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Modulation by the Steroid/Thyroid Hormone Superfamily of TGF-β-Stimulated VEGF Release From Vascular Smooth Muscle Cells

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Abstract We previously reported that transforming growth factor-β (TGF-β) stimulates the release of vascular endothelial growth factor (VEGF) from aortic smooth muscle A10 cells via activation of p38 mitogen-activated protein (MAP) kinase. In the present study, we investigated whether nuclear hormone receptor superfamily members affect TGF-β-stimulated VEGF release from A10 cells. Retinoic acid or 1,25-dihydroxyvitamin D_3 enhanced TGF-β-induced VEGF release in a concentration-dependent manner, whereas dexamethasone or corticosterone suppressed TGF-β-induced VEGF release. 1,25-Dihydroxyvitamin D_3 and TGF-β stimulated phosphorylation of p38 MAP kinase in an additive manner. SB203580, an inhibitor of p38 MAP kinase, decreased the VEGF release induced by TGF-β or 1,25-dihydroxyvitamin D_3. However, retinoic acid, dexamethasone, or corticosterone did not affect phosphorylation of p38 MAP kinase. These results indicate that retinoic acid, 1,25-dihydroxyvitamin D_3, and glucocorticoids affect TGF-β-stimulated VEGF release from aortic smooth muscle cells. The stimulatory effect of 1,25-dihydroxyvitamin D_3 occurs, in part, via modification of TGF-β-induced activation of p38 MAP kinase. J. Cell. Biochem. 99: 187–195, 2006.

Key words: steroid/thyroid hormone superfamily; TGF-β; VEGF; p38 MAP kinase; vascular smooth muscle cells

Regulation of vascular smooth muscle cell proliferation and differentiation is critical for vasculogenesis, angiogenesis, and the maintenance of homeostasis in mature vessel walls [Hungerford and Little, 1999; Ross, 1999]. Proliferation and differentiation of these cells are central importance in the pathogenesis of atherosclerosis, hypertension, and restenosis after procedural revascularization [Hungerford and Little, 1999; Ross, 1999]. Vascular smooth muscle cells and vascular endothelial cells interact with each other; for example, vasoactive agents produced by aortic smooth muscle cells, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), and interleukin (IL)-1β, affect endothelial cell function [Hungerford and Little, 1999; Ross, 1999]. VEGF is a heparin-binding angiogenic growth factor that is highly specific for endothelial cells; VEGF binds to tyrosine kinase receptors expressed almost exclusively in endothelial cells and stimulates endothelial cell proliferation, migration, and inhibition of apoptosis [Gospodarowicz et al., 1989; Neufeld et al., 1999]. VEGF also induces angiogenesis, increases blood vessel permeability, and plays a central

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role in the regulation of vasculogenesis [Neufeld et al., 1999]. Deregulated VEGF expression contributes to the development of solid tumors by promoting tumor angiogenesis and contributes to the development of diseases characterized by abnormal angiogenesis [Neufeld et al., 1999]. Vascular smooth muscle cells are the predominant source of VEGF [Tischer et al., 1991]. Platelet-derived growth factor (PDGF) BB, TGF-β, hypoxia, endothelin, and bFGF induce VEGF production in these cells [Brogi et al., 1994; Stavri et al., 1995; Pedram et al., 1997]. However, the mechanisms underlying VEGF synthesis in vascular smooth muscle cells have not been fully elucidated.

The TGF-β superfamily comprises a large number of structurally related polypeptide growth factors, each capable of regulating an array of cellular processes including cell proliferation, lineage determination, differentiation, motility, adhesion, and death [Massagué, 1998; Miyazono et al., 2000]. In regard to the vascular system, TGF-β plays a pivotal role in promoting alterations in vessel structure [Massagué, 1998]. Recent evidence suggests that alterations in the local abundance of TGF-β in the arterial wall promote vascular cell transdifferentiation, vascular wall remodeling, arterial lesion growth, and lesion regression associated with apoptosis [Schulick et al., 1998]. In these processes, TGF-β stimulates VEGF synthesis in vascular smooth muscle cells and exerts angiogenic effects [Brogi et al., 1994; Stavri et al., 1995]. We previously reported that TGF-β stimulates the release of VEGF from aortic smooth muscle A10 cells at least in part via p38 mitogen-activated protein (MAP) kinase [Yamamoto et al., 2001].

Recently, the nuclear hormone receptor superfamily has been shown to modulate vascular tone and vascular smooth muscle cell proliferation and differentiation [Kornel, 1993; Miano and Berk, 2000; Mizuma et al., 2001; Dubey et al., 2002; Rebsamen et al., 2002]. This superfamily includes receptors for retinoids, vitamin D, steroid hormones, and thyroid hormone [Evans, 1988; Carlberg, 1995; Miano and Berk, 2000]. These receptors are ligand-activated transcription factors that bind discrete *cis* elements within the regulatory regions of a growing list of target genes [Evans, 1988; Carlberg, 1995; Miano and Berk, 2000]. We previously reported that among nuclear hormone receptor superfamily members, 1,25-dihydroxyvitamin D_3 or retinoic acid alone stimulates the release of VEGF from A10 cells [Yamamoto et al., 2002; Tanabe et al., 2004]. In the present study, we investigated the effect of nuclear hormone receptor superfamily members on TGF-β-induced VEGF release from A10 cells.

MATERIALS AND METHODS

Materials

A mouse VEGF enzyme-linked immunosorbent assay (ELISA) kit (recognizing both 120- and 164-amino acid forms of murine VEGF) and active TGF-β were purchased from R&D Systems (Minneapolis, MN). All-*trans* retinoic acid (retinoic acid), 9α-fluoro-16α-methylprednisolone (dexamethasone), 4-pregnene-11β, 21-diol-3, 20-dione (corticosterone), 4-pregnene-3,20-dione (progesterone), 4-androsten-17β-ol-3-one (testosterone), 1,3,5[10]-estratriene-3,17β-diol (estradiol), and 3,3′,5-triiodo-L-thyronine (T_3) were obtained from Sigma-Aldrich (St. Louis, MO). 1α,25-Dihydroxyvitamin D_3 (vitamin D_3) and SB203580 were obtained from Calbiochem-Novabiochem Corp. (La Jolla, CA). Antibodies against phospho-p38 MAP kinase and p38 MAP kinase were from New England BioLabs, Inc. (Beverly, MA). The ECL Western blot detection system was purchased from Amersham Japan (Tokyo, Japan). Other materials and chemicals were obtained from commercial sources. Retinoic acid, vitamin D_3, dexamethasone, corticosterone, progesterone, testosterone, and estradiol were dissolved in ethanol. T_3 was dissolved in 0.1 N NaOH. SB203580 was dissolved in dimethyl sulfoxide. The maximum concentration of ethanol or dimethyl sulfoxide was 0.1%, which did not affect VEGF immunoassay or Western blot results.

Cell Culture

A10 cells derived from fetal rat aortic smooth muscle [Kimes and Brandt, 1976] were obtained from American Type Culture Collection (Manassas, VA). Cells were seeded into 35-mm (1×10^5 cells) or 90-mm (5×10^5 cells) diameter dishes and maintained at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air in Dulbecco's Modified Eagle's medium (DMEM) containing 10% fetal calf serum. After 5 days, the medium was replaced with serum-free DMEM. Cells were used for experiments 48 h thereafter.

VEGF Assay

Cultured cells were pretreated with various agents belonging to the nuclear hormone receptor superfamily members in serum-free DMEM for 9 h and were then stimulated with TGF-β for the indicated periods. When indicated, the cells were pretreated with SB203580 for 60 min prior to stimulation with vitamin D_3. The conditioned medium was then collected, and the VEGF in the medium was measured with a VEGF ELISA kit that recognizes rat VEGF (according to the manufacturer), as described previously [Seko et al., 1999].

Western Blot Analysis of p38 MAP Kinase

Cultured cells were pretreated with retinoic acid, vitamin D_3, dexamethasone, or corticosterone in serum-free DMEM for 9 h and were then stimulated with TGF-β for 45 min. When indicated, the cells were pretreated with SB203580 for 60 min prior to stimulation with vitamin D_3. Stimulated cells were rinsed twice with phosphate-buffered saline, then lysed, homogenized, and sonicated in lysis buffer containing 62.5 mM Tris/Cl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 50 mM dithiothreitol, and 10% glycerol. The cytosolic fraction was collected as a supernatant after centrifugation at 125,000*g* for 10 min at 4°C. The supernatant was used for analysis of p38 MAP kinase by Western blotting. SDS–polyacrylamide gel electrophoresis (PAGE) was performed by the Laemmli method in 10% polyacrylamide gels [Laemmli, 1970]. Western blot analysis was performed as described previously [Yamamoto et al., 2001, 2002] with phospho-specific p38 MAP kinase antibody or p38 MAP kinase antibody with peroxidase-labeled goat anti-rabbit IgG as a secondary antibody. Peroxidase activity was visualized on X-ray film by ECL.

Other Methods

The absorbance of ELISA samples at 450 nm was measured with a Multiskan JX ELISA reader (Thermo Labsystems, Helsinki, Finland). Absorbance was correlated with concentration by means of a standard curve. Densitometric analysis was performed with Molecular Analyst Software for Macintosh (BioRad, Hercules, CA).

Statistical Analysis

Data were analyzed by ANOVA followed by the Bonferroni method for multiple comparisons between pairs. $P < 0.05$ was considered significant. All data are presented as mean ± SD of triplicate determinations from three independent experiments.

RESULTS

Effect of Retinoic Acid or Vitamin D_3 on TGF-β-Induced VEGF Release

We previously reported increased VEGF release by A10 cells after 12 h or more of TGF-β stimulation, and the effect of TGF-β was significant at concentrations greater than 1 ng/ml [Yamamoto et al., 2001]. In addition, retinoic acid alone significantly stimulates VEGF release in time-and concentration-dependent manners [Tanabe et al., 2004]. In the present study, retinoic acid and TGF-β additively induced VEGF release in a time-dependent manner up to 60 h (Fig. 1A), the effect being significant after 24 h or more of stimulation. The additive effect of retinoic acid on TGF-β-stimulated VEGF release was concentration-dependent from 0.1 nM to 0.1 μM (Fig. 1B). The effect was significant at concentrations greater than 10 nM.

We previously reported that vitamin D_3 alone at concentrations greater than 10 pM stimulates VEGF release from A10 cells [Yamamoto et al., 2002]. Vitamin D_3 and TGF-β synergistically induced VEGF release in a time-dependent manner up to 60 h (Fig. 1C), the effect being significant after 12 h or more of stimulation. The synergistic effect of vitamin D_3 was concentration-dependent from 0.1 to 10 nM (Fig. 1D). The effect was significant at concentrations greater than 0.1 nM.

Effect of Dexamethasone or Corticosterone on TGF-β-Induced VEGF Release

Dexamethasone alone had little effect on the basal level of VEGF release but significantly decreased TGF-β-induced VEGF release in a time-dependent manner up to 60 h (Fig. 2A), the effect being significant after 12 h or more of stimulation. The inhibitory effect of dexamethasone was concentration-dependent from 0.1 to 10 nM (Fig. 2B). The effect was significant at concentrations greater than 1 nM.

Corticosterone, another glucocorticoid, which alone did not affect the basal level of VEGF release, significantly decreased TGF-β-stimulated VEGF release. The inhibitory effect of corticosterone was concentration-dependent

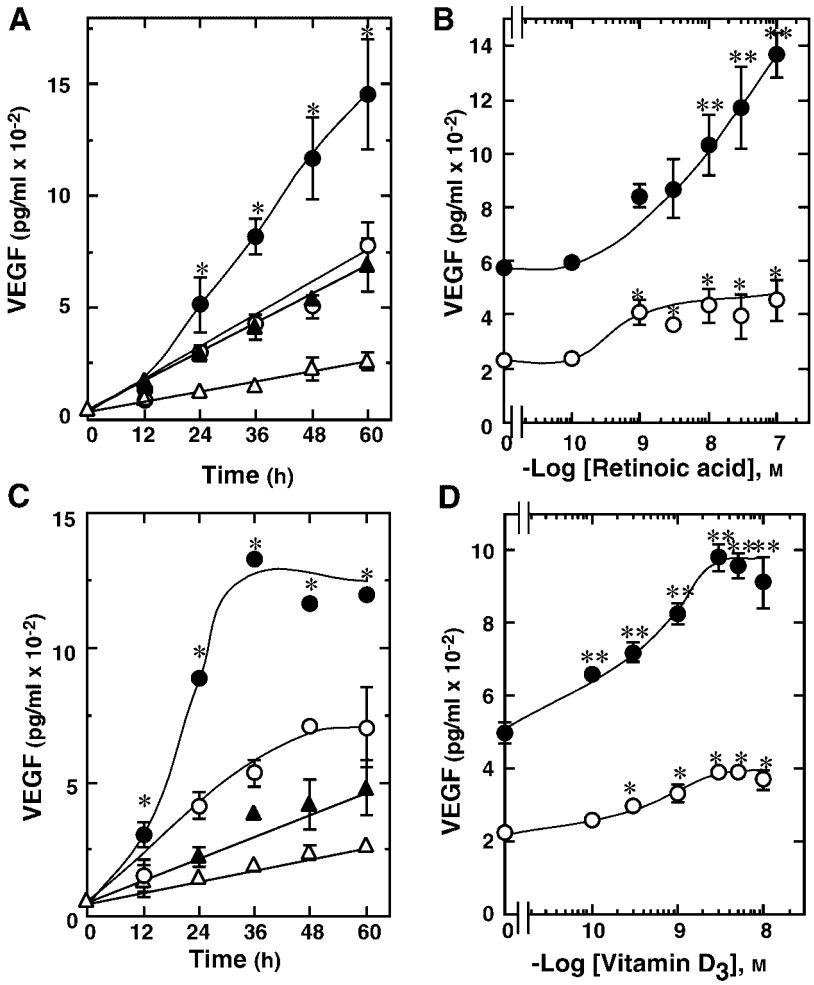

Fig. 1. Effects of retinoic acid or vitamin D_3 on the TGF-β-induced vascular endothelial growth factor (VEGF) release from A10 cells. **A**: Time-course of VEGF release after TGF-β-stimulation. Cultured cells were treated by 0.1 μM retinoic acid (●, ▲) or vehicle (○, △) for 9 h, and then stimulated by 5 ng/ml TGF-β (circles) or vehicle (triangles) for the indicated periods. $^*P < 0.05$ compared with TGF-β or retinoic acid alone. **B**: Dose-dependent effect of retinoic acid on the TGF-β-induced VEGF release. Cultured cells were treated by various doses of retinoic acid for 9 h, and then stimulated by 5 ng/ml TGF-β (●) or vehicle (○) for 48 h. $^*P < 0.05$ compared with vehicle alone. $^{**}P < 0.05$ compared with TGF-β or retinoic acid alone. **C**: Time-course of VEGF release after TGF-β-stimulation. Cultured cells were treated by 10 nM vitamin D_3 (●, ▲) or vehicle (○, △) for 9 h, and then stimulated by 5 ng/ml TGF-β (circles) or vehicle (triangles) for the indicated periods. $^*P < 0.05$ compared with TGF-β or vitamin D_3 alone. **D**: Dose-dependent effect of vitamin D_3 on the TGF-β-induced VEGF release. Cultured cells were treated by various doses of vitamin D_3 for 9 h, and then stimulated by 5 ng/ml TGF-β (●) or vehicle (○) for 48 h. $^*P < 0.05$ compared with vehicle alone. $^{**}P < 0.05$ compared with TGF-β or vitamin D_3 alone. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained in two other cell preparations.

from 0.1 to 10 nM (Fig. 2B). The effect was significant at 10 nM.

Effect of Sex Hormones or T_3 on TGF-β-Induced VEGF Release

Other hormones of the steroid/thyroid hormone superfamily, including the sex hormones progesterone, testosterone, and estradiol and T_3, had no affect on TGF-β-induced VEGF release from A10 cells (Fig. 2C,D).

Effect of Retinoic Acid, Vitamin D_3, Dexamethasone, or Corticosterone on TGF-β-Induced Phosphorylation of p38 MAP Kinase

We previously reported that TGF-β stimulates the release of VEGF from A10 cells at least in part via activation of p38 MAP kinase, with a maximum effect at 5 ng/ml TGF-β [Yamamoto et al., 2001]. Therefore, we investigated whether retinoic acid, vitamin D_3, dexamethasone, or

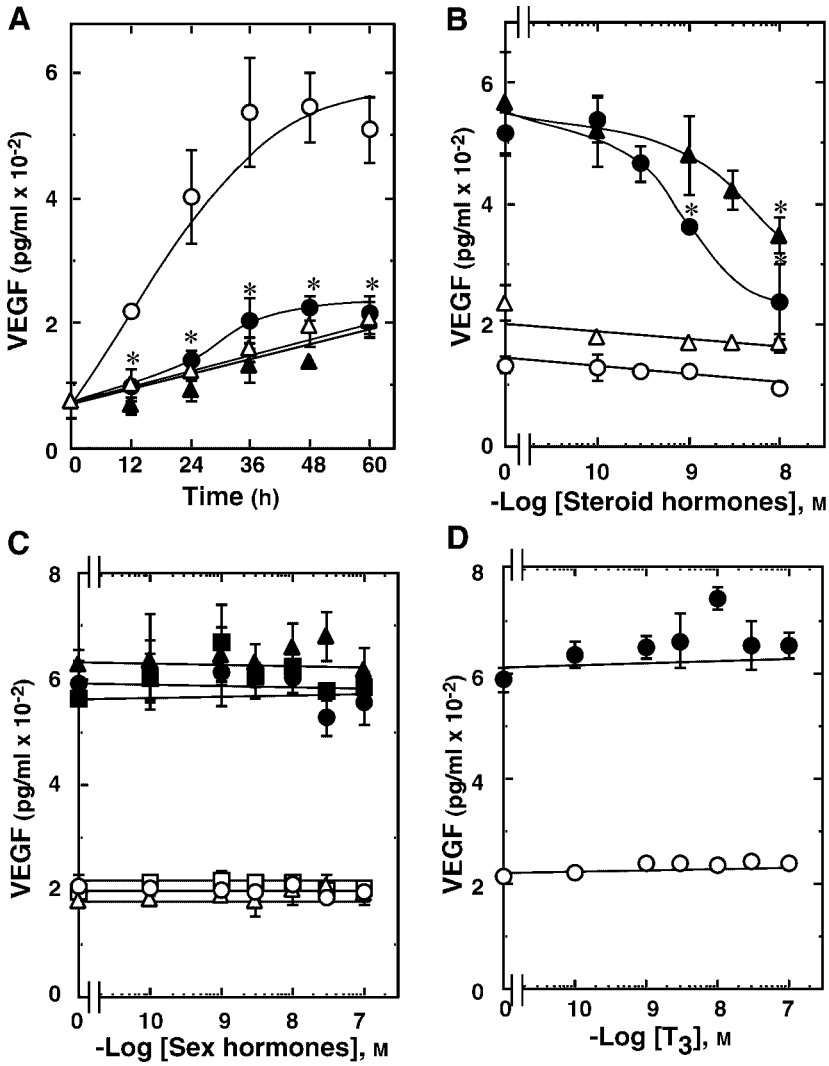

Fig. 2. Effects of dexamethasone, corticosterone, progesterone, testosterone, estradiol, or T_3 on the TGF-β-induced VEGF release from A10 cells. **A**: Time-course of VEGF release after TGF-β-stimulation. Cultured cells were treated by 10 nM dexamethasone (●, ▲) or vehicle (○, △) for 9 h, and then stimulated by 5 ng/ml TGF-β (circles) or vehicle (triangles) for the indicated periods. **B**: Dose-dependent effects of dexamethasone or corticosterone on the TGF-β-induced VEGF release. Cultured cells were treated by various doses of dexamethasone (circles) or corticosterone (triangles) for 9 h, and then stimulated by 5 ng/ml TGF-β (●, ▲) or vehicle (○, △) for 48 h. $^*P < 0.05$ compared with TGF-β alone. **C**: Cultured cells were treated by various doses of progesterone (circles), testosterone (triangles), or estradiol (squares) for 9 h, and then stimulated by 5 ng/ml TGF-β (closed symbols) or vehicle (open symbols) for 48 h. **D**: Cultured cells were treated by various doses of T_3 for 9 h, and then stimulated by 5 ng/ml TGF-β (●) or vehicle (○) for 48 h. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained in two other cell preparations.

corticosterone affects TGF-β (5 ng/ml)-induced p38 MAP kinase phosphorylation in these cells. Retinoic acid (0.1 μM), which by itself had little effect on p38 MAP kinase phosphorylation, had no affect on TGF-β-induced phosphorylation of p38 MAP kinase (Fig. 3A). We previously showed that vitamin D_3 markedly induces p38 MAP kinase phosphorylation, with a maximum effect at 10 nM vitamin D_3 [Yamamoto et al., 2002]. TGF-β and vitamin D_3 (10 nM) induced phosphorylation of p38 MAP kinase in an additive manner (Fig. 3B). Dexamethasone (10 nM), which by itself had little effect on p38 MAP kinase phosphorylation, had no affect on TGF-β-induced phosphorylation (Fig. 3C). In addition, corticosterone (10 nM) had no effect on TGF-β-induced phosphorylation of p38 MAP kinase (data not shown).

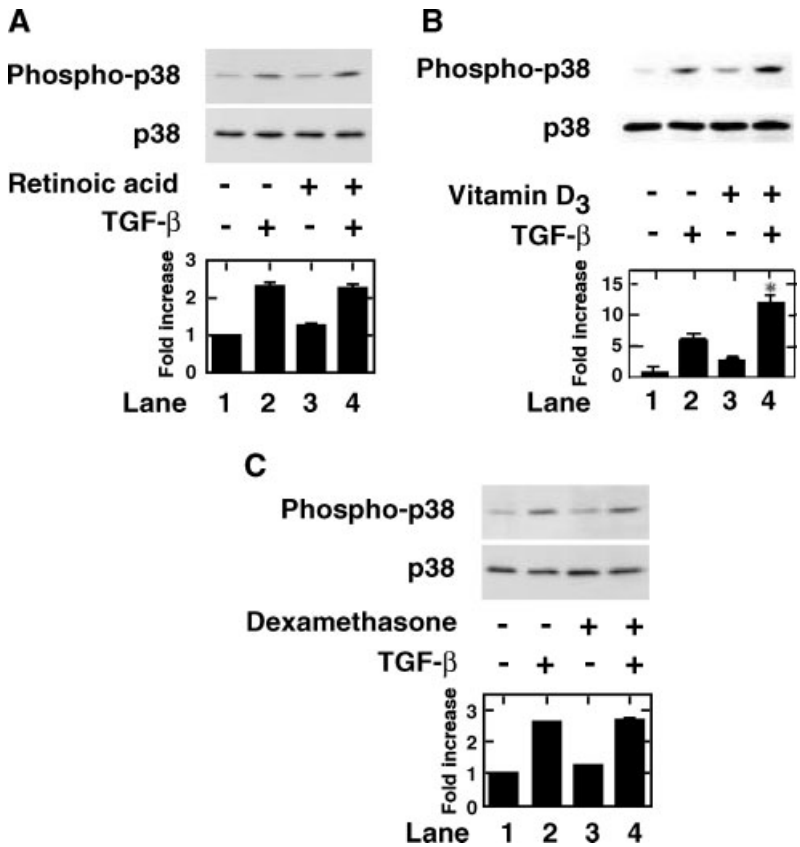

Fig. 3. Effects of retinoic acid, vitamin D_3, or dexamethasone on TGF-β-induced phosphorylation of p38 mitogen-activated protein (MAP) kinase in A10 cells. Cultured cells were treated with 0.1 μM retinoic acid (**A**), 10 nM vitamin D_3 (**B**), 10 nM dexamethasone (**C**), or vehicle for 9 h, and then stimulated by 5 ng/ml TGF-β or vehicle for 45 min. Cell lysates were subjected to SDS–PAGE followed by Western blot analysis using antibodies against phospho-specific p38 MAP kinase or p38 MAP kinase. The histogram shows quantitative representations of the phosphorylation level of p38 MAP kinase obtained from laser densitometric analysis. $^*P < 0.05$ compared with the value in TGF-β alone. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained in two other cell preparations.

Effect of SB203580 on TGF-β and Vitamin D_3-induced VEGF Release and Phosphorylation of p38 MAP Kinase

We examined the effect of SB203580, an inhibitor of p38 MAP kinase [Cuenda et al., 1995], on VEGF release and p38 MAP kinase phosphorylation induced by TGF-β in A10 cells in the absence or presence of vitamin D_3. SB203580 alone had little effect on the basal level of VEGF release but inhibited VEGF release induced by TGF-β or vitamin D_3. The enhancement of VEGF release induced by TGF-β plus vitamin D_3 was also inhibited by SB203580 (Table I). However, SB203580 alone had no affect on the basal level of phosphorylation of p38 MAP kinase and also had little effect on phosphorylation induced by TGF-β plus vitamin D_3 (Fig. 4).

TABLE I. Effects of SB203580 on Vitamin D_3 or/and TGF-β-induced Vascular Endothelial Growth Factor (VEGF) Release From A10 Cells

SB203580 (30 μM)	Vitamin D_3(10 nM)	TGF-β (5 ng/ml)	VEGF (pg/ml)
−	−	−	185 ± 11
−	−	+	583 ± 47*
−	+	−	467 ± 35*
−	+	+	1174 ± 88*
+	−	−	180 ± 10
+	−	+	298 ± 28**
+	+	−	302 ± 25**
+	+	+	496 ± 38**

Cultured cells were pretreated with 30 μM SB203580 or vehicle for 60 min and then stimulated by 10 nM vitamin D_3 for 9 h and/or 5 ng/ml TGF-β for 48 h. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained in two other cell preparations.
$^*P < 0.05$ compared with vehicle alone.
$^{**}P < 0.05$ compared with vitamin D_3 and/or TGF-β.

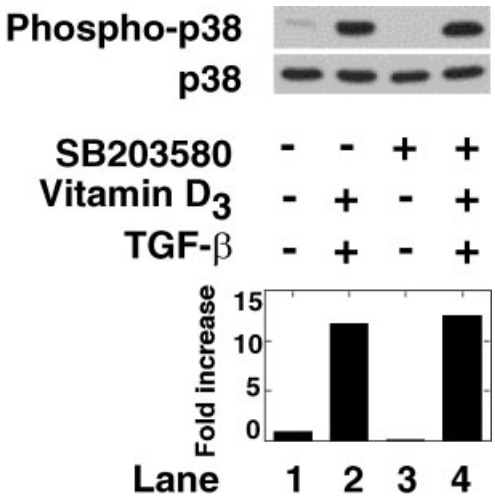

Fig. 4. Effect of SB203580 on a combination with TGF-β and vitamin D_3-induced phosphorylation of p38 MAP kinase in A10 cells. Cultured cells were treated with 30 μM SB203580 or vehicle for 60 min and then stimulated by 10 nM vitamin D_3 for 9 h and 5 ng/ml TGF-β for 45 min. Cell lysates were subjected to SDS–PAGE followed by Western blot analysis using antibodies against phospho-specific p38 MAP kinase or p38 MAP kinase. The histogram shows quantitative representations of the phosphorylation level of p38 MAP kinase obtained from laser densitometric analysis. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained in two other cell preparations.

DISCUSSION

TGF-β family members are multifunctional agonists whose effects depend on the state of responsiveness of target cells [Massagué, 1998]. Alterations in the local abundance of TGF-β in the arterial wall promote vascular cell transdifferentiation, vascular wall remodeling, arterial lesion growth, and lesion regression associated with apoptosis [Schulick et al., 1998]. One possible explanation for the angiogenic effect of TGF-β is that TGF-β is an indirect mitogen that acts via the production of other angiogenic factors. TGF-β promotes VEGF synthesis in vascular smooth muscle cells and exerts angiogenic effects [Brogi et al., 1994; Stavri et al., 1995]. We previously reported that TGF-β stimulates the release of VEGF from aortic smooth muscle A10 cells via activation of the p38 MAP kinase pathway [Yamamoto et al., 2001].

In the present study, both retinoic acid and vitamin D_3, which alone stimulate VEGF release from A10 cells [Yamamoto et al., 2002; Tanabe et al., 2004], induced VEGF release with TGF-β in an additive manner. Retinoic acid or vitamin D_3 modulate growth and differentiation of vascular smooth muscle cells [Miano and Berk, 2000; Neuville et al., 2000; Rebsamen et al., 2002]. In vitro, it has been shown that in the absence of growth factor, retinoic acid stimulates vascular smooth muscle cell proliferation but attenuates growth factor-stimulated proliferation [Miano and Berk, 2000]. In addition, retinoic acid decreases the size of neointimal masses, elicits favorable remodeling, and increases lumen diameter and area of injured arteries in vivo [Neuville et al., 2000]. In vascular endothelial cells, retinoic acid enhances the expression of the TGF-β receptor, potentiates TGF-β-induced inhibition of fibrinolytic activity and cell proliferation, and modulates endothelial cell growth and morphology [Kojima and Rifkin, 1993; Yoshizawa et al., 1998; Miano and Berk, 2000]. VEGF also induces endothelial cell proliferation [Neufeld et al., 1999]. Thus, it is likely that retinoic acid, along with TGF-β, modulates endothelial cell growth. With respect to vitamin D_3, it plays an important role in regulatory calcium homeostasis, cell differentiation, and proliferation [Kato, 2000]. In the vascular system, vitamin D_3 contributes to the development of hypertension, induces vascular calcification, and modulates vascular smooth muscle cell growth [Rebsamen et al., 2002]. Vitamin D_3 also inhibits VEGF-induced angiogenesis by inhibiting vascular endothelial cell proliferation [Mantell et al., 2000]. Our results suggest that vitamin D_3 modulates endothelial cell function by working with TGF-β to stimulate VEGF release.

We also showed that dexamethasone and corticosterone inhibited TGF-β-induced VEGF release in A10 cells. Glucocorticoids inhibit angiogenesis [Auerbach and Auerbach, 1994], and dexamethasone, hydrocortisone, or cortisone inhibit PDGF-induced VEGF gene expression and VEGF release in vascular smooth muscle cells [Nauck et al., 1998]. Our present study is consistent with this report. Thus, it is likely that dexamethasone and corticosterone inhibit angiogenesis, at least in part, by inhibiting TGF-β-induced VEGF release from vascular smooth muscle cells.

We also investigated whether the stimulatory and inhibitory effects of retinoic acid, vitamin D_3, dexamethasone, or corticosterone on VEGF release are mediated via TGF-β-induced p38 MAP kinase activation in A10 cells. Retinoic

acid, dexamethasone, or corticosterone had no affect on TGF-β-induced phosphorylation of p38 MAP kinase. On the contrary, we showed that the maximal concentration of vitamin D_3 (10 nM) plus that of TGF-β (5 ng/ml) on p38 MAP kinase phosphorylation was additive. Therefore, it is likely that vitamin D_3 plus TGF-β stimulates VEGF release in A10 cells via enhancement of p38 MAP kinase activation. SB203580, a p38 MAP kinase inhibitor [Cuenda et al., 1995], inhibited TGF-β-induced VEGF release in A10 cells in the absence or presence of vitamin D_3. SB203580 did not affect p38 MAP kinase phosphorylation induced by TGF-β plus vitamin D_3. SB203580 is not an inhibitor of the upstream kinase of p38 MAP kinase but is a direct inhibitor of p38 MAP kinase. Therefore, it is likely that p38 MAP kinase, at least in part, mediates TGF-β-induced VEGF release in A10 cells in the absence or presence of vitamin D_3.

Other members of the nuclear hormone receptor superfamily (e.g., progesterone, testosterone, estradiol, and T_3) are known to modulate blood pressure and vascular smooth muscle cell growth [Mizuma et al., 2001; Dubey et al., 2002]. However, these factors had no affect on TGF-β-induced VEGF release from A10 cells. It has been reported that deoxycorticosterone and pregnenolone have no effect on VEGF gene expression or release in vascular smooth muscle cells [Nauck et al., 1998]. Thus, it is likely that retinoic acid, vitamin D_3, or glucocorticoids regulate the vascular system, at least in part, by modulating the release of endogenous and TGF-β-induced VEGF from vascular smooth muscle cells.

A10 cells derived from fetal rat aorta express many characteristics of vascular smooth muscle cells such as the production of spontaneous action potentials and increase myokinase and creatine phosphokinase activities [Kimes and Brandt, 1976]. VEGF which is synthesized and released predominantly by vascular smooth muscle cells plays a central role in the regulation of angiogenesis in both physiologic and pathologic states, such as wound healing and tumorigenesis, through the induction of vascular endothelial cell proliferation, migration, and inhibition of apoptosis [Neufeld et al., 1999]. It is likely that the modulation by steroid hormones of TGF-β-induced VEGF synthesis in vascular smooth muscle cells plays a role in pathophysiologic angiogenesis. However, discrepancies between in vivo and in vitro findings may occur. Further investigations are necessary to elucidate these effects in vivo.

In conclusion, nuclear hormone receptor superfamily members, including retinoic acid, vitamin D_3, and glucocorticoids, affect TGF-β-stimulated VEGF release from aortic smooth muscle cells. The stimulatory effect of vitamin D_3 occurs, in part, via up-regulation of TGF-β-induced p38 MAP kinase activation.

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