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(−)-Epigallocatechin Gallate Enhances Prostaglandin $F_{2\alpha}$-Induced VEGF Synthesis Via Upregulating SAPK/JNK Activation in Osteoblasts

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Abstract Catechin, one of the major flavonoids presented in plants such as tea, reportedly suppresses bone resorption. We previously reported that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) stimulates the synthesis of vascular endothelial growth factor (VEGF) via p44/p42 mitogen-activated protein (MAP) kinase in osteoblast-like MC3T3-E1 cells. To clarify the mechanism of catechin effect on osteoblasts, we investigated the effect of (−)-epigallocatechin gallate (EGCG), one of the major green tea flavonoids, on the VEGF synthesis by $PGF_{2\alpha}$ in MC3T3-E1 cells. The $PGF_{2\alpha}$-induced VEGF synthesis was significantly enhanced by EGCG. The amplifying effect of EGCG was dose dependent between 10 and 100 μM. EGCG did not affect the $PGF_{2\alpha}$-induced phosphorylation of p44/p42 MAP kinase. SB203580, a specific inhibitor of p38 MAP kinase, and SP600125, a specific inhibitor of stress-activated protein kinase/c-*Jun* N-terminal kinase (SAPK/JNK), reduced the $PGF_{2\alpha}$-induced VEGF synthesis. EGCG markedly enhanced the phosphorylation of SAPK/JNK induced by $PGF_{2\alpha}$ without affecting the $PGF_{2\alpha}$-induced phosphorylation of p38 MAP kinase. SP600125 markedly reduced the amplification by EGCG of the SAPK/JNK phosphorylation. In addition, the $PGF_{2\alpha}$-induced phosphorylation of c-Jun was amplified by EGCG. These results strongly suggest that EGCG upregulate $PGF_{2\alpha}$-stimulated VEGF synthesis resulting from amplifying activation of SAPK/JNK in osteoblasts. J. Cell. Biochem. 100: 1146–1153, 2007. © 2006 Wiley-Liss, Inc.

Key words: catechin; $PGF_{2\alpha}$; VEGF; MAP kinase; osteoblast

It is generally recognized that compounds in foods such as vegetables and fruits have beneficial properties to human being. Among them, flavonoids reportedly show antioxidative, antibacterial, and antitumor effects [Jankun et al., 1997; Harbourne and Williams, 2000]. Catechins are one of the major flavonoids, which are present in various species of plants such as tea [Harbourne and Williams, 2000]. In bone metabolism, it has been reported that catechin suppresses bone resorption [Delaisse et al., 1986]. Bone metabolism is regulated by two functional cells, osteoblasts, and osteoclasts, responsible for bone formation and bone resorption, respectively [Nijweide et al., 1986]. The

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formation of bone structures and bone remodeling results from the coupling process, bone resorption by activated osteoblasts with subsequent deposition of new matrix by osteoblasts. As for osteoblasts, it has been shown that catechin stimulates alkaline phosphatase activity, a mature osteoblast phenotype and reduces bone-resorptive cytokine production in osteoblast-like MC3T3-E1 cells [Choi and Hwang, 2003]. However, the exact role of catechin in osteoblasts has not yet been clarified.

Vascular endothelial growth factor (VEGF) has been characterized as a heparin-binding angiogenic growth factor displaying high specificity for vascular endothelial cells [Ferrara and Davis-Smyth, 1997]. It is well recognized that VEGF, which is produced and secreted from a variety of cell types, increases capillary permeability and stimulates proliferation of endothelial cells [Ferrara and Davis-Smyth, 1997]. As for bone metabolism, it has been shown 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]. Accumulating evidence indicates that osteoblasts among bone cells produce and secrete VEGF in response to various humoral factors [Goad et al., 1996; Wang et al., 1996; Ferrara and Davis-Smyth, 1997; Schalaeppi et al., 1997]. 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. 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 cells cooperatively regulate bone metabolism [Erlebacher et al., 1995]. Thus, there is no doubt that VEGF secreted from osteoblasts plays an important role in the regulation of bone metabolism. However, the mechanism underlying VEGF synthesis in osteoblasts has not yet been fully clarified.

It is well known that prostaglandins (PGs) act as autocrine/paracrine modulators in osteoblasts and play crucial roles in the regulation of bone metabolism [Nijweide et al., 1986; Pilbeam et al., 1996]. Among them, $PGF_{2\alpha}$ is known as a potent bone-resorptive agent and stimulates the proliferation of osteoblasts and inhibits their differentiation [Pilbeam et al., 1996]. In our previous studies [Miwa et al., 1990; Kozawa et al., 1994], we have reported that $PGF_{2\alpha}$ stimulates both phosphoinositide-hydrolyzing phospholipase C (PI-PLC) and phosphatidylcholine-hydrolyzing phospholipase D (PC-PLD), recognized to be two major pathways of physiological protein kinase C (PKC) activation [Nishizuka, 1992; Exton, 1999], in osteoblast-like MC3T3-E1 cells. In addition, we have recently shown that $PGF_{2\alpha}$ stimulates the VEGF synthesis through PKC-dependent activation of p44/p42 mitogen-activated protein (MAP) kinase in these cells [Tokuda et al., 2003]. In the present study, we investigated the effect of (–)-epigallocatechin gallate (EGCG), one of the major green tea flavonoids, on the $PGF_{2\alpha}$-induced VEGF synthesis in osteoblast-like MC3T3-E1 cells. We here show that EGCG upregulates $PGF_{2\alpha}$-stimulated VEGF synthesis via enhancing SAPK/JNK activation among the MAP kinase superfamily in these cells.

MATERIALS AND METHODS

Materials

$PGF_{2\alpha}$ was purchased from Sigma Chemical Co. (St. Louis, MO). Mouse VEGF enzyme immunoassay kit was purchased from R&D Systems, Inc. (Minneapolis, MN). (–)-Epigallocatechin gallate (EGCG), SB203580, and SP600125 were obtained from Calbiochem-Novabiochem Co. (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, and c-Jun antibodies were purchased from Cell Signaling Technology (Beverly, MA). ECL Western blotting detection system was purchased from Amersham Japan (Tokyo, Japan). Other materials and chemicals were obtained from commercial sources. $PGF_{2\alpha}$ was dissolved in ethanol. SB203580 and SP600125 were dissolved in dimethyl sulfoxide. The maximum concentration of ethanol or dimethyl sulfoxide was 0.1%, which did not affect the assay for VEGF or the analysis of Western blot.

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 diameter dishes (5×10^4/dish) or 90-mm diameter dishes (5×10^5/dish) 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.

VEGF Assay

The cultured cells were stimulated by $PGF_{2\alpha}$ in 1 ml of α-MEM containing 0.3% FCS for the indicated periods. When indicated, the cells were pretreated with EGCG, SB203580, or SP600125 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

The cultured cells were stimulated by $PGF_{2\alpha}$ 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 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, or c-Jun 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. The pretreatment of EGCG or SP600125 was performed for 60 min before the addition of $PGF_{2\alpha}$.

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 ± SEM of triplicate determinations. Each experiment was repeated three times with similar results.

RESULTS

Effect of EGCG on the $PGF_{2\alpha}$-Stimulated VEGF Synthesis in MC3T3-E1 Cells

We have previously shown that $PGF_{2\alpha}$ stimulates VEGF synthesis in osteoblast-like MC3T3-E1 cells [Tokuda et al., 2003]. We first examined the effect of EGCG on the $PGF_{2\alpha}$-stimulated VEGF synthesis. EGCG, which by itself had little effect on the VEGF levels, significantly enhanced the $PGF_{2\alpha}$-stimulated synthesis of VEGF (Table I). The amplifying effect of EGCG was dose dependent between 10 and 100 μM (Table I).

Effect of EGCG on the Phosphorylation of p44/p42 MAP Kinase Induced by $PGF_{2\alpha}$ in MC3T3-E1 Cells

We have previously reported that the $PGF_{2\alpha}$-stimulated VEGF synthesis is regulated by $PGF_{2\alpha}$-activated p44/p42 MAP kinase in MC3T3-E1 cells [Tokuda et al., 2003]. In order to investigate whether EGCG effect on the $PGF_{2\alpha}$-stimulated VEGF synthesis is mediated

TABLE I. Effect of EGCG on the $PGF_{2\alpha}$-Stimulated VEGF Synthesis in MC3T3-E1 Cells

EGCG (μM)	$PGF_{2\alpha}$	VEGF (pg/ml)
—	−	19 ± 10
—	+	580 ± 51
10	−	14 ± 10
10	+	630 ± 85
30	−	15 ± 10
30	+	2680 ± 167*
100	−	20 ± 10
100	+	4100 ± 249*

The cultured cells were pretreated with various doses of EGCG for 60 min, and then stimulated by 10 μM $PGF_{2\alpha}$ or vehicle for 24 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 $PGF_{2\alpha}$ alone.

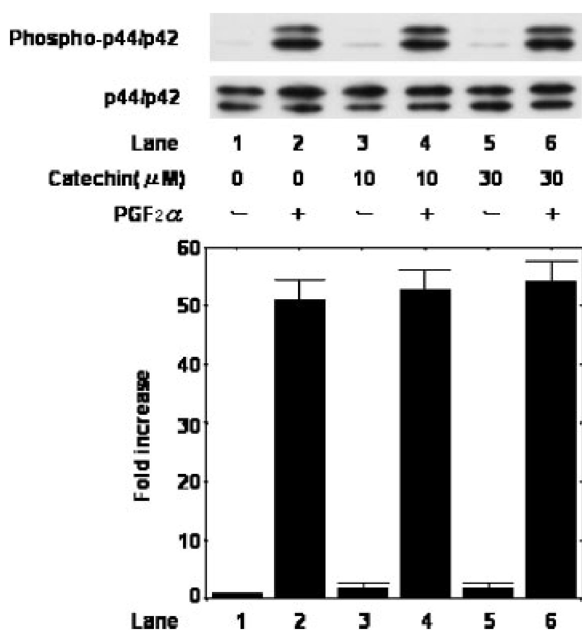

Fig. 1. Effect of EGCG on the phosphorylation of p44/p42 MAP kinase induced by $PGF_{2\alpha}$ in MC3T3-E1 cells. The cultured cells were pretreated with the indicated doses of EGCG or vehicle for 60 min, and then stimulated by 10 μM $PGF_{2\alpha}$ or vehicle for 30 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific p44/p42 MAP kinase or p44/p42 MAP kinase. The histogram shows quantitative representations of the levels of $PGF_{2\alpha}$-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.

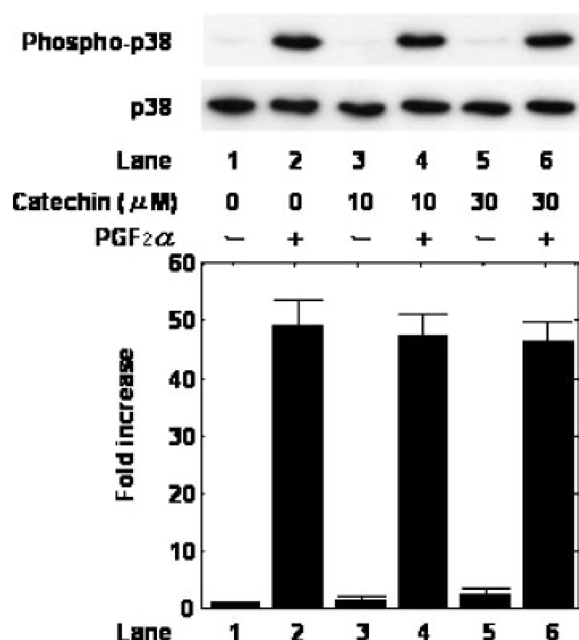

Fig. 2. Effect of EGCG on the phosphorylation of p38 MAP kinase induced by $PGF_{2\alpha}$ in MC3T3-E1 cells. The cultured cells were pretreated with the indicated doses of EGCG or vehicle for 60 min, and then stimulated by 10 μM $PGF_{2\alpha}$ or vehicle for 10 min. The extracts of cells were subjected 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 $PGF_{2\alpha}$-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.

through p44/p42 MAP kinase activation in MC3T3-E1 cells, we next examined the effect of EGCG on the $PGF_{2\alpha}$-induced phosphorylation of p44/p42 MAP kinase. However, EGCG hardly affected the $PGF_{2\alpha}$-induced phosphorylation of p44/p42 MAP kinase (Fig. 1).

Effect $PGF_{2\alpha}$ of on the Phosphorylation of p38 MAP Kinase and SAPK/JNK in MC3T3-E1 Cells

Among the MAP kinase superfamily, p38 MAP kinase and SAPK/JNK in addition to p44/p42 MAP kinase play a crucial role as intracellular components to transduce the various signals of agonists [Widmann et al., 1999]. Therefore, in order to investigate whether activates p38 MAP kinase or SAPK/JNK in MC3T3-E1 cells, we next examined the effect of $PGF_{2\alpha}$ on the phosphorylation of p38 MAP kinase and SAPK/JNK. $PGF_{2\alpha}$ induced both the phosphorylation of p38 MAP kinase (lane 2 in Fig. 2) and SAPK/JNK (lane 2 in Fig. 3).

Effects of SB203580 or SP600125 on the $PGF_{2\alpha}$-Stimulated VEGF Synthesis in MC3T3-E1 Cells

In order to clarify the involvement of the MAP kinases in the $PGF_{2\alpha}$-stimulated VEGF synthesis in MC3T3-E1 cells, we examined the effect of SB203580, a specific inhibitor of p38 MAP kinase [Cuenda et al., 1995] or SP600125, a specific SAPK/JNK inhibitor [Bennett et al., 2001], on the VEGF synthesis. SB203580 or SP600125, which by itself had little effect on the VEGF levels, significantly suppressed the $PGF_{2\alpha}$-stimulated synthesis of VEGF (Table II).

Effect of EGCG on the Phosphorylation of p38 MAP Kinase or SAPK/JNK Induced by $PGF_{2\alpha}$ in MC3T3-E1 Cells

In order to clarify whether p38 MAP kinase is involved in EGCG effect on the $PGF_{2\alpha}$-

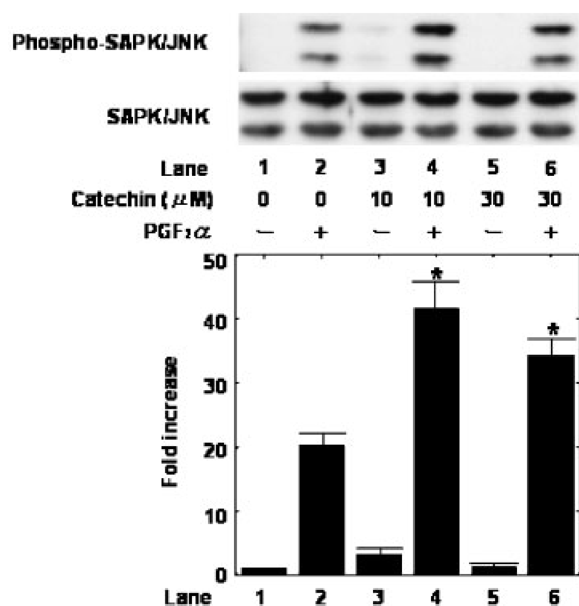

Fig. 3. Effect of EGCG on the phosphorylation of SAPK/JNK induced by $PGF_{2\alpha}$ in MC3T3-E1 cells. The cultured cells were pretreated with the indicated doses of EGCG or vehicle for 60 min, and then stimulated by 10 μM $PGF_{2\alpha}$ or vehicle for 20 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific SAPK/JNK or SAPK/JNK. The histogram shows quantitative representations of the levels of $PGF_{2\alpha}$-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 to the value of $PGF_{2\alpha}$ alone.

stimulated VEGF synthesis in MC3T3-E1 cells, we examined the effect of EGCG on the $PGF_{2\alpha}$-induced phosphorylation of p38 MAP kinase. However, EGCG had little effect on the $PGF_{2\alpha}$-induced phosphorylation of p38 MAP kinase (Fig. 2). On the contrary, EGCG markedly enhanced the $PGF_{2\alpha}$-induced phosphorylation of SAPK/JNK (Fig. 3). According to the densitometric analysis, EGCG (10 μM) caused about 100% enhancement of the $PGF_{2\alpha}$ effect on the SAPK/JNK phosphorylation.

TABLE II. Effects of SB203580 or SP600125 on the $PGF_{2\alpha}$-Stimulated VEGF Synthesis in MC3T3-E1 Cells

Inhibitor (μM)	$PGF_{2\alpha}$	VEGF (pg/ml)
Vehicle	−	20 ± 10
Vehicle	+	559 ± 51
SB203580 (3)	−	13 ± 10
SB203580 (3)	+	221 ± 35*
SB203580 (10)	−	15 ± 10
SB203580 (10)	+	46 ± 12*
SP600125 (1)	−	15 ± 10
SP600125 (1)	+	399 ± 37*
SP600125 (30)	−	13 ± 10
SP600125 (30)	+	162 ± 29*

The cultured cells were pretreated with SB203580, SP600125, or vehicle for 60 min, and then stimulated by 10 μM $PGF_{2\alpha}$ or vehicle for 24 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 $PGF_{2\alpha}$ alone.

Effect of SP600125 on the Enhancement by EGCG of $PGF_{2\alpha}$-Induced Phosphorylation of SAPK/JNK in MC3T3-E1 Cells

SP600125 [Bennett et al., 2001], which by itself did not affect the basal levels of phosphorylation of SAPK/JNK, significantly reduced the enhancement by EGCG of $PGF_{2\alpha}$-induced SAPK/JNK phosphorylation (Fig. 4). The enhanced phosphorylation levels by EGCG of $PGF_{2\alpha}$-induced SAPK/JNK were suppressed by

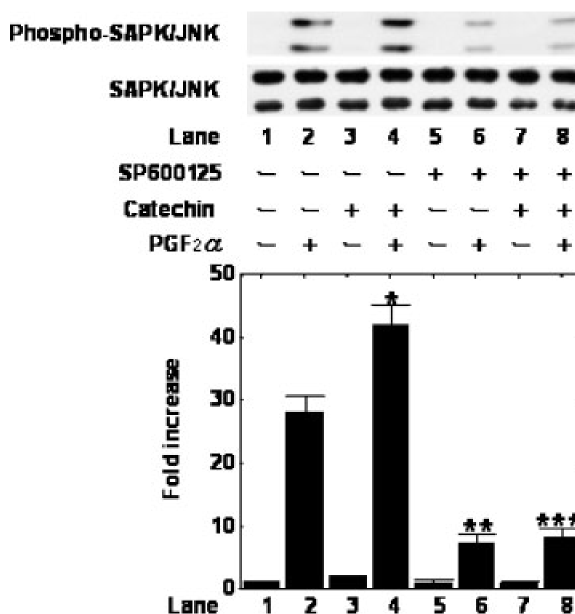

Fig. 4. Effect of SP600125 on the enhancement by EGCG of the $PGF_{2\alpha}$-induced phosphorylation of SAPK/JNK in MC3T3-E1 cells. The cultured cells were pretreated with 30 μM SP600125 or vehicle for 60 min, and then incubated by 10 μM EGCG for 60 min. The cells were stimulated by 10 μM $PGF_{2\alpha}$ or vehicle for 20 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific SAPK/JNK or SAPK/JNK. The histogram shows quantitative representations of the levels of $PGF_{2\alpha}$-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 to the value of $PGF_{2\alpha}$ alone. $^{**}P < 0.05$, compared to the value of $PGF_{2\alpha}$ alone. $^{***}P < 0.05$, compared to the value of $PGF_{2\alpha}$ with EGCG pretreatment.

SP600125 similar to the levels by $PGF_{2\alpha}$ with SP600125 treatment.

Effect of EGCG on the Phosphorylation of c-Jun Induced by $PGF_{2\alpha}$ in MC3T3-E1 Cells

It is well known that c-Jun acts as a downstream effector of SAPK/JNK [Widmann et al., 1999; Weston and Davis, 2002]. Thus, in order to investigate whether EGCG effect on the $PGF_{2\alpha}$-stimulated VEGF synthesis is mediated through c-Jun activation in MC3T3-E1 cells, we next examined the effect of EGCG on the $PGF_{2\alpha}$-induced phosphorylation of c-Jun. We found that $PGF_{2\alpha}$ time-dependently phosphorylated c-Jun and that SP600125 suppressed the $PGF_{2\alpha}$-induced phosphorylation of c-Jun in these cells (data not shown). EGCG markedly amplified the $PGF_{2\alpha}$-induced phosphorylation of c-Jun (Fig. 5). According to the densitometric analysis, EGCG (10 μM) caused about 80% enhancement of the $PGF_{2\alpha}$ effect on the c-Jun phosphorylation.

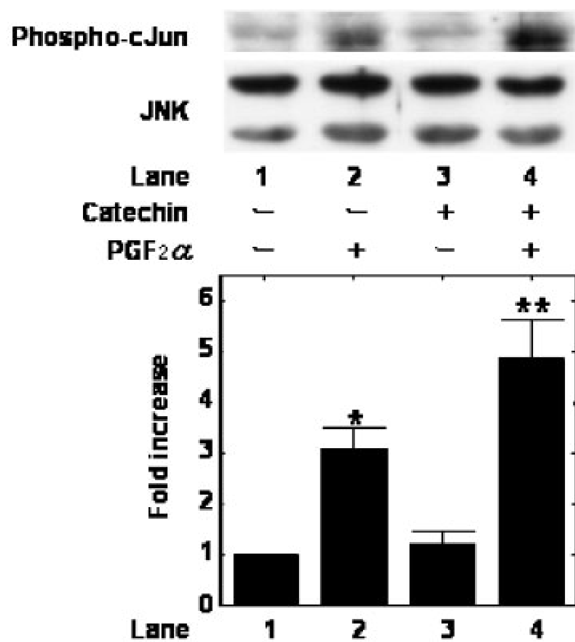

Fig. 5. Effect of EGCG on the phosphorylation of c-Jun induced by $PGF_{2\alpha}$ in MC3T3-E1 cells. The cultured cells were pretreated with the indicated doses of EGCG or vehicle for 60 min, and then stimulated by 10 μM $PGF_{2\alpha}$ or vehicle for 20 min. The extracts of cells were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against c-Jun or SAPK/JNK. The histogram shows quantitative representations of the levels of $PGF_{2\alpha}$-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 to the control value. $**P < 0.05$, compared to the value of $PGF_{2\alpha}$ alone.

DISCUSSION

In the present study, we demonstrated that EGCG, which alone did not affect the levels of VEGF, significantly enhanced the $PGF_{2\alpha}$-stimulated VEGF synthesis in osteoblast-like MC3T3-E1 cells. We next investigated the mechanism of EGCG behind the amplifying effect on the VEGF synthesis. It is well recognized that the MAP kinase superfamily mediates intracellular signaling of extracellular agonists and plays a crucial role in cellular functions including proliferation, differentiation, and cell death in a variety of cells [Widmann et al., 1999]. Three major MAP kinase, p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK are known as central elements used by mammalian cells to transduce the diverse messages [Widmann et al., 1999]. In our previous study [Tokuda et al., 2003], we have shown that $PGF_{2\alpha}$-activated p44/p42 MAP kinase acts as a positive regulator in $PGF_{2\alpha}$-induced VEGF synthesis in osteoblast-like MC3T3-E1 cells. In the present study, we showed that EGCG failed to affect the $PGF_{2\alpha}$-induced phosphorylation of p44/p42 MAP kinase. Thus, it seems unlikely that EGCG amplifies the $PGF_{2\alpha}$-induced VEGF synthesis through upregulating the activation of p44/p42 MAP kinase in osteoblast-like MC3T3-E1 cells.

We further investigated the involvement of p38 MAP kinase and SAPK/JNK in the amplifying effect of EGCG. We found that $PGF_{2\alpha}$ induced both the phosphorylation of p38 MAP kinase and SAPK/JNK. It has been shown that MAP kinases are activated by phosphorylation of threonine and tyrosine residues by dual specificity MAP kinase kinases [Widmann et al., 1999]. Therefore, our findings strongly suggest that $PGF_{2\alpha}$ activates p38 MAP kinase and SAPK/JNK in addition to p44/p42 MAP kinase in osteoblast-like MC3T3-E1 cells. In addition, we showed that the $PGF_{2\alpha}$-stimulated VEGF synthesis was suppressed by a specific p38 MAP kinase inhibitor, SB203580 [Cuenda et al., 1995], or a specific SAPK/JNK inhibitor, SP600125 [Bennett et al., 2001]. Based on our findings, it is probable that $PGF_{2\alpha}$ stimulates the synthesis of VEGF via the three MAP kinases in osteoblast-like MC3T3-E1 cells.

However, EGCG had little effect on the $PGF_{2\alpha}$-induced p38 MAP kinase phosphorylation. Therefore, it seems unlikely that the EGCG-induced enhancement of $PGF_{2\alpha}$-stimulated VEGF synthesis is due to the upregulation of p38 MAP kinase activation. On the contrary, we showed that the $PGF_{2\alpha}$-induced SAPK/JNK phosphorylation was markedly amplified by EGCG. Furthermore, SP600125 [Bennett et al., 2001] markedly reduced the enhancement by EGCG almost to the levels of $PGF_{2\alpha}$ alone with SP600125 in the phosphorylation of SAPK/JNK. These results suggest that EGCG upregulates the $PGF_{2\alpha}$-stimulated activation of SAPK/JNK. Additionally, we demonstrated that EGCG strengthened the $PGF_{2\alpha}$-induced phosphorylation of c-Jun, well known as a downstream effector of SAPK/JNK [Widmann et al., 1999; Weston and Davis, 2002], as well as SAPK/JNK. Taking our findings into account as a whole, it is most likely that EGCG upregulates $PGF_{2\alpha}$-stimulated VEGF synthesis through enhancing the activation of SAPK/JNK in osteoblast-like MC3T3-E1 cells. Further investigations are necessary to clarify the precise mechanism of catechin behind the amplification of VEGF synthesis in osteoblasts.

It is generally recognized that the expansion of capillary network providing microvasculature is an essential process of bone remodeling [Erlebacher et al., 1995]. Since VEGF is a specific mitogen of vascular endothelium [Ferrara and Davis-Smyth, 1997], it is speculated that VEGF synthesized by osteoblasts functions as a pivotal intercellular mediator between osteoblasts and vascular endothelial cells. Moreover, it has been reported that VEGF is involved in trabecular bone formation and expansion of the hypertrophic chondrocyte zone in epiphyseal growth plate of mouse [Gerber et al., 1999], supporting the importance of VEGF in bone metabolism. On the other hand, it has been shown that catechin exerts an inhibitory effect on bone resorption [Delaisse et al., 1986]. Additionally, in osteoblasts, production of bone-resorptive cytokines such as tumor necrosis factor-α and interleukin-6 has been reported to be suppressed by catechin [Choi and Hwang, 2003]. Based on our results as a whole, it is probable that catechin-enhanced VEGF synthesized from osteoblasts acts a crucial role in the process of bone remodeling via regulating the capillary endothelial cells proliferation. Further investigations are required to elucidate the role of catechin in bone metabolism.

In conclusion, our present results strongly suggest that catechin upregulates $PGF_{2\alpha}$-stimulated VEGF synthesis via enhancing activation of SAPK/JNK among the MAP kinase superfamily in osteoblasts.

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