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Thromboxane A_2 Regulation of Endothelial Cell Migration, Angiogenesis, and Tumor Metastasis

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Prostaglandin endoperoxide H synthases and their arachidonate products have been implicated in modulating angiogenesis during tumor growth and chronic inflammation. Here we report the involvement of thromboxane A_2, a downstream metabolite of prostaglandin H synthase, in angiogenesis. A TXA_2 mimetic, U46619, stimulated endothelial cell migration. Angiogenic basic fibroblast growth factor (bFGF) or vascular endothelial growth factor (VEGF) increased TXA_2 synthesis in endothelial cells three- to fivefold. Inhibition of TXA_2 synthesis with furegrelate or CI reduced HUVEC migration stimulated by VEGF or bFGF. A TXA_2 receptor antagonist, SQ29,548, inhibited VEGF- or bFGF-stimulated endothelial cell migration. *In vivo,* CI inhibited bFGF-induced angiogenesis. Finally, development of lung metastasis in C57Bl/6J mice intravenously injected with Lewis lung carcinoma or B16a cells was significantly inhibited by thromboxane synthase inhibitors, CI or furegrelate sodium. Our data demonstrate the involvement of TXA_2 in angiogenesis and development of tumor metastasis.

Key Words: **thromboxane A_2 (TXA_2); angiogenesis; eicosanoid; endothelial cell; tumor metastasis.**

Angiogenesis, the formation of new capillary blood vessels, is a tightly regulated process involving endothelial cell proliferation, migration, and tube differentiation (1). Persistent angiogenesis underscores many pathological conditions in adults such as tumor growth and metastasis, diabetic retinopathy, and chronic inflammations (2). During angiogenesis, angiogenic factors such as VEGF and bFGF can prompt endothelial cells to exit quiescence and undergo various angiogenic responses such as proliferation, migration, and survival (3). An understanding of the signaling mechanism which underlies angiogenesis is important since it will provide potential therapeutic targets to inhibit or enhance angiogenesis.

One key aspect of cellular signaling involves mobilization of arachidonic acid and subsequent formation of bioactive eicosanoids through cyclooxygenase (COX), lipoxygenase (LOX), or P450 epoxygenase pathways. In a previous study of prostate cancer, we found that platelet-type 12-LOX stimulated angiogenesis and tumor growth (4). In addition to 12-LOX, it has been shown that COX-1 and COX-2 was up-regulated in endothelial and tumor cells during angiogenesis (5, 6) and that expression of COX was associated with angiogenesis by human gastric endothelial cells (7). Inhibition of COX activity by non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to reduce angiogenesis and tumor growth (for review, ref. 8). Among the products of the cyclooxygenase pathway, PGE_1 and PGE_2 are reported to promote angiogenesis (9–11). In contrast, 15-deoxy-$\Delta^{12,14}$-PGJ_2, a product from PGD_2, induces endothelial cell apoptosis by activation of PPARγ (12) and inhibits angiogenesis (13). It seems, therefore, that the actual profile of the downstream COX metabolites, rather than the level of COX protein or activity, is more relevant in angiogenesis regulation.

The purpose of the present study is to examine the possible involvement of the downstream eicosanoid products of the COX pathway in angiogenesis. Here we report that TXA_2, an eicosanoid metabolite from the sequential activities of COX and thromboxane synthase, is an important mediator for angiogenesis. A TXA_2 mimetic, U46619, stimulated endothelial cell migration while a TXA_2 receptor antagonist, SQ29,548, inhibited endothelial cell migration in response to VEGF or bFGF. We also present evidence that inhibi-

Abbreviations used: COX, cyclooxygenase; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2; PG, prostaglandin; PGI_2, prostacyclin; CI, carboxyheptal imidazole; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; RV-ECT, rat vascular endothelial cell-tube forming.

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tion of TXA_2 biosynthesis reduced VEGF- or bFGF-stimulated endothelial cell migration *in vitro* and bFGF-induced angiogenesis *in vivo*. Our data suggest a functional role for TXA_2 in angiogenesis and also implicate the modulation of TXA_2 production or function as a potential therapeutic target for diverse diseases in which vascular endothelial cells play an important role.

MATERIALS AND METHODS

Materials. Prostaglandin E_2, $PGF_{2\alpha}$, carbacyclin, arachidonic acid, and SQ29,548 were purchased from Cayman Chemical Co. (Ann Arbor, MI). U46619, carboxyheptal imidazole (CI), furegrelate sodium were purchased from Biomol (Plymouth Meeting, PA). Recombinant human VEGF-A was purchased from R&D (Minneapolis, MN). Recombinant human bFGF was purchased from Sigma (St. Louis, MO).

Endothelial cell culture. The cord-forming angiogenic endothelial cell line established from rat brain resistance vessels, RV-ECT, was obtained from Dr. Clement Diglio at the Department of Pathology, Wayne State University (Detroit, MI). RV-ECT cells were cultured in DMEM with 10% FBS. Human umbilical vein endothelial cells (HUVEC) and human dermal microvascular endothelial cells (HMVEC) were purchased from Clonetics (San Diego, CA) and multiplied in EGM-2 and used between passage 4 to 10.

Immunoblot analysis of thromboxane synthase expression. Semi-confluent confluent (70–80%) HUVEC, HMVEC, or RV-ECT endothelial cells were rinsed with ice-cold PBS, scraped into lysis buffer containing 20 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.5 mM EGTA, 0.5 mM PMSF, 0.5 mM leupeptin, 0.15 mM pepstatin A, 1 mM dithiothreitol and 1% NP-40. Protein concentration was measured using BCA protein assay kit (Pierce, Rockford, IL). Seventy-five micrograms of protein from each samples were loaded into a minigel for electrophoresis separation. The proteins in the gel were then transferred onto a PVDF membrane and processed for immunodetection using a thromboxane synthase polyclonal antibody (Cayman Chemical Co., Ann Arbor, MI). Horseradish peroxidase conjugated goat anti-rabbit IgG antibodies and enhanced chemiluminescent (ECL) reagent was purchased from Amersham (Arlington Heights, IL).

Thromboxane A_2 production. RV-ECT cells were grown to 80–90% confluency in DMEM supplemented with 10% FBS and then serum starved overnight in serum-free DMEM. Fresh serum-free DMEM with 1 μM arachidonic acid was added one hour prior to VEGF or bFGF treatment. Thromboxane synthase inhibitor CI was added to a final concentration of 20 μM 15 min prior to VEGF or bFGF treatment. Recombinant human VEGF or bFGF was added in a final concentration of 10 ng/ml. After 20 min of treatment, cells were washed in PBS once and harvested using cell scrapers. After centrifugation, the cell pellets were resuspended in ice-cold 100% ethanol and sonicated. An aliquot was saved for determination of protein level. The samples were purified using BAKERBOND spe Octadecyl (C18) columns (J.T. Baker, Phillipsburg, NJ) and TXB_2 levels were measured using EIA kit as per the manufacturer's instruction (Cayman Chemical, Ann Arbor, MI). Experiments were performed using two different types of endothelial cells, RV-ECT and HUVEC.

Endothelial cell migration assay. Endothelial cell migration assay was conducted using modified Boyden Chamber essentially as previously described (4). HUVE cells were harvested and resuspended in EBM-2 with 2% FBS at density of 5×10^5 cells per ml. Cells (0.5 ml) were placed in the upper chamber and migration was initiated by placing 1 ml of same media containing various treatments in the bottom chamber. After 12–18 h, the cells on the upper side of the membrane were removed by cotton swab and the membrane was cut out, fixed, stained, and mounted in Permount. Cells migrated were enumerated in a double blind approach. For stimulation of endothelial cell migration, usually VEGF (10 ng/ml) or bFGF (10 ng/ml) was placed in the lower chamber. The concentrations of test compounds were described in text. For each treatment, at least three chambers were used unless otherwise indicated.

Wound healing assay. A confluent monolayer of HUVE cell culture was wounded with a pipet tip and the media changed to EBM-2 with 2% FBS. For treatment, VEGF or bFGF was added at the final concentration of 10 ng/ml. SQ29,548 was added to the final concentration of 4 μM. The closure of the wound was monitored and recorded every 6 h.

Matrigel implantation assay for angiogenesis. The Matrigel implantation assay was performed as described by Ito *et al.* (14) with the following modifications. An aliquot (0.4 ml) of Matrigel (Becton Dickinson, Bedford, MA) alone or with test additives was injected s.c. into nude mice (4 mice/group). Mice were sacrificed 5 days after injection and dissected to expose the implants for recording using an SP SZ-4060 stereomicroscope (Olympus America, Melville, NY). The amount of blood retained in the Matrigel was further assessed by measuring the hemoglobin levels using Drabkin's reagent (Sigma Diagnostics, St. Louis, MO).

Tumor cell lines, in vivo maintenance, and isolation of cell subpopulations. The B16 amelanotic melanoma and Lewis lung carcinoma, were originally obtained from the Division of Cancer Treatment, National Institutes of Health (Frederick, MD), and passaged in male C57BL/6J syngeneic mice (15). Monodispersed cells were prepared by enzymatic (collagenase) dispersion, and cell populations were isolated by centrifugal elutriation (16). Isolated cells typically consisted of 100% dispersed cells, >95% tumor cells, with 90–95% viability, and no detectable cellular debris (17).

Experimental metastasis assay. Elutriated cells were re-suspended in MEM at 7.5×10^5 cells/ml, and maintained at 4°C. Five to 10 min prior to injection, the cell suspension was warmed to 25°C. Cells (3.75×10^4) were injected into the lateral tail vein of unanesthetized C57BL/6J male mice (8–9 weeks old). A minimum of 12 animals were used per experimental group. Three days post injection, when all cells had cleared the vasculature as previously described (18, 19), animals were administered thromboxane synthase inhibitors (p.o.) daily for 18 days, after which they were sacrificed by cervical dislocation. Lungs were removed and fixed for minimum of 24 h in Bouin's fixative, and visible tumor colonies were enumerated as described previously (20). *In vivo* experiments with the B16A cells were repeated three times and with 3LL subpopulations, four times with reproducible results.

RESULTS

Stimulation of Endothelial Cell Migration by TXA_2 Receptor Agonist U46619

It has been shown in a number of studies that COX-1 and COX-2 were up-regulated in both endothelial and tumor cells during angiogenesis (5, 6). In addition, both COX-1 and COX-2 inhibitors are reported to inhibit angiogenesis (5–7). Since the immediate product of COX-1 and COX-2 activities, PGH_2, is used as substrate for the biosynthesis of PGE_2, $PGF_{2\alpha}$, PGI_2, TXA_2, and other eicosanoids by downstream enzymes, we examined the effects of these eicosanoids from the COX pathway on endothelial cell migration. As shown in Fig. 1, at 300 nM, PGE_2 and $PGF_{2\alpha}$ had no significant

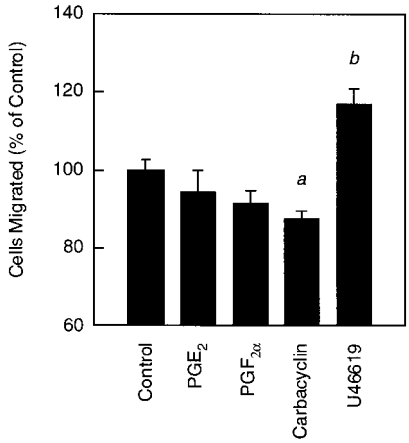

FIG. 1. Modulation of endothelial cell migration by major products of the COX pathway. Endothelial cell migration assay was conducted using modified Boyden Chamber. HUVE cells were harvested and resuspended in EBM-2 with 2% FBS at density of 5×10^5 cells/ml. Cells (0.5 ml) were placed in the upper chamber and migration was initiated by placing 1 ml of same media containing various treatments in the bottom chamber. After 12 h, the cells on the upper side of the membrane were removed by cotton swab and the membrane was cut out, fixed, stained, and mounted in Permount. Cells migrated were enumerated in a double blind approach. The concentrations of all testing compounds were 300 nM. Columns, average cell migration as compared to the basal control; bars, SE. a, $P = 0.002$; b, $P = 0.001$ (Student's t test).

effect on HUVEC migration. At the same concentration, carbacyclin, a PGI_2 stable analog inhibited cell migration while U46619, a TXA_2 receptor agonist, significantly stimulated HUVEC migration by ~20% ($P < 0.01$). The optimal dose for stimulation of endothelial cell migration by U46619 is approximately 300 nM. An increase in U46619 concentration to 3 μM or 30 μM was found to inhibit rather stimulate endothelial cell migration (data not shown), suggesting a biphasic response of the TXA_2 receptor, possibly due to receptor desensitization. The study suggests TXA_2 as a potential mediator of COX in the regulation of angiogenesis.

Stimulation of TXA_2 Production in Endothelial Cells by VEGF and bFGF and Its Involvement in Endothelial Cell Migration

When stimulated with xenoactive antibodies and complement, endothelial cells can release TXA_2 (21), suggesting the presence of thromboxane synthase in endothelial cells. To further confirm the expression of thromboxane synthase, we analyzed its expression in HUVE and HMVE cells by immunoblot analysis. As shown in Fig. 2A, both HMVE and HUVE cells express thromboxane synthase. In addition to endothelial cells of human origin, we also found that RV-ECT cells, an endothelial cell line originated from rat brain, express thromboxane synthase (data not shown).

To examine whether there is an increase in TXA_2 output during angiogenesis, we treated RV-ECT endothelial cells with bFGF or VEGF and measured the level of TXB_2, which is the stable product of TXA_2 after its rapid inactivation. As shown in Fig. 2B, when endothelial cells were treated by VEGF, the production of TXA_2 was increased by 3- to 4-fold. Pretreatment with a thromboxane synthase inhibitor, CI (20 μM), significantly reduced the TXA_2 biosynthesis. A similar increase in TXA_2 synthesis also was observed in RV-ECT cells when treated with bFGF (2.5-fold). We also studied the effect of VEGF or bFGF treatment on TXA_2 biosynthesis in HUVE cells and found that 20 min of VEGF or bFGF treatment increase the level of TXA_2 levels by 4.5- and 4.9-fold, respectively.

Next we examined the role of the increased synthesis of TXA_2 in VEGF- or bFGF-stimulated endothelial cell migration. As shown in Fig. 2C, both thromboxane synthase inhibitors, CI or furegrelate sodium, reduced

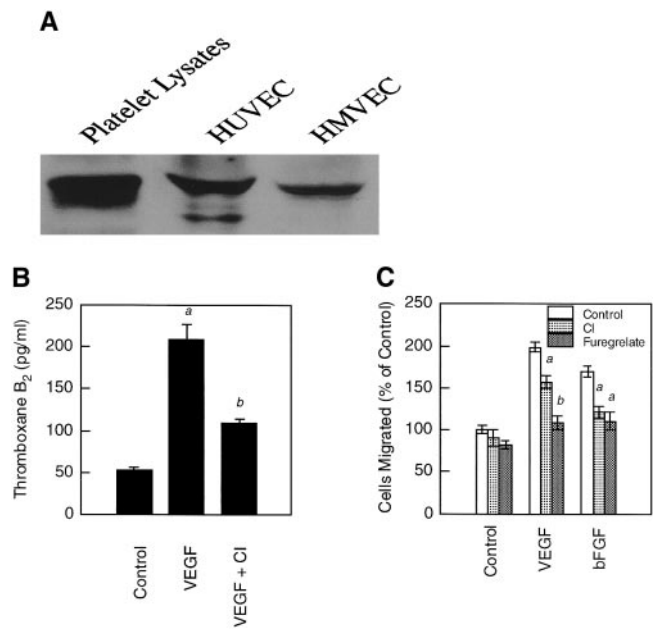

FIG. 2. Expression of thromboxane synthase, TXA_2 biosynthesis, and endothelial cell migration. (A) Western blot analysis of thromboxane synthase expression in various endothelial cells. Platelet lysates were used as the positive control. (B) Stimulation of TXA_2 biosynthesis by bFGF and VEGF. TXA_2 synthesis was measured by the levels of TXB_2 in RV-ECT endothelial cells after bFGF or VEGF treatment as described under Materials and Methods. A representative from three independent experiments is shown. Columns, average level of TXB_2 from triplicate samples; bars, SE. a, $P < 0.01$ when compared to the control; b, $P < 0.01$ when compared to VEGF-treated group (Student's t test). (C) inhibition of VEGF- or bFGF-stimulated endothelial cell migration by thromboxane synthase inhibitors. VEGF- or bFGF-stimulated endothelial cell migration assay was conducted as described under Materials and Methods. Columns, average cell migration as compared to the basal control; bars, SE. a, $P < 0.05$; b, $P < 0.01$ when compared to their respective control (within VEGF- or bFGF-treated group) (Student's t test).

endothelial cell migration stimulated by VEGF or bFGF, suggesting that the increased TXA_2 production is involved for VEGF- or bFGF-stimulated endothelial cell migration.

Inhibition of Endothelial Cell Migration by a TXA_2 Antagonist, SQ29,548

TXA_2 is synthesized within cells and exported immediately to the extracellular miliu. To study whether endogenously synthesized TXA_2 can stimulate endothelial cell migration in an autocrine manner, we next examined the effect of a TXA_2 receptor antagonist, SQ29,548 (22), on VEGF- or bFGF-stimulated wound healing of HUVEC monolayers, a process involving cell migration and proliferation. As shown in Fig. 3A, SQ29,548 inhibited VEGF- or bFGF-stimulated wound closure. SQ29,548 also attenuated endothelial cell migration stimulated by VEGF or bFGF (Fig. 3B). The data suggest that the involvement of TXA_2 in VEGF- or bFGF-stimulated endothelial cell migration, as described above, requires the activation of the TXA_2 receptor since SQ29,548 functions as an antagonist of the TXA_2 receptor.

Inhibition of Angiogenesis in Vivo by Thromboxane Synthase Inhibitor

The Matrigel implantation assay was used to study whether inhibition of TXA_2 synthesis compromises angiogenesis *in vivo.* As shown in the left panel of Fig. 4A, bFGF stimulated angiogenesis as evidenced by the penetration of blood vessels into and the accumulation of blood within the Matrigel plugs. Inclusion of CI in the gels significantly reduced angiogenesis (Fig. 4A, right panel) and reduced the accumulation of hemoglobin in the Matrigel plug (Fig. 4B), suggesting that inhibition of thromboxane synthesis compromised angiogenesis *in vivo.*

Inhibition of Tumor Metastasis by Thromboxane Synthase Inhibitors

Angiogenesis is required for the growth of any solid tumors including primary tumor and tumor metastasis (2). In order to demonstrate whether inhibition of TXA_2 biosynthesis can block the development of metastasis, we injected (tail vein) elutriated B16a or 3LL cells into C57Bl/6J mice and initiated oral administration with thromboxane synthase inhibitors three days post injection. As shown in Figs. 5A and 5B, oral administration of CI significantly reduced the number and the size of lung metastasis from B16a cells. Similar results were obtained with another thromboxane synthase inhibitor, furegrelate sodium. Both thromboxane synthase inhibitors also significantly inhibited the formation of lung metastasis from 3LL cells (Fig. 5B). Taken together, the data suggest that thromboxane synthase activity is required for development of tumor metastasis and that anti-angiogenic thromboxane synthase inhibitors are potential anti-metastatic agents.

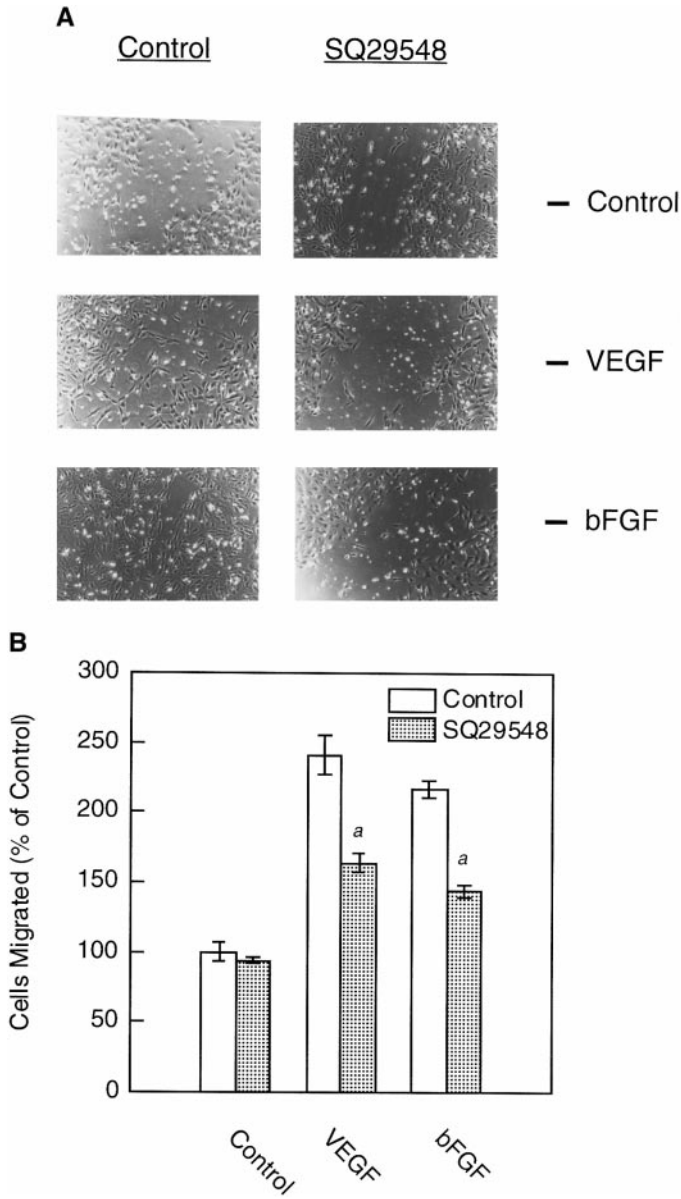

FIG. 3. Inhibition of VEGF- or bFGF-stimulated endothelial cell migration by SQ29,548, a TXA_2 receptor antagonist. (A) Effect of SQ29,548 on HUVEC monolayer wound healing. HUVEC culture monolayer was wounded with a pipet tip and changed into EBM-2 with 2% FBS. VEGF or bFGF was added at the final concentration of 10 ng/ml. SQ29,548 was added in the final concentration of 4 μM. Representatives from each group 36 hours after treatment are shown. (B) Inhibition of VEGF- or bFGF-stimulated endothelial cell migration by SQ29,548. The VEGF- or bFGF-stimulated endothelial cell migration assay was conducted as described under Materials and Methods. The concentration of SQ29,548 was 4 μM. Columns, average cell migration as compared to the basal control; bars, SE. a, $P < 0.05$ when compared to their respective controls (Student's t test).

DISCUSSION

In the present study, our data demonstrate that (i) among various major eicosanoid products from COX activity, the TXA_2 mimetic U46619 stimulates, while the PGI_2 analog carbacyclin reduces, endothelial cell migration, (ii) biosynthesis of TXA_2 is stimulated by bFGF or VEGF in endothelial cells, (iii) inhibition of endogenous TXA_2 synthesis reduced VEGF- or bFGF-stimulated endothelial cell migration, (iv) blocking TXA_2 function by SQ29,548 decreased VEGF- or bFGF-stimulated endothelial cell migration, (v) inhibition of thromboxane synthase activity by CI reduced bFGF-stimulated angiogenesis *in vivo,* and (vi) inhibition of thromboxane synthase activity by CI or furegrelate sodium reduced the development of experimental pulmonary metastasis from B16a or 3LL cells.

TXA_2 is a potent stimulator of platelet aggregation and smooth muscle constriction and is regarded as a mediator of myocardial infarction, atherosclerosis, and bronchial asthma (23). It is the main product of arachidonic acid metabolism via the COX pathway in platelets. The pro-angiogenic function of TXA_2 implicates the possible involvement of platelets in angiogenesis during tumor growth and metastasis. Indeed, platelets are intimately involved in tumor angiogenesis (24, 25) and platelet aggregation stimulates the release of VEGF (26). Clinically, 30–60% of advanced cancer patients possess platelet abnormalities, such as thrombocytosis and many other thromboembolic disorders. Also, activated platelets have been frequently associated with many malignant tumors (27). Pharmacologically, many anti-platelet agents, including thromboxane synthase inhibitors, have been shown to possess anti-metastatic effects (28). These original observations were believed to be due to the effect of thromboxane synthase inhibitors/receptor antagonists on platelet TXA_2 production. In the present study, thromboxane synthase inhibitors were administered 3 days post injection. This time interval between injection and treatment was chosen because of previous studies from our laboratory, which demonstrated that intravenously injected tumor cells attach to the endothelium, induce endothelial cell retraction, and extravasate from the vasculature into the lung parenchymal tissues within 24 hours post injection (18, 19). Therefore, any effect observed with these inhibitors could not be explained by inhibition of tumor/platelet/endothelial cell interactions or tumor cell extravasation. The inhibition of development of metastasis in the experiments presented here suggests that the thromboxane synthase inhibitors affected the ability of tumor cells to grow at the site of distant metastasis. One possible explanation could be a reduction in angiogenesis due to an inhibition of endothelial cell TXA_2 synthase.

Although it is generally accepted that TXA_2 is mainly produced by platelets, TXA_2 can also be synthesized by numerous cells in response to various physiological and pathological stimuli (23, 29). Synthesized TXA_2 is rapidly secreted from cells and acts as a local hormone in the immediate vicinity of its site of production. The human TXA_2 receptor (TP) is a typical G-protein coupled receptor with seven transmembrane segments (30). Activation of TP by TXA_2, or more stable synthetic agonists, evokes the activation of phospholipase C and a subsequent rise in the intracellular calcium ion concentration. Depending on cell type, TP activation will result in platelet aggregation (31), contraction of vascular smooth muscle cells (32) or release of prostacyclin from endothelial cells (33).

In this study, we found that putative angiogenic factors such as VEGF or bFGF can increase TXA_2 biosynthesis in endothelial cells and the increased TXA_2 biosynthesis mediates the stimulation of endothelial cell migration by VEGF or bFGF. The newly synthesized TXA_2 probably acts through its G protein-coupled receptor to modulate endothelial cell migration, since a TXA_2 receptor antagonist, SQ29,548, decreases VEGF- or bFGF-stimulated endothelial cell migration. The results suggest the activation of TXA_2 receptor is required for full stimulation of endothelial cell migration by VEGF or bFGF. However, it should be noted that neither thromboxane synthase inhibitors nor SQ29,548 are able to completely abolish the stimulation of endothelial cell migration by VEGF or bFGF, suggesting that VEGF or bFGF can activate multiple pathways, including TXA_2, to effect endothelial cell motility.

When included in bFGF-containing Matrigel implants, thromboxane synthase inhibitor CI inhibited bFGF-induced angiogenesis *in vivo.* Since CI can inhibit thromboxane synthase activity in proximal smooth muscle cells and platelets, as well as in endothelial cells, the exact contribution of TXA_2 synthesized by endothelial cells in angiogenesis remains to be defined. Nevertheless, the observed anti-angiogenic activity of a thromboxane synthase inhibitor, along with the recent observation that the TXA_2 receptor antagonist SQ29,548 inhibited corneal angiogenesis (34), suggests TXA_2 is an important factor in bFGF-induced angiogenesis *in vivo.*

It should be noted that although TXA_2 is involved in angiogenesis, we did not find that the activation of TXA_2 receptor by U46619 alone leads to *de novo* angiogenesis (Nie *et al.*, unpublished observation), suggesting TXA_2 must act in concert with other factors to promote angiogenesis. Indeed, angiogenesis is a complex process which involves extensive interplay between cell, extracellular matrix, and a plethora of angiogenic factors such as bFGF, VEGF, and angiogenin. For example, bFGF is a stronger endothelial cell mito-

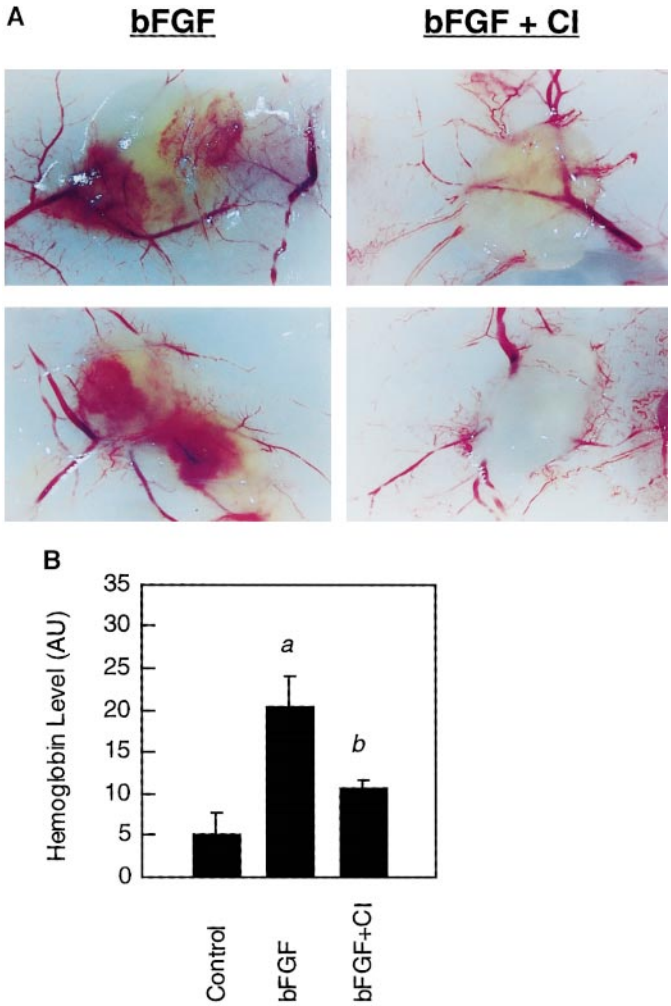

FIG. 4. Inhibition of bFGF-induced angiogenesis *in vivo* by a thromboxane synthase inhibitor, CI. Matrigel implantation assay for angiogenesis was conducted as described under Materials and Methods. (A) Matrigel implantation angiogenesis assay. Left panel: bFGF (5 μg/ml of Matrigel) *in situ;* Right panel: bFGF plus CI (2.5 mg/ml of Matrigel) *in situ*. (B) Hemoglobin levels in resected implants. The hemoglobin was measured by Drabkin's reagent. Columns, average hemoglobin levels (AU); bars, SE from quadruplicate samples. a, $P < 0.05$ when compared to the control; b, $P < 0.05$ when compared to the bFGF-treated group.

gen than VEGF while VEGF is stronger chemotactic stimulant than bFGF (35). For bFGF to induce angiogenesis, it stimulates VEGF expression in endothelial cells and together with VEGF, induces angiogenesis (36). Neutralization of VEGF function can block bFGF-induced angiogenesis, suggesting that different angiogenic factors must work together to induce angiogenesis (36). Currently, we are actively exploring the possible additive or synergistic interaction between TXA_2 and other angiogenic factors during angiogenesis.

In summary, here we identified TXA_2 as an important factor in angiogenesis. Modulation of the function of this eicosanoid presents a means to control angiogenesis in various diseases in which vascular endothelial cells play a prominent role. The identification of another lipid which regulates angiogenesis, in addition to 12(S)-HETE as described in our previous study of prostate cancer (4), provide a new paradigm that, in

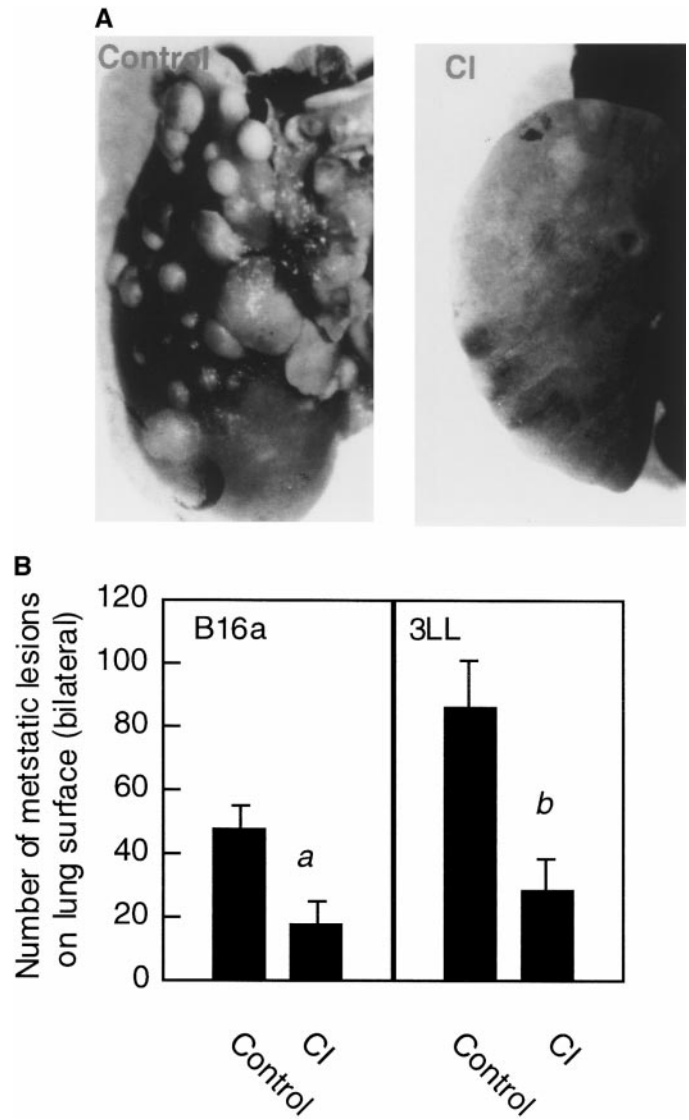

FIG. 5. Inhibition of development of experimental metastasis by thromboxane synthase inhibitor. Experimental metastasis study was conducted by injecting mice with 3.75×10^4 elutriated B16a or 3LL cells. Daily oral administration of CI was initiated 3 days post injection with the dose of 4 mg/kg mouse weight. After 18 days of treatment, mice were sacrificed and the number of metastatic lesions on lung surface was assessed as described under Materials and Methods. (A) Gross morphology of resected mouse lungs 21 days after tail vein injection of B16a cells. Left, a typical mouse lung in control group; right, a representative lung from the mice treated with CI for 18 days. (B) reduction of the formation of metastatic lesions by CI. Columns, average number of metastatic lesions per mouse lung (bilateral); Bars, S.E. from 12 samples. a and b, $P < 0.05$ when compared to their respective control.

addition to proteinaceous factors, angiogenesis is further regulated by small bioactive lipids such as 12(S)-HETE and TXA_2. Manipulation of the function or biosynthesis of these pro-angiogenic bioactive lipids provides a novel approach for development of anti-angiogenesis therapy for many diseases including cancer.

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