Journal of Cellular Biochemistry 89:500–506 (2003)

Thrombospondin-1-Induced Vascular Smooth Muscle Cell Chemotaxis: The Role of the Type 3 Repeat and Carboxyl Terminal Domains

Taeseung Lee,[1,2] Susan M. Nesselroth,[1,2] Eric T. Olson,[1,2] Nowokere Esemuede,[1,2] Jack Lawler,[3] Bauer E. Sumpio,[1,2] and Vivian Gahtan[1,2]*

[1]Section of Vascular Surgery, Yale University School of Medicine, New Haven, Connecticut
[2]VA Connecticut Healthcare System, West Haven, Connecticut
[3]Department of Pathology, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, Massachusetts

Abstract Thrombospondin-1 (TSP-1), an acute phase reactant implicated in vascular disease, is a matricellular glycoprotein with six domains that confer different functions. The authors have shown TSP-1 induces vascular smooth muscle cell (VSMC) chemotaxis via extracellular signal-regulated kinases-1 and -2 (ERK) and p38 kinase (p38) and that a fusion protein of the carboxyl terminal (COOH) and type 3 repeat (T3) domains independently induce VSMC chemotaxis. The purpose of this study was to determine whether COOH-, T3-induced VSMC chemotaxis, or both, is dependent upon ERK or p38 activation. To determine if the T3, COOH, or type 2 repeat domain (T2, control domain not associated with chemotaxis) activate ERK, p38, or both, VSMCs were exposed to each fusion protein (20 μg/ml for 15, 30, 60, or 120 min), serum-free media (SFM, negative control), or TSP-1 (20 μg/ml for 30 min, positive control). Western immunoblotting was performed for activation studies. Using a microchemotaxis chamber, VSMCs pre-incubated in SFM, DMSO (vehicle control), PD98059 (10 μM), or SB202190 (10 μM) were exposed to each domain, TSP-1, or SFM. After 4 h (37°C), migrated VSMCs were recorded as cells/five fields (400 ×) and analyzed by paired *t*-test. ERK was activated by T2, T3, and COOH. However, p38 was activated by T3 and COOH, but not T2. T3 and COOH-induced VSMC chemotaxis were inhibited by PD98059 or SB202190, but more completely by SB202190. The T2 domain had no effect on VSMC chemotaxis. These results suggest activation of the p38 pathway may be more specific than ERK for COOH- and T3-induced VSMC chemotaxis. J. Cell. Biochem. 89: 500–506, 2003.

Key words: thrombospondin; ERK; p38 kinase; smooth muscle cell; chemotaxis

Vascular smooth muscle cell (VSMC) chemotaxis—a form of directed cell migration along a gradient in response to a soluble chemoattractant—contributes to development of vascular pathologic states, including intimal hyperplasia and atherosclerotic plaque formation. Thrombospondin-1 (TSP-1)—a matricellular glycoprotein chemotactic for VSMCs—is not integral to arterial wall structure, but is expressed locally after acute arterial injury, in atherosclerotic plaque, and early intimal hyperplasia [Miano et al., 1993; Yabkowitz et al., 1993; Reed et al., 1995; Roth et al., 1998]. "Matricellular refers to a group of modular, matrix proteins that do not contribute to structural integrity, but whose functions are accomplished by binding to matrix proteins, cell-surface receptors, cytokines, and proteases, all of which interact with the cell surface to exert different effects" [Bornstein, 1995].

The structure of TSP-1, 420 kDa in size, consists of the following six domains: amino terminal or heparin binding (NH2), procollagen homology, properdin or type 1 repeat, type 2 repeat (T2), type 3 repeat (T3), and carboxyl terminal (COOH). Several receptors also have

Grant sponsor: American Heart Association; Grant number: 9951011T; Grant sponsor: VA Merit Review Entry Program; Grant sponsor: Heart, Lung, and Blood Institute of the NIH; Grant number: HL68003.

*Correspondence to: Vivian Gahtan, MD, Yale University School of Medicine, Section of Vascular Surgery, PO Box 208062, FMB 137, New Haven, CT 06520.
E-mail: vivian.gahtan@yale.edu

Received 10 January 2003; Accepted 20 February 2003
DOI 10.1002/jcb.10524

been identified and interacted with domains or peptide sequences from TSP-1. An RGD sequence in the T3 binds to the integrin $\alpha_v\beta_3$ [Lawler et al., 1988]. Two sequences from the carboxyl-terminal domain that contain a VVM motif bind CD47 and regulate activity of the integrins αIIbβ3, αvβ3, and α2β1 in specific cell types [Gao et al., 1996; Chung et al., 1997; Wang and Frazier, 1998]. Differential expression of the receptors for these domains and the varying accessibility of these domains within the intact TSP-1 molecule are potential mechanisms for regulating the effects of TSP-1 on cell behavior. Responses of cells to TSP-1 include adhesion, spreading, chemotaxis, and haptotaxis through cooperative interactions with multiple domains [Roberts et al., 1987]. The focus of this research is on the mechanism of TSP-1-induced VSMC chemotaxis. A fusion protein synthesized to the COOH domain has been determined to be the predominant domain that induces chemotaxis [Nesselroth et al., 2001]. The NH2 and T3 domains also independently induce VSMC chemotaxis.

TSP-1 has been reported to induce VSMC chemotaxis through extracellular signal-regulated protein kinases-1 and -2 (ERK) [Gahtan et al., 1999] and p38 kinase (p38) [Wang et al., 1999]. Whether the effect of the T3 and COOH domains on VSMC chemotaxis is through ERK, p38 activation, or both was examined, based on the observation that specific domains of TSP-1 independently induce chemotaxis.

METHODS

VSMC Culture

Bovine aortic VSMCs were isolated and seeded in a culture medium containing serum free medium (SFM, Dulbecco's modified Eagle's medium, Gibco-BRL, Gaithersburg, MD), 10% fetal bovine serum (FBS), and antibiotics ($1\times$ penicillin G sodium, streptomycin sulfate, and amphotericin B, Gibco-BRL) in primary culture [Gahtan et al., 1999]. Subconfluent monolayers of VSMCs in early passage (2–5) were used for each experiment.

Chemoattractants and Inhibitors

Purified TSP-1 from human platelets and fusion proteins to T2, T3, and COOH domains were prepared as described previously [Legrand et al., 1992; Adams and Lawler, 1994]. The cDNAs for the type two repeats (amino acids 559 to 669), the type three repeats (amino acids 674 to 925), and the C-terminal domain (amino acids 926 to 1152) of TSP-1 were prepared by PCR. The sequence of the PCR products was verified and they were cloned into pGEX vectors (Amersham Pharmacia Biotech, Piscataway, NJ). All sequencing was done by the chain termination method of Sanger [Sanger et al., 1977]. The fusion proteins were expressed and purified using glutathione-sepharose following protocols that were provided by the manufacturer. The concentration of the TSP-1, T2, T3, and COOH proteins was 20 μg/ml. This concentration was chosen, because peak chemotaxis occurred by this concentration [Gahtan et al., 1999; Wang et al., 1999].

PD98059 and SB202190 were purchased (Calbiochem, La Jolla, CA). The concentration, 10 μM, was used for both inhibitors as this concentration has been previously established for TSP-1 [Gahtan et al., 1999; Wang et al., 1999]. The concentration 10 μM was used because it induced inhibition without causing toxic or nonspecific effects of the inhibitors.

Western Blot Analysis

VSMCs were exposed to SFM, T2 (control domain not associated with chemotaxis), T3, or COOH for 0, 15, 30, 60, and 120 min (20 μg/ml) and TSP-1 (20 μg/ml, positive control) for 30 min, using similar methods described previously [Gahtan et al., 1999]. VSMCs were washed twice with ice-cold phosphate-buffered saline solution and scraped in lysis buffer containing 50 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 10% glycerol, 1% Triton X-100, 1.5 mmol/L $MgCl_2$, 1 mmol/L EGTA, 100 mmol/L sodium fluoride, 10 mmol/L sodium orthovanadate, 10 μg/mL leupeptin, and 1 mmol/L phenylmethylsulfonylfluoride. Western blot analysis was performed by SDS–PAGE (10% polyacrylamide) and transferred to a nitrocellulose membrane (Amersham, Arlington Heights, IL). Blocking was performed in a 5% non-fat milk-tris-buffer saline tween-20 (TBST) solution. Blots were incubated in a 5% non-fat milk-TBST solution with anti-p-ERK 1/2 (Cell Signaling, Beverly, MA), anti-p-p38 (Cell Signaling), and a secondary antibody tagged with HRP (Cell Signaling). The fold inductions were normalized by reprobing for total ERK 1/2 or total p38 (Cell Signaling) after stripping its respective membrane. Immunoreactivity was

determined by means of enhanced chemiluminescence (Amersham) and quantified with an UN-SCAN-IT densitometer (Silk Scientific, Orem, UT). To verify that the inhibitors used in the chemotaxis assay were specific to ERK (PD98059) and p38 (SB202190), quiescent cells were exposed to either inhibitor for 30 min and then exposed to T3 or COOH (20 μg/ml) for 30 min and then processed as described above.

Chemotaxis Assay

A 48-well modified Boyden microchemotaxis chamber (NeuroProbe, Inc., Gaithersburg, MD) was used for chemotaxis assays [Nesselroth et al., 2001]. In the lower chamber, 30 μl of one of the following chemoattractants were placed: T2, T3, COOH domain, GST (fusion protein control), or TSP-1 (20 μg/ml). A poretics membrane (8-μm pores) was applied, followed by the gasket and upper chamber. VSMCs (50,000/well) after 30 min exposure to SFM, PD98059 (ERK inhibitor by direct inhibition of MEK1, 10 μM), SB202190 (p38 inhibitor, 10 μM), or DMSO (vehicle) were placed into the top wells. Assays were conducted for 4 h at 37°C. The membrane was then removed from the chamber, fixed in 70% ethyl alcohol, stained with hematoxylin, and mounted on a slide. Results were recorded as the total number of cells migrated to the undersurface of the membrane for five high-power fields (400×) per well.

Statistical Analysis

For the activation studies, differences in densitometry data were assessed by paired *t*-test. For the chemotaxis experiments, the number of migrated VSMCs per five fields under high power (400×) per well was calculated for each experimental group. Each experiment was performed in triplicate. The data were analyzed by paired *t*-test. A P value less than 0.05 was considered significant.

RESULTS

ERK and p38 Activation Studies

The T3 domain induced ERK activation by 15 min [ERK1, 10.62 ± 3.79 (fold increase compared to SFM, mean ± SEM), $P = 0.042$; ERK2, 4.5 ± 1.4, $P = 0.04$], to a level similar to that seen after a 30-min exposure to TSP-1 (Fig. 1A). After 30 min, ERK activation declined. Peak activation of p38 was seen at 30 min of treatment with T3 (3.25 ± 0.42, $P = 0.006$; Fig. 1B).

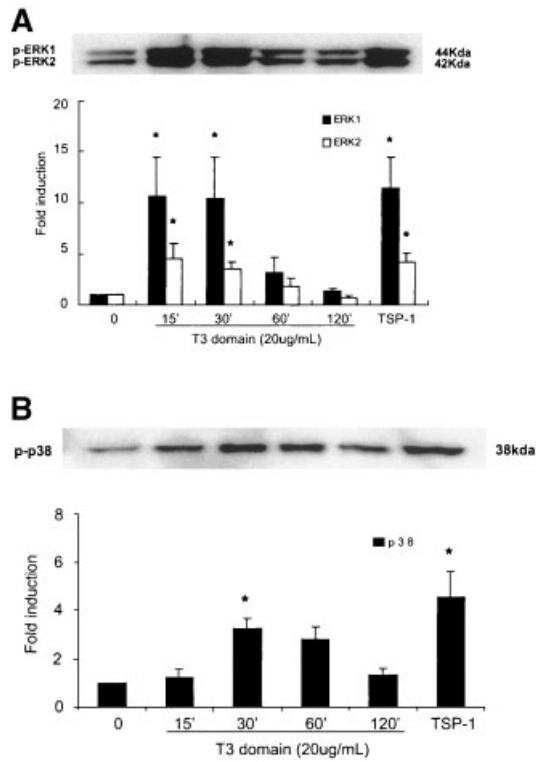

Fig. 1. Time course of ERK and p38 activation after T3 exposure. Total cell lysates were analyzed by Western blotting by use of antibodies against phospho ERK1/ERK2 or p38. Representative Western blots and a summary of the densitometry data for three separate experiments are shown. **A**: Activation of ERK is seen at 15 and 30 min of exposure; (**B**) peak activation of p38 is seen at 30 min. *$P < 0.05$.

COOH induced activation of ERK occurred after 15 min of exposure (ERK1, 8.87 ± 1.08, $P = 0.009$; ERK2, 10.61 ± 1.5, $P = 0.028$ Fig. 2A). Activation continued at 30 min, and declined thereafter with activation still present at 60 and 120 min for ERK1. Activation of p38 occurred by 15 min of COOH exposure and was sustained during subsequent intervals examined (30, 60, and 120 min; at 60 min, 6.25 ± 0.73, $P = 0.0007$; Fig. 2B). PD98059 inhibited ERK activation and SB202190 inhibited p38 activation of COOH (Fig. 2C).

The T2 domain induced ERK activation by 15 min (10.9 ± 4.6, $P = 0.034$; Fig. 3A). Activation declined thereafter and was not significant at the other time points studied (30, 60, and 120 min). No activation of p38 by T2 was noted (at 15 min, 1.41 ± 0.36, $P = 0.17$; Fig. 3B). Isolated GST protein had no effect on ERK or p38 activation (data not shown).

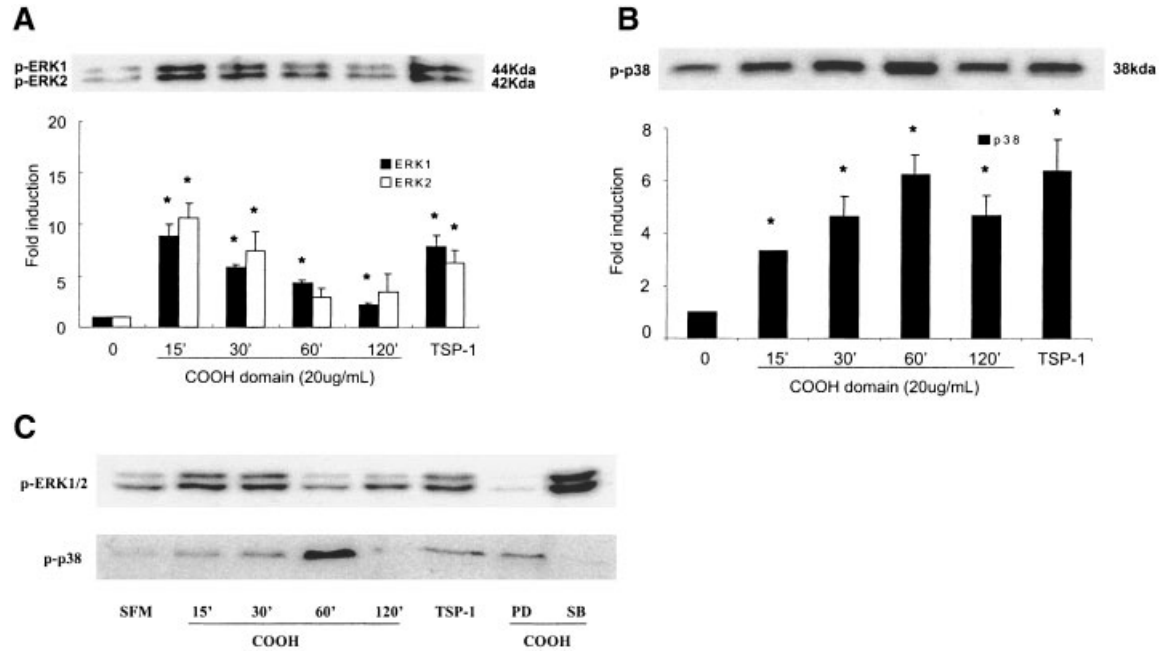

Fig. 2. Time course of ERK and p38 activation after COOH exposure. Total cell lysates were analyzed by Western blotting by use of antibodies against phospho ERK1/ERK2 or p38. Representative Western blots and a summary of the densitometry data are shown (n = 3). **A**: Peak activation of ERK is seen by 15 min of exposure; (**B**) peak activation of p38 is seen by 60 min. **C**: The inhibitors PD98059 and SB202190 were confirmed to be specific for ERK and p38, respectively. PD98059, but not SB202190, inhibited COOH-induced ERK activation. Similarly, SB202190, but not PD98059, inhibited COOH-induced p38 activation. $^*P < 0.05$.

T3- and COOH-Induced VSMC Chemotaxis Is Functionally Dependent on ERK and p38

A significant increase in VSMC chemotaxis was observed in response to T3 (106 ± 4.6, $P = 0.004$), COOH (146 ± 3.7, $P = 0.002$), or TSP-1 (162 ± 4.2, $P = 0.0015$), in comparison to SFM (21.3 ± 1.8; Fig. 4A). Both PD98059 and SB202190 independently inhibited T3-, COOH-, and TSP-1-induced VSMC chemotaxis (T3, $P = 0.01$ and 0.002; COOH, $P = 0.0004$ and 0.0003; TSP-1, $P = 0.00005$ and 0.00004). SB202190 inhibited chemotaxis more than PD98059 for each chemoattractant studied (T3, $P = 0.005$; COOH, $P = 0.021$; TSP-1, $P = 0.014$). When making comparisons to the negative control, chemotaxis was still significant in the PD98059 treated groups (T3, $P = 0.008$; COOH, $P = 0.005$; TSP-1, $P = 0.002$); however, chemotaxis was abolished by SB202190 (T3, $P = 0.11$; COOH, $P = 0.17$; TSP-1, $P = 0.053$). The degree of chemotaxis was less for T3 than COOH ($P = 0.029$). Similarly, the degree of chemotaxis was less for COOH than for TSP-1 ($P = 0.042$). DMSO had no intrinsic effect on T3 and COOH-induced VSMC chemotaxis. T2 ($P = 0.097$) and isolated GST protein did not induce chemotaxis (Fig. 4B). Morphologically, neither the inhibitors nor GST induced any obvious toxic effects in the cells.

DISCUSSION

TSP-1 is a matricellular protein because it has the ability to bind both extracellular matrix proteins and cellular receptors, and the many effects of this molecule are the result of these bindings. Multiple, often contradictory, functions have been attributed to TSP-1, varying by both cell type and species. This variability is thought to be due to differences in the extracellular milieu and cell-surface receptors present in various different cell types [Murphy-Ullrich et al., 1993; Hogg, 1994; Gao et al., 1996; Qian et al., 1997]. For human [Patel et al., 1997] and bovine [Nesselroth et al., 2001] VSMCs, TSP-1 has been observed to induce VSMC chemotaxis, relevant to vessel remodeling after acute arterial injury and in the subsequent development of atherosclerotic plaque and intimal hyperplasia.

The cell-signal-transduction events by which TSP-1 induces VSMC chemotaxis have not been well characterized. A functional role for

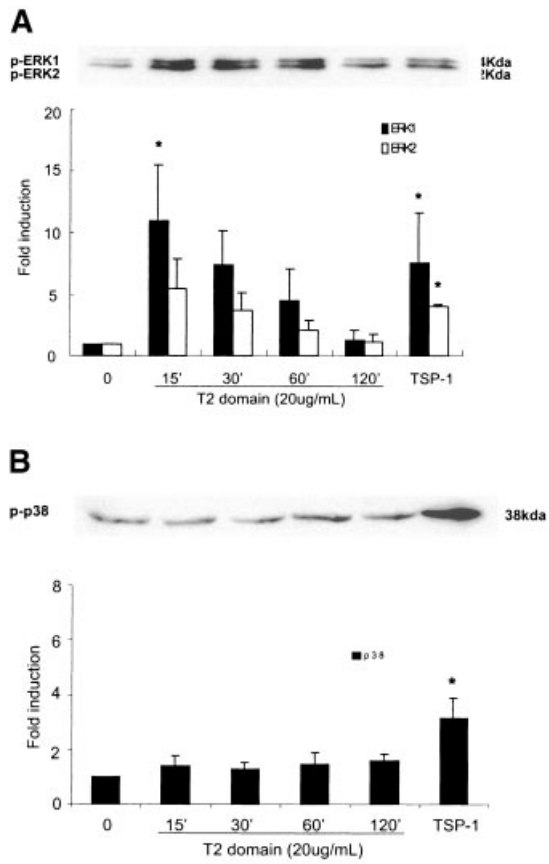

Fig. 3. Time course of ERK and p38 activation after T2 exposure. Total cell lysates were analyzed by Western blotting by use of antibodies against phospho ERK1/ERK2 or p38. Representative Western blots and a summary of the densitometry data are shown (n = 3). **A**: Peak activation of ERK is seen after 15 min of exposure; (**B**) T2 does not induce p38 activation. $^*P < 0.05$.

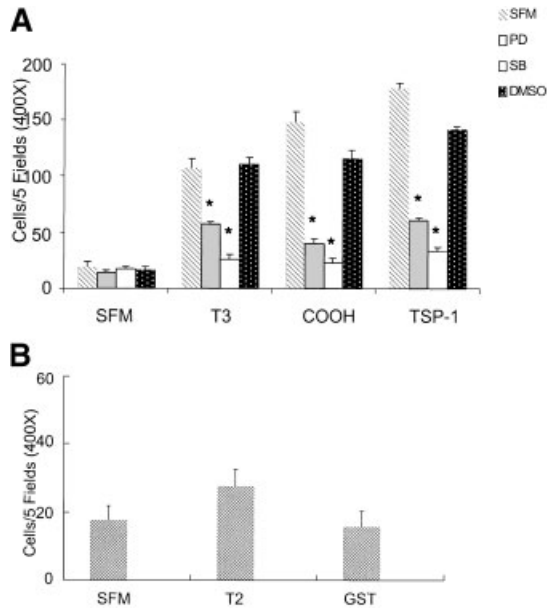

Fig. 4. **A**: T3- and COOH-induced VSMC chemotaxis is dependent on ERK and p38. A significant increase in VSMC chemotaxis was observed in response to T3 and COOH versus SFM. Preincubation of VSMCs with PD98059 (10 μM) or SB202190 (10 μM) inhibited T3- and COOH-induced chemotaxis. $^*P < 0.05$ (n = 3); (**B**) T2 domain did not induce VSMC chemotaxis. No difference in VSMC chemotaxis was observed in response to the T2 domain versus SFM and the GST control fusion protein.

TSP-1-induced VSMC chemotaxis was demonstrated by a number of different integrally related cell signaling pathways, including protein tyrosine kinases, focal adhesion kinase, ERK, p38, p21Ras, phosphatidylinositol 3-kinase, and protein kinase C [Patel et al., 1997; Wang et al., 1999; Willis et al., 1999, 2000]. One set of pathways focused on in the study of TSP-1-induced VSMC chemotaxis, are the mitogen-activated protein kinase (MAPK) pathways. MAPK, a family of serine/threonine protein kinases, consists of three main pathways: ERK, p38, and c-jun-N-terminal kinase (JNK) [Robinson and Cobb, 1997]. The exact mechanism by which these pathways affect cell chemotaxis is not fully understood. Actin, myosin, and microtubules are involved in cytoskeleton remodeling and cell migration. Although less is known about remodeling of smooth muscle cell myosin and actin, other studies showed that myosin light chain kinase, which phosphorylates and reorganizes cytoskeletal components that facilitate cell movement, also is a substrate for MAPK [Klemke et al., 1997].

Both ERK and p38 have been associated with VSMC migration, as well as cell proliferation, apoptosis, differentiation, cytoskeletal remodeling, and cell cycle function [Bennett and Tonks, 1997; Graf et al., 1997; Hedges et al., 1999]. Since specific inhibitors of ERK (PD98059) and p38 (SB202190) cascades were first described, they have been widely used to investigate their involvement in intracellular signal transduction pathways. PD98059 is a specific inhibitor of the mammalian MEK1/2, which is just upstream of ERK [Dudley et al., 1995]. SB202190 selectively inhibits p38 α-, β-isoforms, but not γ-, δ-isoforms [Kumar et al., 1997]. The authors showed previously that TSP-1-activated ERK, and PD98059 inhibited TSP-1-induced VSMC chemotaxis [Gahtan et al., 1999]. Similarly, p38 was activated in TSP-1-stimulated VSMCs, and the p38 inhibitor, SB202190, inhibited

TSP-1-induced VSMC chemotaxis [Wang et al., 1999]. These inhibitors were specific in their ability to selectively block ERK or p38 as demonstrated in our control experiments for ERK and p38 activation (Fig. 2C).

Through the use of fusion proteins to each of the domains of TSP-1, we have shown that T3 and COOH domains are involved in VSMC chemotaxis [Nesselroth et al., 2001]. The COOH domain contains a binding site for the integrin-associated protein (CD47) that is associated with cell attachment, platelet aggregation, cell migration, and VSMC chemotaxis [Klemke et al., 1997]. One previous study shows that a peptide agonist to CD47, 4N1K, independently induced VSMC chemotaxis [Guo et al., 1998]. Consistent with this, we noted that anti-CD47 antibodies inhibited TSP-1-induced VSMC chemotaxis (unpublished data). The authors demonstrated previously that a fusion protein to the T3 domain also independently induced VSMC chemotaxis [Nesselroth et al., 2001]. In addition, TSP-1-induced VSMC chemotaxis can be blocked by RGD (the major binding site for T3)-containing peptides [Patel et al., 1997]. The activity of the RGD containing peptide implies involvement of the $\alpha_v\beta_3$ integrin, which is associated with development of arterial lesions, including intimal hyperplasia. Since $\alpha_v\beta_3$ also associates with CD47, it is possible with $\alpha_v\beta_3$ concomitantly binds to the RGD site on the T3 domain and the CD47 on the COOH domain to stimulate VSMC chemotaxis.

Since the COOH and T3 domains are the primary sites mediating induction of TSP-1-induced VSMC chemotaxis [Nesselroth et al., 2001], we sought to determine whether the COOH and T3 domains independently activated the same cell-signaling pathways as the whole TSP-1 protein. The findings of the current study confirmed that COOH and T3 domains independently activated ERK and p38 in VSMCs. Complete inhibition of either p38 or ERK was able to inhibit VSMC chemotaxis. These findings suggest when complete inhibition of one pathway is present, activation of the other pathway may not be sufficient to induce chemotaxis, or that cross-talk between ERK and p38 is essential. Previous studies have addressed cross-talk between ERK and p38 pathways in a specific and nonspecific manner [Singh et al., 1999; Birkenkamp et al., 2000]. The findings of this study suggest that p38 may be more specific for chemotaxis, as there was p38 activation by COOH and T3, but not T2. In contrast, each of the three fusion proteins induced a similar degree of ERK activation. In addition, a more sustained degree of p38 activation by COOH in comparison to T3 occurred (see Figs. 1B and 2B). This observation also correlated closely with VSMC chemotaxis, in which chemotaxis was abolished after SB202190 exposure (see Fig. 4A). After inhibition with PD98059, a small degree of VSMC chemotaxis remained, which was significant.

The N-terminal domain of TSP-1 is essential to support attachment of various cultured cells including VSMCs and endothelial cells [Asch et al., 1991]. Others reported that the N-terminal A2.5 binding domain of TSP contains an important binding site for sulfated glycoconjugates and appears to have an important role in tumor cell chemotaxis [Taraboletti et al., 1987]. Our previous study showed that the NH2 domain also is involved in VSMC chemotaxis, but the degree of chemotaxis is much less than that observed for COOH [Nesselroth et al., 2001]. The cell signaling pathways involved in the induction of chemotaxis by NH2 is an area for future study.

In conclusion, this study indicates activation of the ERK and p38 pathways by T3, COOH, or both, and regulates VSMC chemotaxis. Our findings further suggest activation of the p38 pathway may be more specific than ERK for COOH- and T3-induced VSMC chemotaxis. Selective inhibition of T3 or the COOH domain of TSP-1 may be a helpful adjunct in prevention of VSMC chemotaxis, a process associated with development of arterial lesions.

REFERENCES

Adams JC, Lawler J. 1994. Cell-type specific adhesive interactions of skeletal myoblasts with thrombospondin-1. Mol Biol Cell 5:423–437.

Asch AS, Tepler J, Silbiger S, Nachman RL. 1991. Cellular attachment to thrombospondin. Cooperative interactions between receptor systems. J Biol Chem 266:1740–1745.

Bennett AM, Tonks NK. 1997. Regulation of distinct stages of skeletal muscle differentiation by mitogen-activated protein kinases. Science 278:1288–1291.

Birkenkamp KU, Tuyt LM, Lummen C, Wierenga AT, Kruijer W, Vellenga E. 2000. The p38 MAP kinase inhibitor SB203580 enhances nuclear factor-kappa B transcriptional activity by a non-specific effect upon the ERK pathway. Br J Pharmacol 131:99–107.

Bornstein P. 1995. Diversity of function is inherent in matricellular proteins: An appraisal of thrombospondin 1. J Cell Biol 130:503–506.

Chung J, Gao AG, Frazier WA. 1997. Thrombspondin acts via integrin-associated protein to activate the platelet integrin alphaIIbbeta3. J Biol Chem 272:14740–14746.

Dudley DT, Pang L, Decker SJ, Bridges AJ, Saltiel AR. 1995. A synthetic inhibitor of the mitogen-activated protein kinase cascade. Proc Natl Acad Sci USA 92: 7686–7689.

Gahtan V, Wang XJ, Willis AI, Tuszynski GP, Sumpio BE. 1999. Thrombospondin-1 regulation of smooth muscle cell chemotaxis is extracellular signal-regulated protein kinases 1/2 dependent. Surgery 126:203–207.

Gao AG, Lindberg FP, Dimitry JM, Brown EJ, Frazier WA. 1996. Thrombospondin modulates alpha v beta 3 function through integrin-associated protein. J Cell Biol 135:533–544.

Graf K, Xi XP, Yang D, Fleck E, Hsueh WA, Law RE. 1997. Mitogen-activated protein kinase activation is involved in platelet-derived growth factor-directed migration by vascular smooth muscle cells. Hypertension 29:334–339.

Guo N, Zabrenetzky VS, Chandrasekaran L, Sipes JM, Lawler J, Krutzsch HC, Roberts DD. 1998. Differential roles of protein kinase C and pertussis toxin-sensitive G-binding proteins in modulation of melanoma cell proliferation and motility by thrombospondin 1. Cancer Res 58:3154–3162.

Hedges JC, Dechert MA, Yamboliev IA, Martin JL, Hickey E, Weber LA, Gerthoffer WT. 1999. A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. J Biol Chem 274:24211–24219.

Hogg PJ. 1994. Thrombospondin 1 as an enzyme inhibitor. Thromb Haemost 72:787–792.

Klemke RL, Cai S, Giannini AL, Gallagher PJ, de Lanerolle P, Cheresh DA. 1997. Regulation of cell motility by mitogen-activated protein kinase. J Cell Biol 137:481–492.

Kumar S, McDonnell PC, Gum RJ, Hand AT, Lee JC, Young PR. 1997. Novel homologues of CSBP/p38 MAP kinase: Activation, substrate specificity and sensitivity to inhibition by pyridinyl imidazoles. Biochem Biophys Res Commun 235:533–538.

Lawler J, Weinstein R, Hynes RO. 1988. Cell attachment to thrombospondin: The role of ARG-GLY-ASP, calcium, and integrin receptors. J Cell Biol 107:2351–2361.

Legrand C, Thibert V, Dubernard V, Begault B, Lawler J. 1992. Molecular requirements for the interaction of thrombospondin with thrombin-activated human platelets: Modulation of platelet aggregation. Blood 79:1995–2003.

Miano JM, Vlasic N, Tota RR, Stemerman MB. 1993. Smooth muscle cell immediate-early gene and growth factor activation follows vascular injury. A putative in vivo mechanism for autocrine growth. Arterioscler Thromb 13:211–219.

Murphy-Ullrich JE, Gurusiddappa S, Frazier WA, Hook M. 1993. Heparin-binding peptides from thrombospondins 1 and 2 contain focal adhesion-labilizing activity. J Biol Chem 268:26784–26789.

Nesselroth SM, Willis AI, Fuse S, Olson ET, Lawler J, Sumpio BE, Gahtan V. 2001. The C-terminal domain of thrombospondin-1 induces vascular smooth muscle cell chemotaxis. J Vasc Surg 33:595–600.

Patel MK, Lymn JS, Clunn GF, Hughes AD. 1997. Thrombospondin-1 is a potent mitogen and chemoattractant for human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 17:2107–2114.

Qian X, Wang TN, Rothman VL, Nicosia RF, Tuszynski GP. 1997. Thrombospondin-1 modulates angiogenesis in vitro by up-regulation of matrix metalloproteinase-9 in endothelial cells. Exp Cell Res 235:403–412.

Reed MJ, Iruela-Arispe L, O'Brien ER, Truong T, LaBell T, Bornstein P, Sage EH. 1995. Expression of thrombospondins by endothelial cells. Injury is correlated with TSP-1. Am J Pathol 147:1068–1080.

Roberts DD, Sherwood JA, Ginsburg V. 1987. Platelet thrombospondin mediates attachment and spreading of human melanoma cells. J Cell Biol 104:131–139.

Robinson MJ, Cobb MH. 1997. Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 9:180–186.

Roth JJ, Gahtan V, Brown JL, Gerhard C, Swami VK, Rothman VL, Tulenko TN, Tuszynski GP. 1998. Thrombospondin-1 is elevated with both intimal hyperplasia and hypercholesterolemia. J Surg Res 74:11–16.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467.

Singh RP, Dhawan P, Golden C, Kapoor GS, Mehta KD. 1999. One-way cross-talk between p38(MAPK) and p42/44(MAPK). Inhibition of p38(MAPK) induces low density lipoprotein receptor expression through activation of the p42/44(MAPK) cascade. J Biol Chem 274:19593–19600.

Taraboletti G, Roberts DD, Liotta LA. 1987. Thrombospondin-induced tumor cell migration: Haptotaxis and chemotaxis are mediated by different molecular domains. J Cell Biol 105:2409–2415.

Wang XQ, Frazier WA. 1998. The thrombospondin receptor CD47 (IAP) modulates and associates with alpha2 beta1 integrin in vascular smooth muscle cells. Mol Biol Cell 9:865–874.

Wang XJ, Willis AI, Kito H, Tuszynski GP, Sumpio BE, Gahtan V. 1999. Thrombosondin-1 induces p38 activation in vascular smooth muscle cells. FASEB J J13:A436.

Willis AI, Wang XJ, Kito H, Tuszynski GP, Sumpio BE, Gahtan V. 1999. Ras N17 transfection inhibits TSP-1-induced VSMC migration. Surg Forum L:454–455.

Willis AI, Fuse S, Wang XJ, Chen E, Tuszynski GP, Sumpio BE, Gahtan V. 2000. Inhibition of phosphatidylinositol 3-kinase and protein kinase C attenuates extracellular matrix protein-induced vascular smooth muscle cell chemotaxis. J Vasc Surg 31:1160–1167.

Yabkowitz R, Mansfield PJ, Ryan US, Suchard SJ. 1993. Thrombospondin mediates migration and potentiates platelet-derived growth factor-dependent migration of calf pulmonary artery smooth muscle cells. J Cell Physiol 157:24–32.