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Phosphatidylinositol 3-Kinase/Akt Auto-Regulates PDGF-BB-Stimulated Interleukin-6 Synthesis in Osteoblasts

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Abstract It has been reported that platelet-derived growth factor (PDGF)-BB stimulates the synthesis of interleukin (IL)-6 in osteoblasts. In the present study, we investigated whether the phosphatidylinositol 3-kinase (PI3K)/Akt is involved in the PDGF-BB-induced IL-6 synthesis in osteoblast-like MC3T3-E1 cells. PDGF-BB markedly induced the phosphorylation of Akt and GSK-3β. Akt inhibitor, 1L-6-hydroxymethyl-*chiro*-inositol 2-(*R*)-2-*O*-methyl-3-O-octadecylcarbonate, significantly amplified the synthesis of IL-6 by PDGF-BB. The PDGF-BB-induced GSK-3β phosphorylation was suppressed by the Akt inhibitor. The IL-6 synthesis stimulated by PDGF-BB was markedly enhanced by LY294002 and wortmannin, inhibitors of PI3K. Wortmannin and LY294002 suppressed the PDGF-BB-induced phosphorylation of Akt and GSK-3β. Taken together, these results strongly suggest that PI3K/Akt negatively regulates the PDGF-BB-stimulated IL-6 synthesis in osteoblasts. J. Cell. Biochem. 99: 1564–1571, 2006.

Key words: platelet-derived growth factor (PDGF); interleukin-6 (IL-6); phosphatidylinositol 3-kinase; Akt; osteoblast

It is generally known that platelet-derived growth factor (PDGF) is a mitogenic factor, which mainly acts on connective tissue cells [Heldin and Westermark, 1999; Heldin et al., 2002]. PDGF occurs as five different isoforms [Heldin et al., 2002]. PDGF isoforms were originally isolated from platelets, but have been shown to be produced and released from a variety of cell types including osteosarcoma [Heldin et al., 1986; Heldin and Westermark, 1999]. Bone metabolism is regulated by two functional cells, osteoblasts and osteoclasts, responsible for bone formation and bone resorption, respectively [Nijweide et al., 1986]. As for stimulation of biological activities in bone cells, PDGF-BB is a potent stimulator and induces osteoblast proliferation and collagen synthesis [Canalis et al., 1992]. 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 [Canalis et al., 1992]. PDGF receptor has an intrinsic protein tyrosine kinase activity and associates with SH-2 domain-containing substrates such as phospholipase C and phosphatidylinositol 3-kinase (PI3K) [Heldin and Westermark, 1999]. 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 [Kozawa et al., 1995]. However, the exact role of PDGF in osteoblasts is not precisely known.

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Interleukin-6 (IL-6) is a multifunctional cytokine that has important physiological effects on a wide range of functions, such as promoting B cell differentiation, T cell activation, and inducing acute phase proteins [Akira et al., 1993; Heymann and Rousselle, 2000; Kwan Tat et al., 2004]. As for bone metabolism, IL-6 has been shown to stimulate bone resorption and promote osteoclast formation [Ishimi et al., 1990; Roodman, 1992; Heymann and Rousselle, 2000; Kwan Tat et al., 2004]. It has been reported that bone resorptive agents such as tumor necrosis factor-α (TNF-α) and IL-1 stimulate IL-6 synthesis in osteoblasts [Helle et al., 1988; Ishimi et al., 1990; Littlewood et al., 1991]. Thus, accumulating evidence indicates that IL-6 secreted from osteoblasts plays a key role as a downstream effector of bone resorptive agents. 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 [Franchimont et al., 1999]. However, the exact mechanism underlying PDGF-BB-stimulated IL-6 synthesis in osteoblasts has not yet been precisely clarified.

It is currently known that Akt, also called protein kinase B, is a serine/threonine protein kinase that plays crucial roles in mediating intracellular signaling of variety of agonists including insulin-like growth factor-I, PDGF, and cytokines [Coffer et al., 1998]. It has been shown that Akt regulates biological functions, such as gene expression, survival, and oncogenesis [Coffer et al., 1998]. Accumulating evidence suggests that PI3K functions at a point upstream from Akt [Chan et al., 1999; Cantley, 2002]. Akt containing a pleckstrin homology domain is recruited to the plasma membrane by the lipid product of PI3K and activated. As for osteoblasts, it has been reported that TNF-α and PDGF induce translocation of Akt to the nucleus [Borgatti et al., 2000]. In addition, Akt is reportedly activated by cyclic stretch [Danciu et al., 2003] or androgen [Kang et al., 2004]. However, the exact role of Akt in PDGF-BB-effect on osteoblasts has not yet been clarified.

In the present study, we investigated whether PI3K/Akt is involved in the PDGF-BB-stimulated IL-6 synthesis in osteoblast-like MC3T3-E1 cells. We here show that PI3-kinase/Akt activated by PDGF-BB plays an inhibitory role in the IL-6 synthesis in these cells.

MATERIALS AND METHODS

Materials

PDGF-BB and mouse IL-6 enzyme immunoassay kit were purchased from R&D Systems, Inc. (Minneapolis, MN). Akt inhibitor (1L-6-hydroxymethyl-*chiro*-inositol 2-(*R*)-2-*O*-methyl-3-*O*-octadecylcarbonate), LY294002 and wortmannin were obtained from Calbiochem-Novabiochem Co. (La Jolla, CA). Phospho-specific Akt antibodies, Akt antibodies, phospho-specific GSK-3β antibodies, and GSK-3β antibodies were purchased from Cell Signaling, Inc. (Beverly, MA). ECL Western blotting detection system was purchased from Amersham Japan (Tokyo, Japan). Other materials and chemicals were obtained from commercial sources. Akt inhibitor, wortmannin or LY294002 were dissolved in dimethyl sulfoxide (DMSO). The maximum concentration of DMSO was 0.1%, which did not affect the assay for IL-6 or Western blot analysis.

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-mm 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. The plating density of the cells was about 8×10^5 cells/dish for 35-mm diameter dish, or 7×10^6 cells/dish for 90-mm diameter dish. The media were changed again when the various inhibitors and/or PDGF-BB were subsequently added.

IL-6 Assay

The cultured cells were stimulated by various dose of PDGF-BB in 1 ml of α-MEM containing 0.3% FCS for the indicated periods. When indicated, the cells were pre-treated with Akt inhibitor, wortmannin or LY294002 for 60 min. The conditioned medium was collected at the end of the incubation, and the IL-6 concentration was measured by ELISA kit.

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 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 Akt antibodies, Akt antibodies, phospho-specific GSK-3β antibodies, or GSK-3β antibodies, with peroxidase-labeled antibodies raised in goat against rabbit IgG being used as second antibodies. Peroxidase activity on the PVDF sheet was visualized on X-ray film by means of the ECL Western blotting detection system.

Determination

The absorbance of enzyme immunoassay 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 ± SD of triplicate determinations. Each experiment was repeated three times with similar results.

RESULTS

Effect of PDGF-BB on the Phosphorylation of Akt in MC3T3-E1 Cells

We examined the effect of PDGF-BB on the phosphorylation of Akt in order to investigate whether PDGF-BB activates Akt in osteoblast-like MC3T3-E1 cells. PDGF-BB time-dependently stimulated the phosphorylation of Akt up to 120 min (Fig. 1). The maximum effect of PDGF-BB on the phosphorylation of Akt was observed at 20 min after the stimulation.

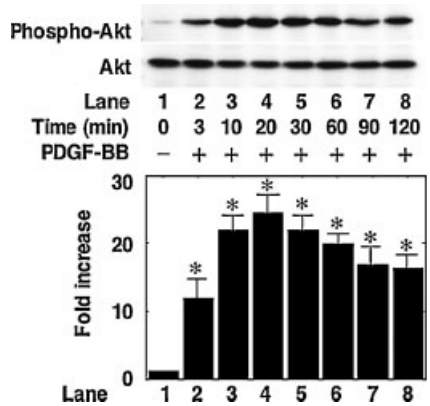

Fig. 1. Effect of PDGF-BB on the phosphorylation of Akt in MC3T3-E1 cells. The cultured cells were stimulated by 50 ng/ml PDGF-BB for the indicated periods. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific Akt or Akt. The histogram shows quantitative representations of the levels of PDGF-BB-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were expressed as the fold increase to the basal levels presented as **lane 1**. The values were calculated as the average values of lane 1 to be 1.0. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}P < 0.05$, compared to the value of control.

Effect of Akt Inhibitor on the IL-6 Synthesis by PDGF-BB in MC3T3-E1 Cells

It has been reported that PDGF-BB induces IL-6 transcription in osteoblasts from fetal rat calvariae [Franchimont et al., 1999]. We found that PDGF-BB stimulated IL-6 synthesis in a dose-dependent manner between 10 ng/ml and 70 ng/ml in osteoblast-like MC3T3-E1 cells (data not shown). In order to clarify the involvement of Akt pathway in the PDGF-BB-stimulated IL-6 synthesis in these cells, we first examined the effect of Akt inhibitor, 1L-6-hydroxymethyl-*chiro*-inositol 2-(*R*)-2-*O*-methyl-3-*O*-octadecylcarbonate [Hu et al., 2000], on the IL-6 synthesis. The Akt inhibitor, which by itself hardly affected the IL-6 levels, significantly enhanced the PDGF-BB-stimulated synthesis of IL-6 (Fig. 2). The amplifying effect of the Akt inhibitor on the IL-6 synthesis was dose-dependent between 1 μM and 3 μM (Fig. 2). The Akt inhibitor at 10 μM caused about 100% enhancement in the PDGF-BB-effect.

Effect of Akt Inhibitor on the Phosphorylation of GSK-3β Induced by PDGF-BB in MC3T3-E1 Cells

It is generally known that GSK-3β is a critical downstream target molecule of the Akt, and its

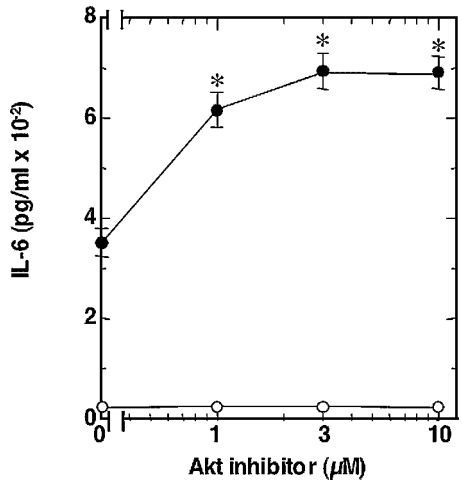

Fig. 2. Effect of Akt inhibitor on the PDGF-BB-stimulated IL-6 synthesis in MC3T3-E1 cells. The cultured cells were pre-treated with various doses of Akt inhibitor for 60 min, and then stimulated by 50 ng/ml PDGF-BB or vehicle for 24 h. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}P < 0.05$, compared to the value of PDGF-BB alone.

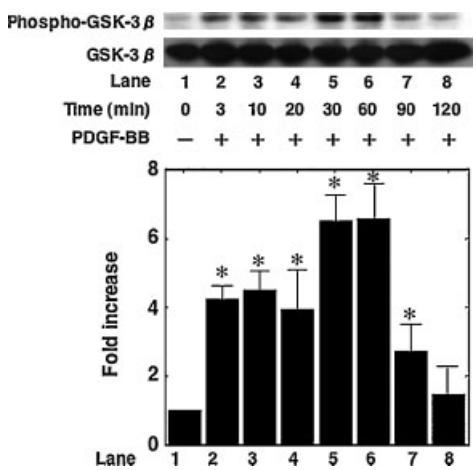

Fig. 3. Effect of PDGF-BB on the phosphorylation of GSK-3β in MC3T3-E1 cells. The cultured cells were stimulated by 50 ng/ml PDGF-BB for the indicated periods. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific GSK-3β or GSK-3β. The histogram shows quantitative representations of the levels of PDGF-BB-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were expressed as the fold increase to the basal levels presented as **lane 1**. The values were calculated as the average values of lane 1 to be 1.0. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}P < 0.05$, compared to the value of control.

activity can be inhibited by Akt-mediated phosphorylation of GSK-3β at Ser9 [Cross et al., 1995; Srivastava and Pandey, 1998]. We found that PDGF-BB truly induced the Akt-mediated phosphorylation of GSK-3β in a time-dependent manner in MC3T3-E1 cells (Fig. 3). The maximum effect of PDGF-BB on the phosphorylation of GSK-3β was observed at 60 min after the stimulation. We next examined the effect of Akt inhibitor on the phosphorylation of GSK-3β induced by PDGF-BB in these cells. Akt inhibitor markedly reduced the PDGF-BB-induced phosphorylation of GSK-3β (Fig. 4).

Effects of LY294002 or Wortmannin on the PDGF-BB-Induced Phosphorylation of Akt in MC3T3-E1 Cells

To clarify whether or not PI3K functions at a point upstream from Akt in MC3T3-E1 cells, we examined the effect of LY294002, a specific inhibitor of PI3-kinase [Vlahos et al., 1994], on the Akt phosphorylation induced by PDGF-BB.

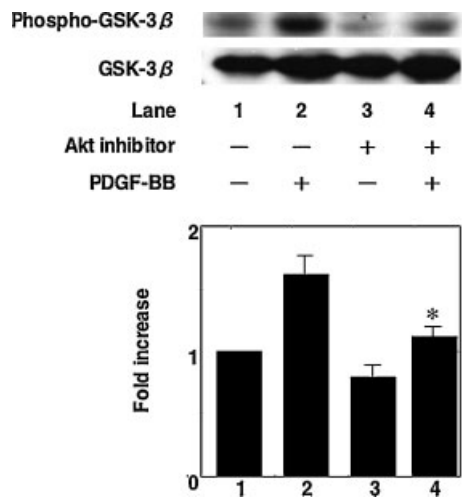

Fig. 4. Effect of Akt inhibitor on the PDGF-BB-induced phosphorylation of GSK-3β in MC3T3-E1 cells. The cultured cells were pre-treated with 50 μM Akt inhibitor for 60 min, and then stimulated by 50 ng/ml PDGF-BB or vehicle for 60 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific GSK-3β or GSK-3β. The histogram shows quantitative representations of the levels of PDGF-BB-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were expressed as the fold increase to the basal levels presented as **lane 1**. The values were calculated as the average values of lane 1 to be 1.0. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}P < 0.05$, compared to the value of PDGF-BB alone.

LY294002 suppressed the PDGF-BB-induced phosphorylation of Akt (Fig. 5). The inhibitory effect of LY294002 was dose-dependent between 10 μM and 50 μM. In addition, the PDGF-BB-induced phosphorylation of Akt was markedly attenuated by wortmannin, another PI3-kinase inhibitor [Arcaro and Wymann, 1993], (Fig. 6).

Effects of LY294002 or Wortmannin on the PDGF-BB-Stimulated IL-6 Synthesis and Phosphorylation of GSK-3β in MC3T3-E1 Cells

LY294002, which by itself hardly affected the IL-6 levels, significantly amplified the PDGF-BB-stimulated synthesis of IL-6 (Fig. 7). The amplifying effect of LY294002 was dose-dependent between 1 μM and 10 μM. The LY294002 at 10 μM caused about 80% enhancement in the PDGF-BB effect. Wortmannin, as well as LY294002, also enhanced the PDGF-BB-stimulated synthesis of IL-6 without affecting IL-6 synthesis alone (data not shown). In addition, we found that the phosphorylation of GSK-3β

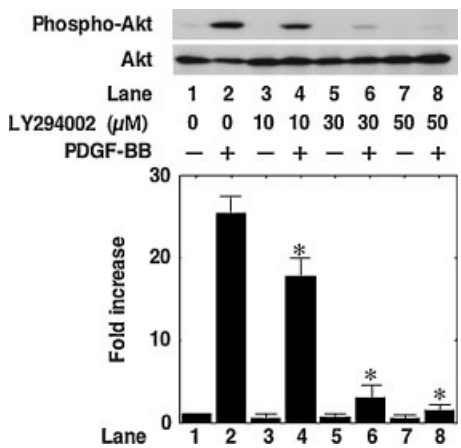

Fig. 5. Effect of LY294002 on the PDGF-BB-induced phosphorylation of Akt in MC3T3-E1 cells. The cultured cells were pre-treated with various doses of LY294002 for 60 min, and then stimulated by 50 ng/ml PDGF-BB or vehicle for 20 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific Akt or Akt. The histogram shows quantitative representations of the levels of PDGF-BB-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were expressed as the fold increase to the basal levels presented as **lane 1**. The values were calculated as the average values of lane 1 to be 1.0. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}P < 0.05$, compared to the value of PDGF-BB alone.

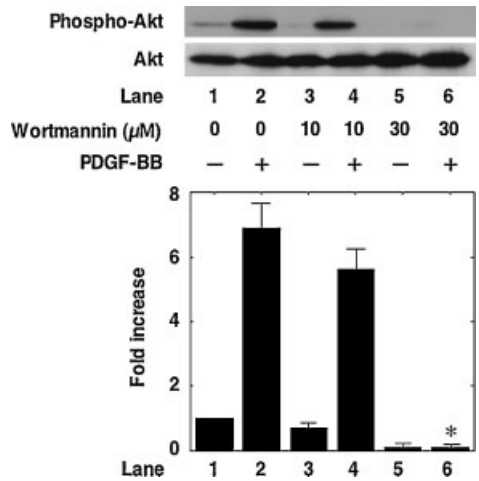

Fig. 6. Effect of wortmannin on the PDGF-BB-induced phosphorylation of Akt in MC3T3-E1 cells. The cultured cells were pre-treated with various doses of wortmannin for 60 min, and then stimulated by 50 ng/ml PDGF-BB or vehicle for 20 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific Akt or Akt. The histogram shows quantitative representations of the levels of PDGF-BB-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were expressed as the fold increase to the basal levels presented as **lane 1**. The values were calculated as the average values of lane 1 to be 1.0. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}P < 0.05$, compared to the value of PDGF-BB alone.

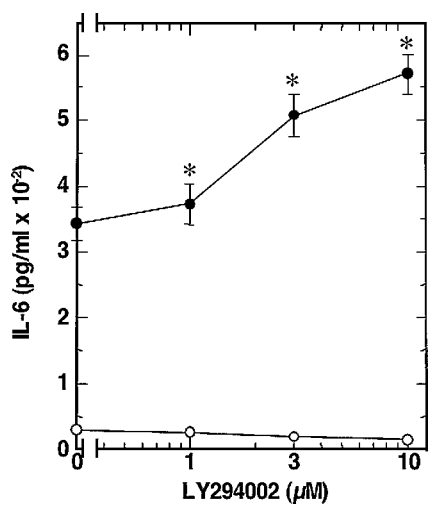

Fig. 7. Effect of LY294002 on the PDGF-BB-stimulated IL-6 synthesis in MC3T3-E1 cells. The cultured cells were pre-treated with various doses of LY294002 for 60 min, and then stimulated by 50 ng/ml PDGF-BB or vehicle for 24 h. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}P < 0.05$, compared to the value of PDGF-BB alone.

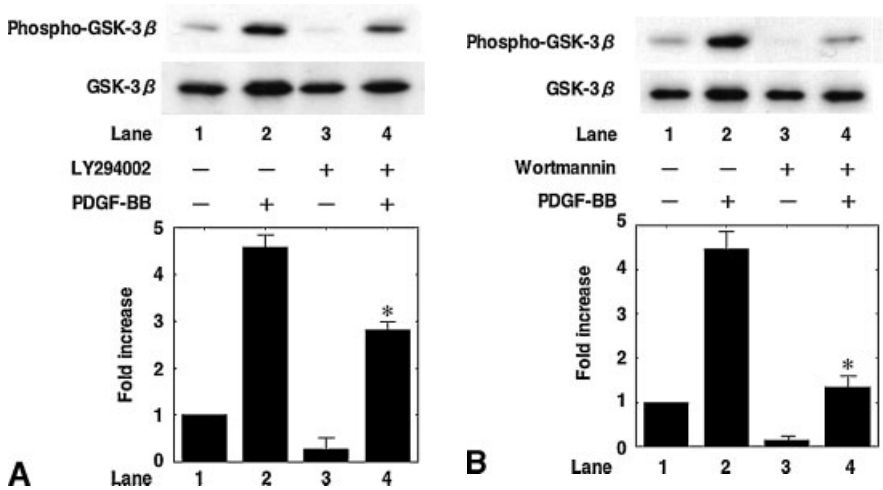

Fig. 8. Effects of LY294002 or wortmannin on the PDGF-BB-induced phosphorylation of GSK-3β in MC3T3-E1 cells. The cultured cells were pre-treated with 30 μM of LY294002 (**A**), 30 μM wortmannin (**B**) or vehicle for 60 min, and then stimulated by 50 ng/ml PDGF-BB or vehicle for 60 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific GSK-3β or GSK-3β. The histogram shows quantitative representations of the levels of PDGF-BB-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were expressed as the fold increase to the basal levels presented as **lane 1**. The values were calculated as the average values of lane 1 to be 1.0. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}P < 0.05$, compared to the value of PDGF-BB alone.

induced by PDGF-BB was significantly reduced by LY294002 or wortmannin (Fig. 8A,B).

DISCUSSION

In the present study, we found that PDGF-BB time-dependently induced the phosphorylation of Akt in osteoblast-like MC3T3-E1 cells. It has been reported that Akt is activated by PDGF-BB in osteoblastic cells [Chaudhary and Hruska, 2001]. In addition, we demonstrated that PI3K inhibitors, such as LY294002 [Vlahos et al., 1994] and wortmannin [Arcaro and Wymann, 1993] attenuated the PDGF-BB-induced phosphorylation of Akt in MC3T3-E1 cells. It has been shown that Akt is activated by phosphorylation of threonine and serine residues [Coffer et al., 1998; Chan et al., 1999]. The Akt signaling pathway is currently recognized to play a critical role in mediating survival signals in a wide range of cell types [Chan et al., 1999]. Therefore, these results suggest that PDGF-BB activates Akt via PI3K in osteoblast-like MC3T3-E1 cells.

We investigated whether or not PI3K/Akt functions in the PDGF-BB-stimulated IL-6 synthesis in osteoblast-like MC3T3-E1 cells. First, Akt inhibitor [Hu et al., 2000] significantly enhanced the PDGF-BB-stimulated synthesis of IL-6. It is generally known that GSK-3β is a downstream target of Akt and phosphorylated by Akt [Cross et al., 1995; Srivastava and Pandey, 1998]. We confirmed that the PDGF-BB-induced phosphorylation of GSK-3β was truly reduced by the Akt inhibitor. These results suggest that the activated Akt has an inhibitory effect on IL-6 release by PDGF-BB in osteoblast-like MC3T3-E1 cells. In addition, we found that wortmannin [Arcaro and Wymann, 1993] and LY294002 [Vlahos et al., 1994] markedly amplified the PDGF-BB-induced IL-6 synthesis. Furthermore, the PDGF-BB-induced phosphorylation of GSK-3β was suppressed by wortmannin or LY294002. Therefore, taking our results into account as a whole, it is most likely that PDGF-BB activates the PI3K/Akt pathway, resulting in the reduction of IL-6 synthesis. It is probable that the PI3K/Akt signaling pathway activated by PDGF-BB limits the PDGF-BB-stimulated IL-6 synthesis. To the best of our knowledge, our present finding is probably the first report to show that the activation of PI3K/Akt leads to the negative-feedback regulation of IL-6 synthesis in osteoblasts.

The PI3K/Akt pathway is recognized to play a crucial role in several cellular functions, such as proliferation and cell survival in a variety of cells [Coffer et al., 1998]. Our present results indicate that the PI3K/Akt pathway in

osteoblasts has an important role in the regulatory mechanism of the production of IL-6 in bone metabolism. As for the PDGF-induced IL-6 production, it has been reported that three isoforms of PDGF including PDGF-BB induces the expression of IL-6 gene, resulting in the proliferation of human fibroblasts, vascular smooth muscle cells, and mesangial cells [Roth et al., 1995]. In addition, hypoxia-induced IL-6 production associated with the cell proliferation is reportedly mediated by PDGF in human lung-derived fibroblasts and vascular smooth muscle cells [Tamm et al., 1998]. However, the role of PI3K/Akt pathway in the PDGF-induced IL-6 production has not yet been clarified. As far as we know, this is probably the first report showing the role of PI3K/Akt as a negative regulator in the IL-6 production elicited by PDGF-BB. It is well recognized that IL-6 is a potent bone resorptive agent and induces osteoclast formation [Kwan Tat et al., 2004]. Therefore, our present results lead us to speculate that PDGF-BB-activated PI3K/Akt signaling acts as a negative regulator against bone resorption. It is possible that the PI3/Akt pathway in osteoblasts might be considered as a new candidate as a molecular target of bone resorption concurrent with various bone diseases. The physiological significance of the auto-regulation by PI3K/Akt of PDGF-BB effect remains still unclear. Further investigation is necessary to clarify the exact role of PI3K/Akt in osteoblasts.

In conclusion, our results strongly suggest that PI3K/Akt plays a crucial role in the regulation of PDGF-BB-stimulated IL-6 synthesis in osteoblasts, and may serve as a negative-feedback mechanism to avoid the over-synthesis of IL-6.

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REFERENCES

Akira S, Taga T, Kishimoto T. 1993. Interleukin-6 in biology and medicine. Adv Immunol 54:1–78.

Arcaro A, Wymann MP. 1993. Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: The role of phosphatidylinositol 3,4,5-trisphosphate in neutrophil responses. Biochem J 296:297–301.

Borgatti P, Martelli AM, Bellacosa A, Casto R, Massari L, Capitani S, Neri LM. 2000. Translocation of Akt/PKB to the nucleus of osteoblast-like MC3T3-E1 cells exposed to proliferative growth factors. FEBS Lett 477:27–32.

Canalis E, Varghese S, McCarthy TL, Centrella M. 1992. Role of platelet derived growth factor in bone cell function. Growth Regul 2:151–155.

Cantley LC. 2002. The phosphoinositide 3-kinase pathway. Science 296:1655–1657.

Chan TO, Rittenhouse SE, Tsichlis PN. 1999. AKT/PKB and other D3 phosphoinositide-regulated kinases: Kinase activation by phosphoinositide-dependent phosphorylation. Annu Rev Biochem 68:965–1014.

Chaudhary LR, Hruska KA. 2001. The cell survival signal Akt is differentially activated by PDGF-BB, EGF, and FGF-2 in osteoblastic cells. J Cell Biochem 81:304–311.

Coffer PJ, Jin J, Woodgett JR. 1998. Protein kinase B (c-Akt): A multifunctional mediator of phosphatidylinositol 3-kinase activation. Biochem J 335:1–13.

Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. 1995. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378:785–789.

Danciu TE, Adam RM, Naruse K, Freeman MR, Hauschka PV. 2003. Calcium regulates the PI3K-Akt pathway in stretched osteoblasts. FEBS Lett 536:193–197.

Franchimont N, Durant D, Rydziel S, Canalis E. 1999. Platelet-derived growth factor induces interleukin-6 transcription in osteoblasts through the activator protein-1 complex and activating transcription factor-2. J Biol Chem 274:6783–6789.

Heldin CH, Westermark B. 1999. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 79:1283–1316.

Heldin CH, Johnsson A, Wennergren S, Wernstedt C, Betsholtz C, Westermark B. 1986. A human osteosarcoma cell line secretes a growth factor structurally related to a homodimer of PDGF A-chains. Nature 319: 511–514.

Heldin CH, Eriksson U, Ostman A. 2002. New members of the platelet-derived growth factor family of mitogens. Arch Biochem Biophys 398:284–290.

Helle M, Brakenhoff JP, De Groot ER, Aarden LA. 1988. Interleukin 6 is involved in interleukin 1-induced activities. Eur J Immunol 18:957–959.

Heymann D, Rousselle AV. 2000. gp130 Cytokine family and bone cells. Cytokine 12:1455–1468.

Hu Y, Qiao L, Wang S, Rong SB, Meuillet EJ, Berggren M, Gallegos A, Powis G, Kozikowski AP. 2000. 3-(Hydroxymethyl)-bearing phosphatidylinositol ether lipid analogues and carbonate surrogates block PI3-K, Akt, and cancer cell growth. J Med Chem 43:3045–3051.

Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y, Yamaguchi A, Yoshiki S, Matsuda T, Hirano T. 1990. IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 145:3297–3303.

Kang HY, Cho CL, Huang KL, Wang JC, Hu YC, Lin HK, Chang C, Huang KE. 2004. Nongenomic androgen activation of phosphatidylinositol 3-kinase/Akt signaling pathway in MC3T3-E1 osteoblasts. J Bone Miner Res 19, 1181–1190.

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

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

and phosphoinositide hydrolysis induced by prostaglandin E2 in osteoblast-like cells. Exp Cell Res 198:130–134.
Kozawa O, Suzuki A, Watanabe Y, Shinoda J, Oiso Y. 1995. Effect of platelet-derived growth factor on phosphatidylcholine-hydrolyzing phospholipase D in osteoblast-like cells. Endocrinology 136:4473–4478.
Kwan Tat S, Padrines M, Theoleyre S, Heymann D, Fortun Y. 2004. IL-6, RANKL, TNF-alpha/IL-1: Interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev 15:49–60.
Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.
Littlewood AJ, Russell J, Harvey GR, Hughes DE, Russell RG, Gowen M. 1991. The modulation of the expression of IL-6 and its receptor in human osteoblasts *in vitro*. Endocrinology 129:1513–1520.
Nijweide PJ, Burger EH, Feyen JH. 1986. Cells of bone: Proliferation, differentiation, and hormonal regulation. Physiol Rev 66:855–886.
Roodman GD. 1992. Interleukin-6: An osteotropic factor? J Bone Miner Res 7:475–478.
Roth M, Nauck M, Tamm M, Perruchoud AP, Ziesche R, Block LH. 1995. Intracellular interleukin-6 mediates platelet-derived growth factor-induced proliferation of nontransformed cells. Proc Natl Acad Sci USA 92:1312–1316.
Srivastava AK, Pandey SK. 1998. Potential mechanism(s) involved in the regulation of glycogen synthesis by insulin. Mol Cell Biochem 182:135–141.
Sudo H, Kodama HA, Amagai Y, Yamamoto S, Kasai S. 1983. *In vitro* differentiation and calcification in a new clonal osteogenic cell line derived from newborn mouse calvaria. J Cell Biol 96:191–198.
Tamm M, Bihl M, Eickelberg O, Stulz P, Perruchoud AP, Roth M. 1998. Hypoxia-induced interleukin-6 and interleukin-8 production is mediated by platelet-activating factor and platelet-derived growth factor in primary human lung cells. Am J Respir Cell Mol Biol 19:653–661.
Vlahos CJ, Matter WF, Hui KY, Brown RF, 1994. A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). J Biol Chem 269:5241–5248.