-1 induces VEGF synthesis via ET_A receptor in osteoblast-like MC3T3-E1 cells, and that the activation of not p44/p42 MAP kinase but p38 MAP kinase is involved in the VEGF synthesis [Kozawa et al., 2000]. It has been reported that interleukin-1β induces VEGF gene transcription through the activation of both p38 MAP kinase and JNK in neonatal rat cardiac myocytes [Tanaka et al., 2000], leading us to speculate the involvement of JNK in ET-1-induced VEGF synthesis in osteoblasts.

In the present study, we investigated the involvement of JNK in the ET-1-induced VEGF synthesis in osteoblast-like MC3T3-E1 cells. We show here that ET-1 activates JNK in addition to p44/p42 MAP kinase and p38 MAP kinase in these cells, and that JNK plays a role at least in part in ET-1-induced VEGF synthesis.

MATERIALS AND METHODS

ET-1 was purchased from Peptide Insitute, Inc. (Minoh, Japan). Mouse VEGF ELISA kit was purchased from R&D Systems, Inc. (Minneapolis, MN). 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole (SB203580) and anthra[1,9-cd]pyrazol-6(2H)-one (SP600125) were obtained from Calbiochem-Novabiochem Co. (La Jolla, CA). Phospho-specific JNK antibodies (rabbit polyclonal IgG, affinity purified), JNK antibodies (rabbit polyclonal IgG, affinity purified), phospho-specific p38 MAP kinase antibodies (rabbit polyclonal IgG, affinity purified), and p38 MAP kinase antibodies (rabbit polyclonal IgG, affinity purified) were purchased from New England BioLabs, Inc. (Beverly, MA). ECL Western blotting detection system was purchased from Amersham Japan (Tokyo, Japan). Other materials and chemicals were obtained from commercial sources. SB203580 and SP600125 were dissolved in dimethyl sulfoxide (DMSO). The maximum concentration of DMSO was 0.1%, which did not affect the assay for VEGF or the analysis of MAP kinases.

Cell Culture

Cloned osteoblast-like MC3T3-E1 cells derived from newborn mouse calvaria [Sudo et al., 1983] were maintained as previously described [Kozawa et al., 1992]. Briefly, the cells were cultured in α-minimum essential medium (α-MEM) 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- or 90-mm diameter dishes in α-MEM containing 10% FCS. After 5 days, the medium was exchanged for α-MEM containing 0.3% FCS. The cells were used for experiments after 48 h.

Assay for VEGF

The cultured cells were stimulated by ET-1 in 1 ml of α-MEM containing 0.3% FCS for the indicated periods. When indicated, the cells were pretreated with SP600125 or SB203580 for 60 min. The reaction was terminated by collecting the medium, and VEGF in the medium was measured by a VEGF ELISA kit.

Analysis of MAP Kinases

The cultured cells were stimulated by ET-1 in α-MEM containing 0.3% FCS 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/HCl, 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 Laemmli [1970] in 10% polyacrylamide gel. Western blotting analysis was performed as described previously [Kato et al., 1996] by using phospho-specific JNK antibodies, JNK antibodies, phospho-specific p38 MAP kinase antibodies or p38 MAP kinase antibodies with, peroxidase-labeled antibodies raised in goat against rabbit IgG being used as second antibodies. Peroxidase activity on the nitrocellulose sheet was visualized on X-ray film by means of the ECL Western blotting detection system. When indicated, the cells were pretreated with SP600125 or SB203580 for 60 min.

Determination

The absorbance of ELISA samples was measured at 450 nm with EL 340 Bio Kinetic Reader (Bio-Tek Instruments, Inc., Winooski, VT). The densitometric analysis was performed using Molecular Analyst/Macintosh (Bio-Rad Laboratories, Hercules, CA).

Statistical Analysis

The data were analyzed by ANOVA followed by the Bonferroni method for multiple comparisons between pairs, and a $P < 0.05$ was considered significant. All data are presented as the mean ± SEM of triplicate determinations. Each experiment was repeated three times with similar results.

RESULTS

Effect of ET-1 on the Phosphorylation of JNK in MC3T3-E1 Cells

We previously reported that ET-1 activates both p44/p42 MAP kinase and p38 MAP kinase in osteoblast-like MC3T3-E1 cells [Kawamura et al., 1999a,b]. To clarify whether ET-1 activates JNK or not in these cells, we examined the effect of ET-1 on the phosphorylation of JNK.

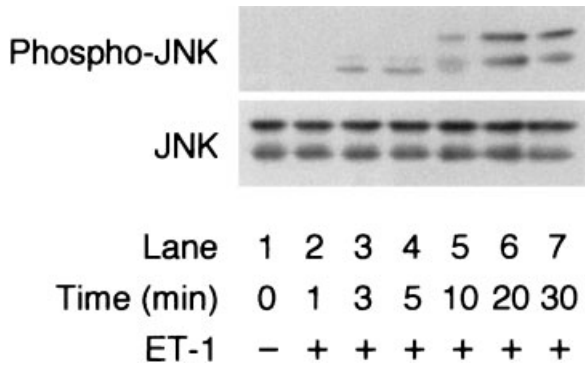

Fig. 1. Effect of ET-1 on the phosphorylation of JNK in MC3T3-E1 cells. The cultured cells were stimulated by 100 nM ET-1 for 1 min (**lane 2**), 3 min (**lane 3**), 5 min (**lane 4**), 10 min (**lane 5**), 20 min (**lane 6**), and 30 min (**lane 7**). The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific JNK or JNK. **Lane 1**: control cells.

ET-1 time dependently induced the phosphorylation of JNK up to 30 min (Fig. 1). The effect of ET-1 was dose-dependent in the range between 0.1 and 100 nM (Fig. 2). The maximum effect of ET-1 was observed at 100 nM.

Effect of SP600125 on the ET-1-Induced VEGF Synthesis in MC3T3-E1 Cells

We have recently shown that ET-1 induces VEGF synthesis not through p44/p42 MAP kinase but p38 MAP kinase in MC3T3-E1 cells [Kozawa et al., 2000]. To investigate whether JNK is involved in the ET-1-induced VEGF synthesis, we examined the effect of SP600125, a highly specific inhibitor of JNK [Bennett et al., 2001], on the synthesis. SP600125, which alone hardly affected VEGF synthesis, significantly

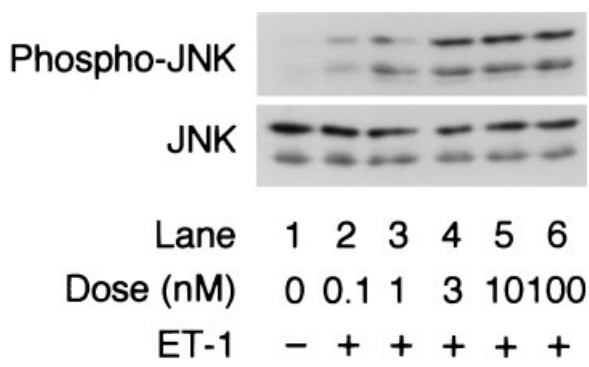

Fig. 2. Dose-dependent effect of ET-1 on the phosphorylation of JNK in MC3T3-E1 cells. The cultured cells were stimulated by various doses of ET-1 for 30 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific JNK or JNK.

reduced the ET-1-induced VEGF synthesis in these cells (Fig. 3). The inhibitory effect of SP600125 on the ET-1-induced VEGF synthesis was dose-dependent in the range between 1 and 10 μM (Fig. 4). The maximum inhibitory effect of SP600125 was observed at 10 μM, which caused about 80% reduction in the ET-1 effect. A combination of SP600125 and SB203580 additively reduced the ET-1-stimulated VEGF synthesis (data not shown).

Effects of SP600125 on the Phosphorylation of JNK and p38 MAP Kinase Induced by ET-1 in MC3T3-E1 Cells

SP600125 truly inhibited the phosphorylation of JNK induced by ET-1 (Fig. 5). The densitometric analysis showed that SP600125 elicited about 50% reduction in the ET-1 effect. On the other hand, the ET-1-induced phosphorylation of p38 MAP kinase was not affected by SP600125 (Fig. 6).

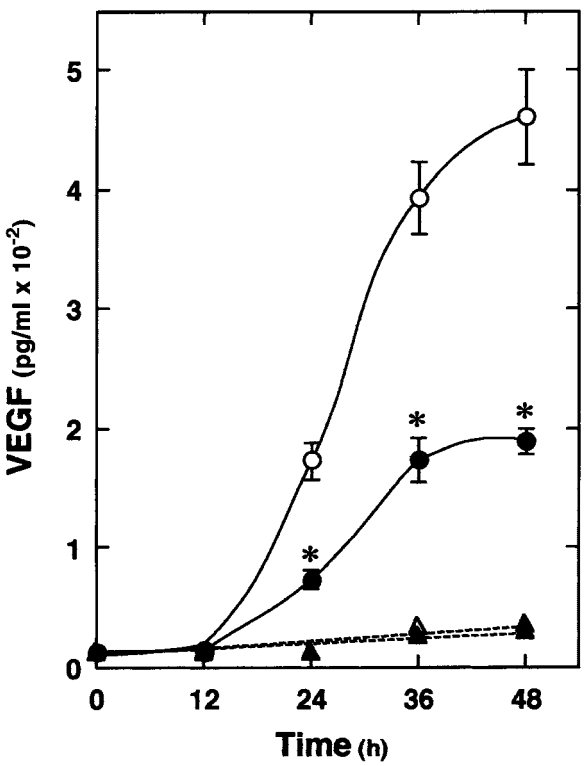

Fig. 3. Effect of SP600125 on the ET-1-induced VEGF synthesis in MC3T3-E1 cells. The cultured cells were pretreated with 3 μM of SP600125 (closed symbols) or vehicle (open symbols) for 60 min, and then stimulated by 100 nM ET-1 (circles) or vehicle (triangles) for the indicated periods. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$, compared to the value of ET-1 alone.

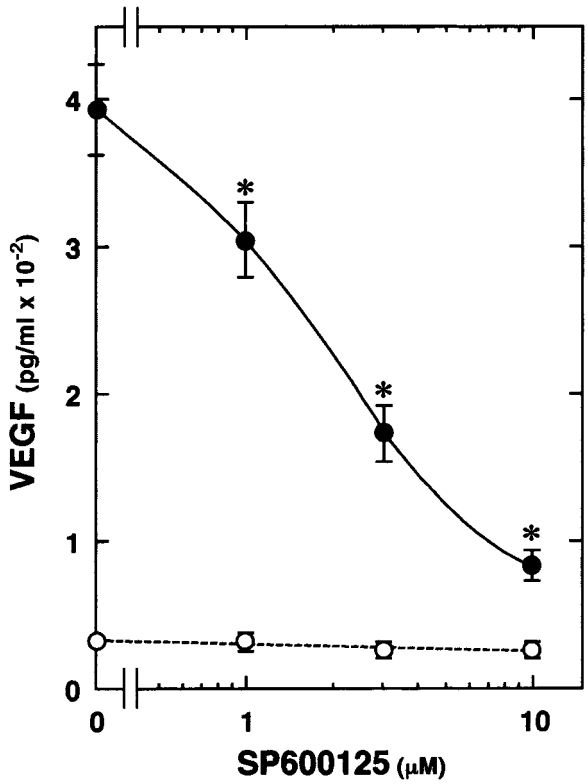

Fig. 4. Effect of SP600125 on the ET-1-induced VEGF synthesis in MC3T3-E1 cells. The cultured cells were pretreated with various doses of SP600125 for 60 min, and then stimulated by 100 nM ET-1 (●) or vehicle (○) for 36 h. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$, compared to the value of ET-1 alone.

Effect of SB203580 on the Phosphorylation of JNK Induced by ET-1 in MC3T3-E1 Cells

We have reported that SB203580, a highly specific inhibitor of p38 MAP kinase [Cuenda et al., 1995], reduces both VEGF synthesis and the phosphorylation of p38 MAP kinase induced by ET-1 in MC3T3-E1 cells [Kozawa et al., 2000]. Thus, we examined the effect of SB203580 on the ET-1-induced JNK phosphorylation in these cells. SB203580, which alone had little effect on the phosphorylation of JNK, failed to affect the ET-1-induced phosphorylation of JNK (Fig. 7).

DISCUSSION

It is generally recognized that the MAP kinase superfamily mediates intracellular signaling of various agonists and plays important roles in cellular functions including proliferation, differentiation, and cell death in a variety

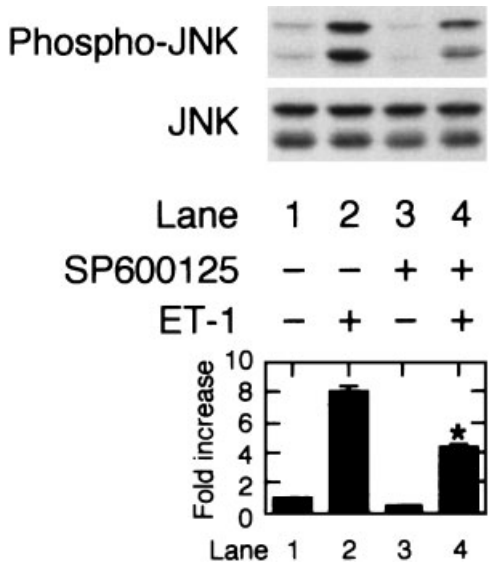

Fig. 5. Effect of SP600125 on the ET-1-induced phosphorylation of JNK in MC3T3-E1 cells. The cultured cells were pretreated with 10 μM SP600125 or vehicle for 60 min, and then stimulated by 100 nM ET-1 or vehicle for 30 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific JNK or JNK. The histogram shows quantitative representations of the levels of ET-1-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$, compared with the value of ET-1 alone.

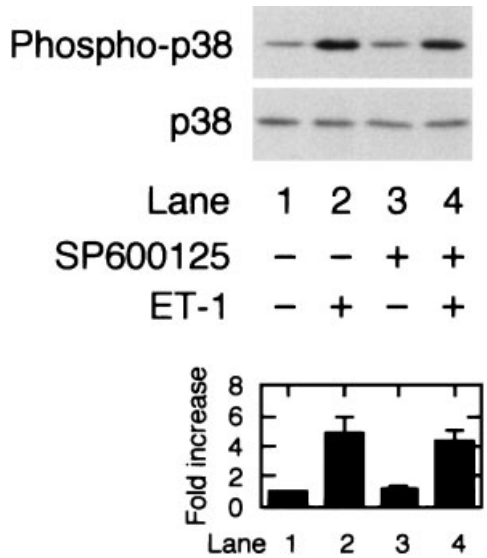

Fig. 6. Effect of SP600125 on the ET-1-induced phosphorylation of p38 MAP kinase in MC3T3-E1 cells. The cultured cells were pretreated with 10 μM SP600125 or vehicle for 60 min, and then stimulated by 3 nM ET-1 or vehicle for 5 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific p38 MAP kinase or p38 MAP kinase. The histogram shows quantitative representations of the levels of ET-1-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

of cells [Nishida and Gotoh, 1993; Widmann et al., 1999]. Three major MAP kinases such as p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK are recognized to transduce signals in mammalian cells [Nishida and Gotoh, 1993; Widmann et al., 1999]. We have previously reported that ET-1 activates both p44/p42 MAP kinase and p38 MAP kinase in osteoblast-like MC3T3-E1 cells [Kawamura et al., 1999a,b]. In addition, we here demonstrated that ET-1 induces the phosphorylation of JNK in these cells. It is well recognized that MAP kinases are activated by phosphorylation of threonine and tyrosine residues by dual specificity MAP kinase kinase [Raingeaud et al., 1995; Widmann et al., 1999]. Therefore, these results strongly suggest that ET-1 activates JNK in addition to p44/p42 MAP kinase and p38 MAP kinase in MC3T3-E1 cells. This is probably a first report showing the ET-1-induced JNK activation in osteoblasts as far as we know.

In the present study, we showed that SP600125, a highly specific inhibitor of JNK [Bennett et al., 2001], significantly suppressed the ET-1-induced VEGF synthesis in MC3T3-E1 cells. We also found that SP600125 actually reduced the phosphorylation of JNK induced by ET-1 in these cells. We have previously reported that the activation of p38 MAP kinase is involved in the ET-1-induced VEGF synthesis in these cells [Kozawa et al., 2000]. However, SP600125 hardly affected the ET-1-induced phosphorylation of p38 MAP kinase. It is probable that the SP600125-induced reduction of ET-1-stimulated VEGF synthesis is due to the attenuation of JNK activation. These results strongly suggest that the activation of JNK is involved in ET-1-stimulated VEGF synthesis in osteoblast-like MC3T3-E1 cells. In addition, we demonstrated that SB203580 had little effect on the phosphorylation of ET-1-induced JNK phosphorylation. Furthermore, the ET-1-stimulated VEGF synthesis was reduced additively by a combination of SP600125 and SB203580. Therefore, it seems that the JNK activation is responsible for the ET-1-stimulated VEGF synthesis independent of the p38 MAP kinase. Taken together, it is most likely

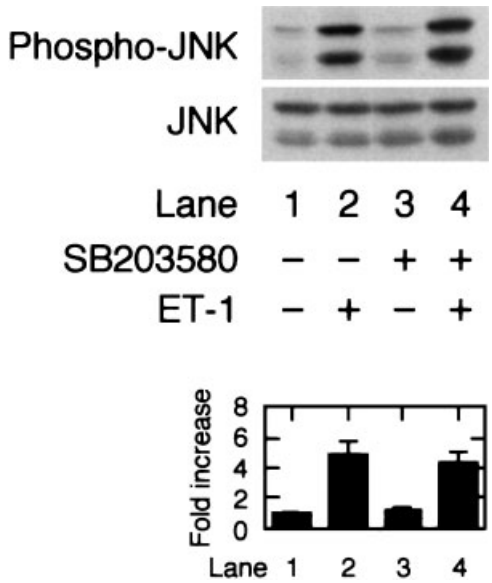

Fig. 7. Effect of SB203580 on the ET-1-induced phosphorylation of JNK in MC3T3-E1 cells. The cultured cells were pretreated with 10 μM SP600125 or vehicle for 60 min, and then stimulated by 100 nM ET-1 or vehicle for 30 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific JNK or JNK. The histogram shows quantitative representations of the levels of ET-1-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

that the VEGF synthesis stimulated by ET-1 is mediated through the activation of both p38 MAP kinase and JNK in osteoblast-like MC3T3-E1 cells.

The expansion of capillary network providing microvasculature is thought to be essential for the promotion of bone remodeling. ET-1 is produced and secreted mainly by endothelial cells [Masaki, 1993; Pollock et al., 1995]. As VEGF is a specific mitogen of vascular endothelial cells [Ferrara and Davis-Smyth, 1997], the VEGF synthesis by osteoblasts seems to be a considerable intercellular mediator toward the vascular endothelial cells providing microvasculature. In addition, VEGF is reportedly involved in trabecular bone formation and expansion of hypertrophic chondrocyte zone in mouse tibial epiphyseal growth plate [Gerber et al., 1999]. Therefore, ET-1-responsive VEGF synthesis by osteoblasts seems to promote the migration of vascular endothelial cells in microenvironment, resulting in the modulation of bone remodeling. It is possible that osteoblasts and vascular endothelial cells cooperatively regulate bone remodeling through ET-1-responsive VEGF synthesis.

In conclusion, our present results strongly suggest that ET-1 activates JNK in osteoblasts, and that JNK plays a part in ET-1-induced VEGF synthesis in addition to p38 MAP kinase.

REFERENCES

Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, Leisten JC, Motiwala A, Pierce S, Satoh Y, Bhagwat SS, Manning AM, Anderson DW. 2001. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal Kinase. Proc Natl Acad Sci USA 98:13681–13686.

Berti-Mattera LN, Wilkins PL, Harwalkar S, Madhun Z, Almhanna K, Mattera R. 2000. Endothelins regulate arachidonic acid release and mitogen-activated protein kinase activity in Schwann cells. J Neurochem 75:2316–2326.

Chevalier D, Thorin E, Allen BG. 2000. Simultaneous measurement of ERK, p38, and JNK MAP kinase cascades in vascular smooth muscle cells. J Pharmacol Toxicol Methods 44:429–439.

Cuenda A, Rouse J, Doza YN, Meier R, Cohen P, Gallagher TF, Young PR, Lee JC. 1995. SB203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. FEBS Lett 364: 229–233.

Erlebacher A, Filvaroff EH, Girelman SE, Derynck R. 1995. Toward a molecular understanding of skeletal development. Cell 80:371–378.

Ferrara N, Davis-Smyth T. 1997. The biology of vascular endothelial growth factor. Endocr Rev 18:4–25.

Gerber H-P, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. 1999. VEGF couples hypertrophic cartilage remodeling, ossification, and angiogenesis during endochondral bone formation. Nature Med 5:623–628.

Goad DL, Rubin J, Wang H, Tashijian AH, Jr., Patterson C. 1996. Enhanced expression of vascular endothelial growth factor in human SaOS-2 osteoblast-like cells and murine osteoblasts induced by insulin-like growth factor I. Endocrinology 137:2262–2268.

Harada S, Nagy JA, Sullivan KA, Thomas KA, Endo N, Rodan GA. 1994. Induction of vascular endothelial growth factor expression by prostaglandin E_2 and E_1 in osteoblasts. J Clin Invest 93:2490–2496.

Kato K, Ito H, Hasegawa K, Inaguma Y, Kozawa O, Asano T. 1996. Modulation of the stress-induced synthesis of hsp27 and αB-crystallin by cyclic AMP in C6 glioma cells. J Neurochem 66:946–950.

Kawamura H, Otsuka T, Tokuda H, Matsuno H, Niwa M, Matsui N, Uematsu T, Kozawa O. 1999a. Involvement of p42/p44 MAP kinase in endothelin-1-induced interleukin-6 synthesis in osteoblast-like cells. Bone 24:315–320.

Kawamura H, Otsuka T, Matsuno H, Niwa M, Matsui N, Kato K, Uematsu T, Kozawa O. 1999b. Endothelin-1 stimulates heat shock protein 27 induction in osteoblasts: Involvement of p38 MAP kinase. Am J Physiol 277: E1046–E1054.

Kozawa O, Tokuda H, Miwa M, Kotoyori J, Oiso Y. 1992. Cross-talk regulation between cyclic AMP production

and phosphoinositide hydrolysis induced by prostaglandin E_2 in osteoblast-like cells. Exp Cell Res 198:130–134.

Kozawa O, Kawamura H, Hatakeyama D, Matsuno H, Uematsu T. 2000. Endothelin-1 induces vascular endothelial growth factor synthesis in osteoblasts: Involvement of p38 mitogen-activated protein kinase. Cell Signal 12:375–380.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Masaki T. 1993. Endothelins: Homeostatic and compensatory actions in the circulatory and endocrine systems. Endocrinol Rev 14:256–268.

Nemoto S, Sheng Z, Lin A. 1998. Opposing effects of Jun kinase and p38 mitogen-activated protein kinases on cardiomyocyte hypertrophy. Mol Cell Biol 18:3518–3526.

Nijweide PJ, Burger EH, Feyen JHM. 1986. Cells of bone: Proliferation, differentiation, and humoral regulation. Physiol Rev 66:855–886.

Nishida E, Gotoh Y. 1993. The MAP kinase cascade is essential for diverse signal transduction pathways. Trends Biochem Sci 18:128–131.

Pollock DM, Keith TL, Highsmith RF. 1995. Endothelin receptors and calcium signaling. FASEB J 9:1196–1204.

Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, David RJ. 1995. Pro-inflammatory cytokines and environmental stress cause p38 MAP kinase activation by dual phosphorylation on tyrosine and threonine. J Biol Chem 270:7420–7426.

Sakurai T, Morimoto H, Kasuya Y, Takuwa Y, Nakauchi H, Masaki T, Goto K. 1992. Level of ETB receptor mRNA is down-regulated by endothelins through decreasing the intracellular stability of mRNA molecules. Biochem Biophys Res Commun 186:342–347.

Schalaeppi JM, Gutzwiller S, Finlenzeller G, Fournier B. 1997. 1,25-dihydroxyvitamin D_3 induces the expression of vascular endothelial growth factor in osteoblastic cells. Endocr Res 23:213–229.

Semler D, Ohlstein EH, Nambi P, Slater C, Stern PH. 1995. Endothelin-1-evoked calcium transients in UMR-106 osteoblastic osteosarcoma cells are mediated through endothelin-A and endothelin-B receptors. J Pharmacol Exp Ther 272:1052–1058.

Stern PH, Tatrai A, Semler DE, Lee SK, Lakatos P, Strieleman PJ, Tarjan G, Sanders JL. 1995. Endothelin receptors, second messengers, and actions in bone. J Nutr 125:2028S–2032S.

Sudo H, Kodama H, Amagai Y, Yamamoto S, Kasai S. 1983. In vivo differentiation and calcification in a new clonal osteogenic cell line derived from newborn mouse calvaria. J Cell Biol 96:191–198.

Suzuki A, Oiso Y, Kozawa O. 1994. Effect of endothelin-1 on phospholipase D activity in osteoblast-like cells. Mol Cell Endocrinol 105:193–196.

Takuwa Y, Ohue Y, Takuwa N, Yamashita K. 1989. Endothelin-1 activates phospholipase C and mobilizes Ca^{2+} from extra- and intracellular pools in osteoblastic cells. Am J Physiol 257:E797–E803.

Takuwa Y, Masaki T, Yamashita K. 1990. The effects of the endothelin family peptides on cultured osteoblastic cells from rat calvariae. Biochem Biophys Res Commun 170: 998–1005.

Tanaka T, Kanai H, Sekiguchi K, Aihara Y, Yokoyama T, Arai M, Kanda T, Nagai R, Kurabayashi M. 2000. 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 32:1955–1967.

Tatrai A, Foster S, Lakatos P, Shankar G, Stern PH. 1992. Endothelin-1 actions on resorption, collagen and non-collagen protein synthesis, and phosphatidylinositol turnover in bone organ cultures. Endocrinology 131: 603–607.

Tatrai A, Lakatos P, Thompson S, Stern PH. 1992. Effects of endothelin-1 on signal transduction in UMR-106 osteoblastic cells. J Bone Miner Res 7:1201–1209.

Wang DS, Yamazaki K, Nohtomi K, Shizume K, Ohsumi K, Shibuya M, Demura H, Sato K. 1996. Increase of vascular endothelial growth factor mRNA expression by 1,25-dihydroxyvitamin D_3 in human osteoblast-like cells. J Bone Miner Res 11:472–479.

Widmann C, Gibson S, Jarpe MB, Johnson GL. 1999. Mitogen-activated protein kinase: Conservation of a three-kinase module from yeast to human. Physiol Rev 79:143–180.

Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Takahashi N, Suda T. 1998. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastgenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 95:3597–3602.