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Limitation by p70 S6 kinase of platelet-derived growth factor-BB–induced interleukin 6 synthesis in osteoblast-like MC3T3-E1 cells

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Abstract

It has been reported that platelet-derived growth factor–BB (PDGF-BB) stimulates interleukin 6 (IL-6) in osteoblasts. In the present study, we investigated the mechanism of IL-6 synthesis induced by PDGF-BB in osteoblast-like MC3T3-E1 cells. Platelet-derived growth factor–BB time-dependently induced the phosphorylation of p44/p42 mitogen-activated protein (MAP) kinase, p38 MAP kinase, stress-activated protein kinase/c-*Jun* N-terminal kinase (SAPK/JNK), and p70 S6 kinase. PD98059 (an inhibitor of MAP kinase/extracellular signal-regulated kinase kinase [MEK]), SB203580 (an inhibitor of p38 MAP kinase), or SP600125 (an inhibitor of SAPK/JNK) suppressed the IL-6 synthesis induced by PDGF-BB. Rapamycin, an inhibitor of p70 S6 kinase, significantly enhanced the PDGF-BB–stimulated IL-6 synthesis. The PDGF-BB–induced phosphorylation of p70 S6 kinase was suppressed by rapamycin. Rapamycin failed to affect the PDGF-BB–induced phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, or SAPK/JNK. These results strongly suggest that PDGF-BB stimulates IL-6 synthesis through activation of 3 MAP kinases in osteoblasts and that p70 S6 kinase negatively regulates the IL-6 synthesis.

1. Introduction

Interleukin 6 (IL-6) is a pleiotropic cytokine that has important physiologic effects on a wide range of functions such as promoting B-cell differentiation and T-cell activation and inducing acute-phase proteins [1-3]. It is generally recognized that bone metabolism is regulated mainly by 2 functional cells, osteoblasts and osteoclasts, responsible for bone formation and bone resorption, respectively [4]. As for bone metabolism, IL-6 has been shown to stimulate bone resorption and promote osteoclast formation [2,3,5,6]. It has been reported that potent bone resorptive agents such as tumor necrosis factor α and IL-1 stimulate IL-6 synthesis in osteoblasts [5,7,8]. Currently, accumulating evidence indicates that IL-6 secreted from osteoblasts plays a pivotal role as a downstream effector of bone-resorptive agents.

It is well known that platelet-derived growth factor (PDGF) is a mitogenic factor, which mainly acts on connective tissue cells [9,10]. Platelet-derived growth factor occurs as 5 different isoforms [10]. Platelet-derived growth factor isoforms were originally isolated from platelets but have been shown to be produced and released from a variety of cell types including osteosarcoma cells [9,11]. As for stimulation of biologic activities in bone cells, PDGF-BB is a potent stimulator and induces osteoblast proliferation and collagen synthesis [12]. It is recognized that PDGF, released during platelet aggregation, has a pivotal role in fracture healing as a systemic factor and that PDGF also regulates bone remodeling as a local factor [12]. Platelet-derived growth factor receptor has an intrinsic protein tyrosine kinase activity and associates with SH-2 domain–containing substrates such as phospholipase C-γ and phosphatidylinositol 3-kinase [9]. We have previously reported that PDGF-BB activates phosphatidylcholine-hydrolyzing phospholipase D via tyrosine kinase activation, resulting in protein kinase C activation in osteoblast-like MC3T3-E1 cells [13]. It has been shown that PDGF-BB induces the transcription of IL-6 through the activator protein 1 complex and activating transcription factor 2 in primary cultured rat osteoblasts [14]. However, the exact mechanism underlying PDGF-BB–stimulated IL-6 synthesis in osteoblasts is not fully known.

It is generally recognized that p70 S6 kinase is a mitogen-activated serine/threonine kinase required for cell

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proliferation and G_1 cell-cycle progression [15]. As for osteoblasts, it has been shown that fluoroaluminate induces an increase in p70 S6 kinase phosphorylation [16]. In our previous study [17], we have reported that p70 S6 kinase plays as a positive regulator in bone morphogenetic protein 4–stimulated synthesis of vascular endothelial growth factor in osteoblast-like MC3T3-E1 cells. In addition, we recently demonstrated that p38 mitogen-activated protein (MAP) kinase, a member of the MAP kinase superfamily, functions at a point upstream from p70 S6 kinase in the synthesis of vascular endothelial growth factor in these cells [18]. However, the exact role of p70 S6 kinase in osteoblasts has not yet been fully clarified.

In the present study, we investigated the mechanism behind PDGF-BB–stimulated IL-6 synthesis in osteoblast-like MC3T3-E1 cells. We here show that PDGF-BB stimulates IL-6 synthesis through activation of 3 MAP kinases, p44/p42 MAP kinase, p38 MAP kinase, and stress-activated protein kinase/c-*Jun* N-terminal kinase (SAPK/JNK), in these cells, and that p70 S6 kinase concomitantly activated by PDGF-BB has an inhibitory role in the IL-6 synthesis.

2. Materials and methods

2.1. Materials

Platelet-derived growth factor–BB and mouse IL-6 and osteocalcin enzyme-linked immunosorbent assay (ELISA) kit were purchased from R&D Systems (Minneapolis, MN). Indomethacin was purchased from Sigma Chemical (St Louis, MO). PD98059, SB203580, SP600125, and rapamycin were obtained from Calbiochem-Novabiochem (La Jolla, CA). Phospho-specific p44/p42 MAP kinase antibodies, p44/p42 MAP kinase antibodies, phospho-specific p38 MAP kinase antibodies, p38 MAP kinase antibodies, phospho-specific SAPK/JNK antibodies, SAPK/JNK antibodies, phospho-specific p70 S6 kinase antibodies (Thr389), and p70 S6 kinase antibodies were purchased from Cell Signaling (Beverly, MA). ECL Western blotting detection system was purchased from Amersham Biosciences (Piscataway, NJ). Other materials and chemicals were obtained from commercial sources. PD98059, SB203580, SP600125, or rapamycin were dissolved in dimethyl sulfoxide. The maximum concentration of dimethyl sulfoxide was 0.1%, which did not affect the assay for IL-6 or Western blot analysis.

2.2. Cell culture

Cloned osteoblast-like MC3T3-E1 cells derived from newborn mouse calvaria [19] were maintained as previously described [20]. 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 hours.

2.3. Interleukin 6 ELISA

The cultured cells were stimulated by various doses of PDGF-BB in 1 mL of α-MEM containing 0.3% FCS for the indicated periods. When indicated, the cells were pretreated

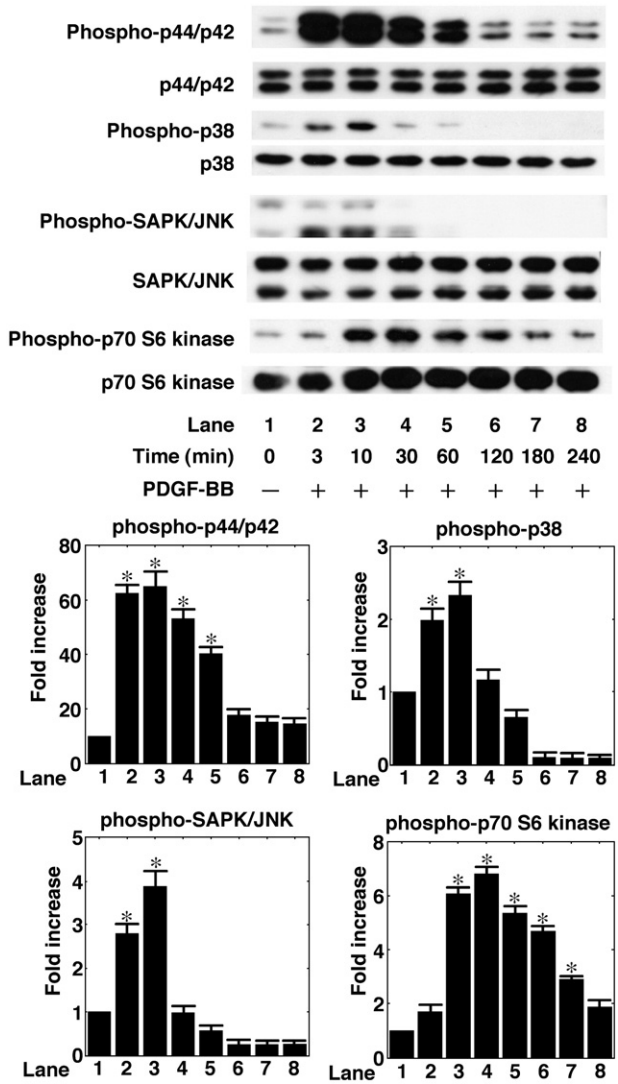

Fig. 1. Effects of PDGF-BB on the phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, SAPK/JNK, or p70 S6 kinase in MC3T3-E1 cells. The cultured cells were stimulated by 50 ng/mL PDGF-BB for the indicated periods. The extracts of cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with subsequent Western blotting analysis with antibodies against phospho-specific p44/p42 MAP kinase, p44/p42 MAP kinase, phospho-specific p38 MAP kinase, p38 MAP kinase, phospho-specific SAPK/JNK, SAPK/JNK, phospho-specific p70 S6 kinase, or p70 S6 kinase. Similar results were obtained with 2 additional and different cell preparations. The histogram shows quantitative representations of the levels of PDGF-BB–induced phosphorylation obtained from laser densitometric analysis of 3 independent experiments. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with 2 additional and different cell preparations. *$P < .05$ compared with the value of control.

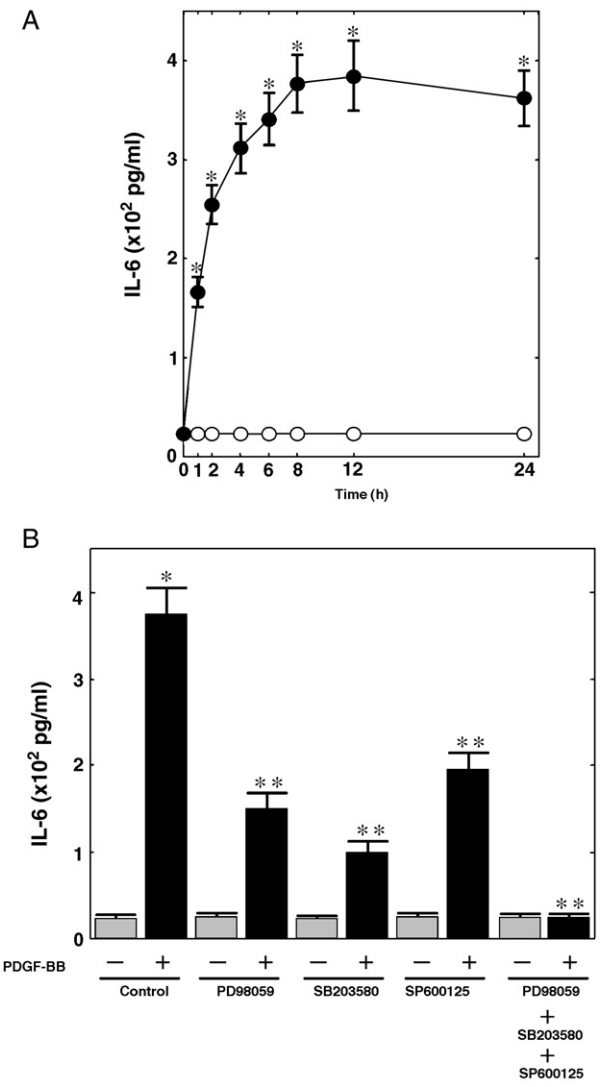

Fig. 2. Time course of PDGF-BB–induced IL-6 synthesis from MC3T3-E1 cells and effects of PD98059, SB203580, or SP600125 on the IL-6 synthesis by PDGF-BB in MC3T3-E1 cells. A, The cultured cells were stimulated by 50 ng/mL PDGF-BB (●) or vehicle (○) for the indicated periods. B, The cultured cells were pretreated with 3 μmol/L PD98059, 3 μmol/L SB203580, 3 μmol/L SP600125, or vehicle for 60 minutes and then stimulated by vehicle (gray bar) or 50 ng/mL PDGF-BB (black bar) for 24 hours. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with 2 additional and different cell preparations. *$P < .05$ compared with the value of control. **$P < .05$ compared with the value of PDGF-BB alone.

with PD98059, SB203580, SP600125, indomathacin, or rapamycin for 60 minutes. The conditioned medium was collected at the end of the incubation, and the IL-6 concentration was measured by ELISA kit.

2.4. Osteocalcin ELISA

The cultured cells were pretreated with various doses of rapamycin for 60 minutes and then stimulated by 50 ng/mL PDGF-BB or vehicle for 24 hours. The conditioned medium was collected at the end of the incubation, and the osteocalcin concentration was measured by ELISA kit.

2.5. Western blot analysis

The cultured cells were stimulated by PDGF-BB 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 (pH 6.8) containing 62.5 mmol/L Tris/HCl, 2% sodium dodecyl sulfate, 50 mmol/L dithiothreitol, and 10% glycerol. The cytosolic fraction was collected as a supernatant after centrifugation at 125 000*g* for 10 minutes at 4°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by Laemmli [21] in 10% polyacrylamide gel. Western blotting analysis was performed as described previously [22] by using phospho-specific p44/p42 MAP kinase antibodies, p44/p42 MAP kinase antibodies, phospho-specific p38 MAP kinase antibodies, p38 MAP kinase antibodies, phospho-specific SAPK/JNK antibodies, SAPK/JNK antibodies, phospho-specific p70 S6 kinase antibodies, or p70 S6 kinase antibodies, with peroxidase-labeled antibodies raised in goat-against-rabbit immunoglobulin G being used as second antibodies. Peroxidase activity on the polyvinylidene difluoride (PVDF) sheet was visualized on x-ray film by means of the ECL Western blotting detection system.

2.6. Determination

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

2.7. Statistical analysis

The data were analyzed by analysis of variance followed by the Bonferroni method for multiple comparisons between pairs, and $P < .05$ was considered significant. All data are presented as the mean ± SEM of triplicate determinations. Each experiment was repeated 3 times, with similar results.

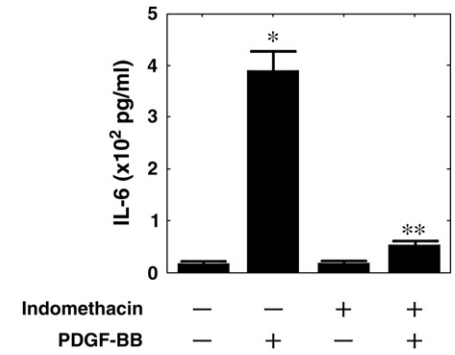

Fig. 3. Effect of indomethacin on the PDGF-BB–stimulated IL-6 synthesis in MC3T3-E1 cells. The cultured cells were pretreated with 10 μmol/L indomethacin or vehicle for 60 minutes and then stimulated by 50 ng/mL PDGF-BB or vehicle for 24 hours. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with 2 additional and different cell preparations. *$P < .05$ compared with the control. **$P < .05$ compared with the value of PDGF-BB alone.

3. Results

3.1. Effects of PDGF-BB on the phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, or SAPK/JNK in MC3T3-E1 cells

It is well recognized that 3 MAP kinases, p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK, are known as central elements used by mammalian cells to transduce the various messages of a variety of agonists [23]. To investigate whether PDGF-BB activates MAP kinases in osteoblast-like MC3T3-E1 cells, we examined the effect of PDGF-BB on the phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, or SAPK/JNK. Platelet-derived growth factor–BB time-dependently induced the phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK. The effect of PDGF-BB on the p44/p42 MAP kinase phosphorylation reached its peak at 10 minutes and continued to 60 minutes after the stimulation of PDGF-BB (Fig. 1). On the other hand, the effect on the phosphorylation of p38 MAP kinase reached its peak at 10 minutes and diminished within 30 minutes after the stimulation of PDGF-BB (Fig. 1). In addition, the maximum effect on the SAPK/JNK phosphorylation was observed at 10 minutes and diminished within 30 minutes after the stimulation of PDGF-BB (Fig. 1).

3.2. Effects of PD98059, SB203580, or SP600125 on the PDGF-BB–stimulated IL-6 synthesis in MC3T3-E1 cells

It has been reported that PDGF-BB induces IL-6 transcription in osteoblasts from fetal rat calvariae [14]. We found that PDGF-BB time-dependently stimulated IL-6 synthesis in osteoblast-like MC3T3-E1 cells (Fig. 2A). To clarify the involvement of the MAP kinase pathway in the PDGF-BB–stimulated IL-6 synthesis in these cells, we first examined the effect of PD98059, a specific inhibitor of MAP kinase/extracellular signal-regulated kinase kinase (MEK, an upstream kinase that activates p44/p42 MAP kinase) [24], on the IL-6 synthesis. PD98059, which by itself had little effect on the IL-6 levels, significantly suppressed the PDGF-BB–stimulated synthesis of IL-6 (Fig. 2B). Similarly, the IL-6 synthesis stimulated by PDGF-BB was markedly reduced by SB203580, a specific inhibitor of p38 MAP kinase [25], or SP600125, a specific SAPK/JNK inhibitor [26] (Fig. 2B). In addition, a combination of PD98059, SB203580, and SP600125 completely suppressed the PDGF-BB–stimulated synthesis of IL-6 (Fig. 2B). To determine whether these inhibitors themselves could affect cell survival, or cell number, the cell viability had been assessed by trypan blue dye exclusion test. We confirmed that the viability of the cells incubated at 37°C for 24 hours in the presence of 3 μmol/L PD980590, 3 μmol/L SB203580, or 3 μmol/L SP600125 was more than 90% compared with that of the control cells.

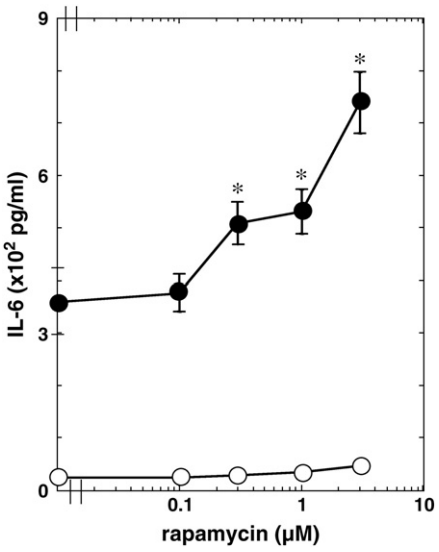

Fig. 4. Effect of rapamycin on the PDGF-BB–stimulated IL-6 synthesis in MC3T3-E1 cells. The cultured cells were pretreated with various doses of rapamycin for 60 minutes and then stimulated by 50 ng/mL PDGF-BB (●) or vehicle (○) for 24 hours. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with 2 additional and different cell preparations. *$P < .05$ compared with the value of PDGF-BB alone.

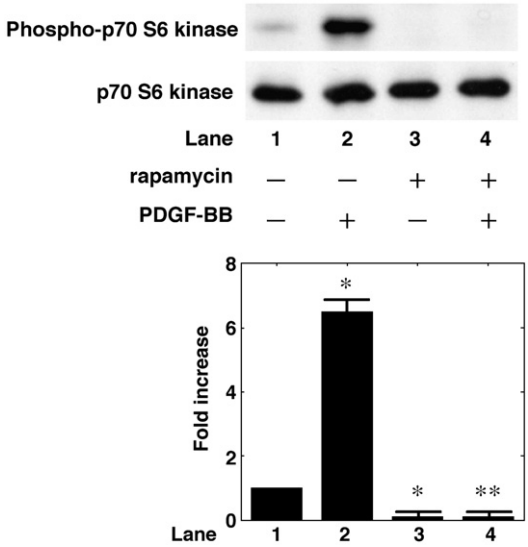

Fig. 5. Effect of rapamycin on the PDGF-BB–induced phosphorylation of p70 S6 kinase in MC3T3-E1 cells. The cultured cells were pretreated with 30 μmol/L rapamycin for 60 minutes and then stimulated by 50 ng/mL PDGF-BB or vehicle for 30 minutes. The extracts of cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with subsequent Western blotting analysis with antibodies against phospho-specific p70 S6 kinase or p70 S6 kinase. The histogram shows quantitative representations of the levels of PDGF-BB–induced phosphorylation obtained from laser densitometric analysis of 3 independent experiments. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with 2 additional and different cell preparations. *$P < .05$ compared with the value of control. **$P < .05$ compared with the value of PDGF-BB alone.

3.3. Effect of indomethacin on the PDGF-BB–stimulated IL-6 synthesis in MC3T3-E1 cells

Because we have previously reported that prostaglandins (PGs) increase IL-6 synthesis in MC3T3-E1 cells [27-30], to address whether endogenous PGs are involved in the

PDGF-BB–induced IL-6 synthesis in MC3T3-E1 cells, we examined the effect of indomethacin, an inhibitor of cyclooxygenase [31], on the IL-6 synthesis. Indomethacin, which by itself had no effect on the IL-6 levels, significantly reduced the PDGF-BB–induced synthesis of IL-6 (Fig. 3). These findings suggest that PGs mediate the stimulatory effect of PDGF-BB on IL-6 synthesis in these cells.

3.4. Effect of PDGF-BB on the phosphorylation of p70 S6 kinase in MC3T3-E1 cells

To clarify whether PDGF-BB activates p70 S6 kinase in MC3T3-E1 cells, we next examined the effect of PDGF-BB on the phosphorylation of p70 S6 kinase. p70 S6 kinase was time-dependently phosphorylated by PDGF-BB (Fig. 1). The maximum effect on the p70 S6 kinase phosphorylation was observed at 30 minutes after the stimulation of PDGF-BB, and the PDGF-BB effect continued 180 minutes after the stimulation.

3.5. Effect of rapamycin on the PDGF-BB–stimulated IL-6 synthesis in MC3T3-E1 cells

To investigate whether p70 S6 kinase is involved in the PDGF-BB–induced synthesis of IL-6 in MC3T3-E1 cells, we examined the effect of rapamycin, a specific inhibitor of p70 S6 kinase [32,33], on the synthesis of IL-6 induced by PDGF-BB. Rapamycin, which alone failed to affect the IL-6 levels, significantly enhanced the PDGF-BB–induced synthesis of IL-6 (Fig. 4). The amplifying effect of rapamycin was dose-dependent in the range between 0.1 and 3 μmol/L. Rapamycin at 3 μmol/L caused approximately 110% enhancement in the PDGF-BB effect.

3.6. Effect of rapamycin on the PDGF-BB–induced phosphorylation of p70 S6 kinase in MC3T3-E1 cells

We examined the effect of rapamycin on the PDGF-BB–induced phosphorylation of p70 S6 kinase. Rapamycin, which itself significantly suppressed the phosphorylation of p70 S6 kinase in itself, truly suppressed the PDGF-BB–induced phosphorylation of p70 S6 kinase (Fig. 5).

3.7. Effect of rapamycin on the proliferation or the differentiation of MC3T3-E1 cells

To determine whether rapamycin could affect cell survival, or cell number, the cell viability had been assessed by trypan blue dye exclusion test. We confirmed that the viability of the cells incubated at 37°C for 24 hours in the

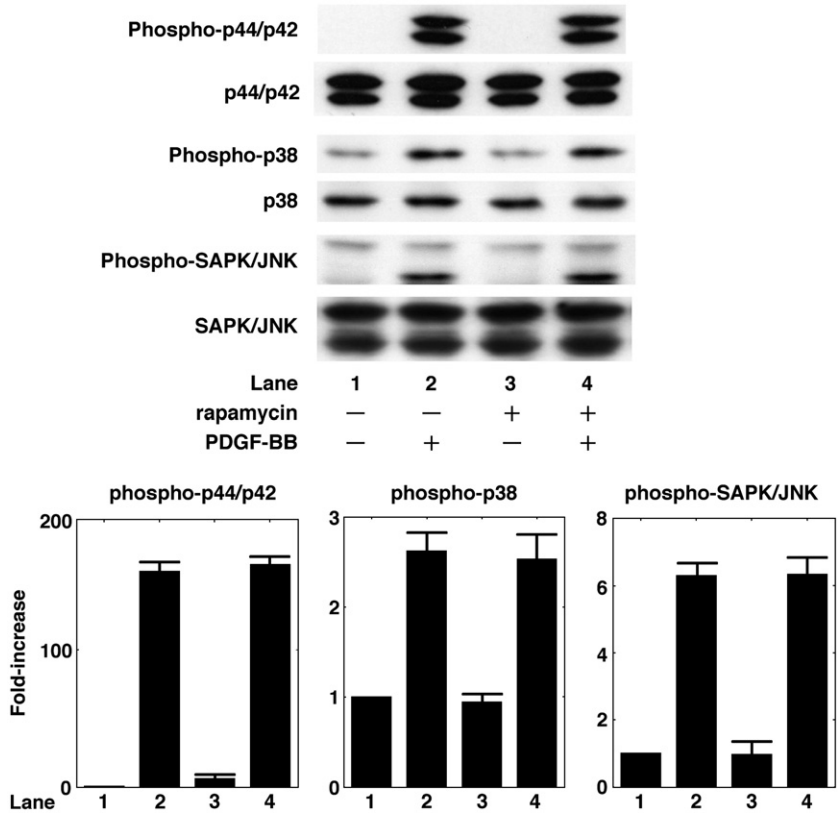

Fig. 6. Effects of rapamycin on the PDGF-BB–induced phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, or SAPK/JNK in MC3T3-E1 cells. The cultured cells were pretreated with 30 μmol/L rapamycin or vehicle for 60 minutes and then stimulated by 50 ng/mL PDGF-BB or vehicle for 10 minutes. The extracts of cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with subsequent Western blotting analysis with antibodies against phospho-specific p44/p42 MAP kinase, p44/p42 MAP kinase, phospho-specific p38 MAP kinase, p38 MAP kinase, phospho-specific SAPK/JNK, or SAPK/JNK. The histogram shows quantitative representations of the levels of PDGF-BB–induced phosphorylation obtained from laser densitometric analysis of 3 independent experiments. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with 2 additional and different cell preparations.

presence of 3 μmol/L rapamycin was more than 90% compared with that of the control cells. To determine whether rapamycin could affect the cell proliferation, we counted the cell number before and after the 24-hour incubation with rapamycin. We confirmed that rapamycin did not affect the cell number at a dose of 3 μmol/L ($9.7 \pm 1.1 \times 10^5$ cells/mL for control; $15.6 \pm 1.6 \times 10^5$ cells/mL for 50 ng/mL PDGF-BB alone; $10.1 \pm 1.4 \times 10^5$ cells/mL for 3 μmol/L rapamycin alone; and $14.7 \pm 1.6 \times 10^5$ cells/mL for 50 ng/mL PDGF-BB with 3 μmol/L rapamycin, as measured during the stimulation for 24 hours).

Next, to determine whether rapamycin affects the differentiation of these cells, we examined the effect of rapamycin on the production of osteocalcin, a mature osteoblast phenotype [34], in MC3T3-E1 cells. Platelet-derived growth factor–BB or rapamycin did not induce osteocalcin production in MC3T3-E1 cells (2.7 ± 0.3 ng/mL for control; 2.6 ± 0.3 ng/mL for 50 ng/mL PDGF-BB alone; 2.5 ± 0.4 ng/mL for 3 μmol/L rapamycin alone; and 2.5 ± 0.3 ng/mL for 50 ng/mL PDGF-BB with 3 μmol/L rapamycin, as measured during the stimulation for 24 hours). These findings as a whole suggest that rapamycin hardly affects the proliferation and the differentiation of osteoblast-like MC3T3-E1 cells within 24 hours.

3.8. Effects of rapamycin on the PDGF-BB–induced phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, or SAPK/JNK in MC3T3-E1 cells

To investigate whether rapamycin's effect on the PDGF-BB–stimulated IL-6 synthesis is dependent on the activation of p44/p42 MAP kinase, p38 MAP kinase, or SAPK/JNK, we next examined the effect of rapamycin on the phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, or SAPK/JNK induced by PDGF-BB in these cells. However, rapamycin failed to affect the PDGF-BB–induced phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, or SAPK/JNK (Fig. 6).

4. Discussion

In the present study, we found that PDGF-BB time-dependently induced the phosphorylation of p70 S6 kinase in osteoblast-like MC3T3-E1 cells, using phospho-specific p70 S6 kinase (Thr389) antibodies. It is generally recognized that the activity of p70 S6 kinase is regulated by multiple phosphorylation events [15]. It has been shown that phosphorylation at Thr389 most strongly correlates with p70 S6 kinase activity [15]. Taking these results into account, it is most likely that PDGF-BB activates p70 S6 kinase in osteoblast-like MC3T3-E1 cells. To the best of our knowledge, this is probably the first report showing the PDGF-BB–induced p70 S6 kinase activation in osteoblasts.

We next demonstrated that PDGF-BB induces the phosphorylation of p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK in these cells. It is well recognized that the MAP kinase superfamily mediates intracellular signaling of extracellular agonists and plays an important role in cellular functions including proliferation, differentiation, and apoptosis in a variety of cells [23]. Three major MAP kinases, p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK, are known as central elements used by mammalian cells to transduce diverse messages [23]. It has been shown that MAP kinases are activated by phosphorylation of threonine and tyrosine residues by dual-specificity MAP kinases [23]. Therefore, our findings strongly suggest that PDGF-BB activates 3 MAP kinases, p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK, in osteoblast-like MC3T3-E1 cells. In addition, we showed that the PDGF-BB–stimulated IL-6 synthesis was suppressed by a MEK inhibitor, PD98059 [24]; a specific p38 MAP kinase inhibitor, SB203580 [25]; or a specific SAPK/JNK inhibitor, SP600125 [26], in these cells. Thus, it is probable that PDGF-BB stimulates the synthesis of IL-6 via the 3 MAP kinases in osteoblast-like MC3T3-E1 cells. We have previously reported that PGs increase IL-6 synthesis in MC3T3-E1 cells [27-30]. In the present study, we found that indomethacin significantly reduced the PDGF-BB–induced synthesis of IL-6. These results suggest that PDGF-BB–induced IL-6 production is mediated, at least in part, by PDGF-BB–stimulated PG production in osteoblast-like MC3T3-E1 cells. In addition, we have previously shown that PGE_2, a major product of eicosanoids in osteoblasts, significantly stimulates IL-6 synthesis after 3 hours in MC3T3-E1 cells [30]. On the contrary, PDGF-BB significantly stimulated the IL-6 production within 3 hours. Taking our findings into account, it is quite likely that there will be PG-dependent and PG-independent effects of PDGF-BB–stimulated IL-6 synthesis, as has been demonstrated for so many growth factors and cytokines in bone cells, and it would be important to be define these. Therefore, experiments using PGE_2 itself instead of PDGF-BB are required.

We investigated whether p70 S6 kinase functions in the PDGF-BB–stimulated IL-6 synthesis in osteoblast-like MC3T3-E1 cells. The PDGF-BB–stimulated synthesis of IL-6 was significantly amplified by rapamycin, a specific inhibitor of p70 S6 kinase [31,32]. We confirmed that rapamycin truly suppressed the PDGF-BB–induced phosphorylation of p70 S6 kinase. It seems that the activated p70 S6 kinase plays an inhibitory role in the IL-6 synthesis by PDGF-BB in osteoblast-like MC3T3-E1 cells. Therefore, taking our results into account, it is most likely that PDGF-BB activates p70 S6 kinase, resulting in down-regulation of IL-6 synthesis. It is probable that the p70 S6 kinase signaling pathway activated by PDGF-BB limits the PDGF-BB–stimulated IL-6 synthesis. As far as we know, our present finding is probably the first report to show that the activation of p70 S6 kinase leads to the negative-feedback regulation of IL-6 synthesis in osteoblasts.

We investigated the relationship between p70 S6 kinase and 3 MAP kinases in the PDGF-BB–stimulated IL-6 synthesis in MC3T3-E1 cells. However, rapamycin failed to enhance the PDGF-BB–induced phosphorylation levels of

p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK. Therefore, it seems unlikely that p70 S6 kinase signaling pathway affects the PDGF-BB–stimulated synthesis of IL-6 through the amplification of activities of 3 MAP kinases, p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK, in osteoblast-like MC3T3-E1 cells.

The p70 S6 kinase pathway is recognized to play a crucial role in various cellular functions, especially cell-cycle progression [15]. Our present results indicate that the p70 S6 kinase pathway in osteoblasts has an important role in the control of the production of IL-6, one of the key regulators of bone metabolism. It is well known that IL-6 produced by osteoblasts is a potent bone resorptive agent and induces osteoclast formation [3,4]. The mitogenic activities of PDGF-BB and its release by platelets suggest an important role in wound healing and fracture repair [35]. It is also possible that PDGF-BB plays a role in acute bone repair after inflammation because the mitogenic actions of PDGF-BB are enhanced in the presence of cytokines [35]. Therefore, our present findings lead us to speculate that PDGF-BB–activated p70 S6 kinase acts as a negative regulator of bone resorption through the fine tuning of the local cytokine network. Thus, the p70 S6 kinase pathway in osteoblasts might be considered to be a new candidate as a molecular target of bone resorption concurrent with various bone diseases. On the contrary, we have previously shown that p70 S6 kinase acts as a positive regulator in bone morphogenetic protein-4–stimulated synthesis of vascular endothelial growth factor in MC3T3-E1 cells [17]. The physiologic significance of regulatory mechanism by p70 S6 kinase in osteoblasts still remains unclear. Further investigation is required to clarify the exact role of p70 S6 kinase in osteoblasts.

In conclusion, our results strongly suggest that p70 S6 kinase plays an important role in the regulation of PDGF-BB–stimulated, MAP kinase–mediated IL-6 synthesis in osteoblasts and may serve as a negative feedback mechanism to prevent from oversynthesizing IL-6 in these cells.

Acknowledgments

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