Thrombospondin-1 antagonizes nitric oxide-stimulated vascular smooth muscle cell responses

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Abstract

Objective: Endothelial-derived nitric oxide (NO), by increasing cGMP, is a major physiological regulator of vascular tone and of vascular smooth muscle cell (VSMC) adhesion, chemotaxis, and proliferation. Thrombospondin-1 is a potent antagonist of NO/cGMP signaling in endothelial cells. Because endothelial and VSMC typically exhibit opposing responses to thrombospondin-1, we examined thrombospondin-1 effects on NO signaling in VSMC.

Methods: Effects of exogenous thrombospondin-1 on human VSMC adhesion, chemotaxis, proliferation, and cGMP signaling were examined. Endogenous thrombospondin-1 function was characterized by comparing NO signaling in VSMC from wild type and thrombospondin-1 null mice.

Results: Picomolar concentrations of exogenous thrombospondin-1 prevented adhesive, chemotactic, and proliferative responses of human aortic VSMC stimulated by low dose NO. A recombinant CD36-binding domain of thrombospondin-1 or antibody ligation of CD36 similarly inhibited NO-stimulated VSMC responses. Thrombospondin-1 and CD36 ligation inhibited NO responses in VSMC by preventing cGMP accumulation. Thrombospondin-1 null VSMC responses to NO and cGMP signaling were enhanced relative to wild type murine VSMC.

Conclusions: In the presence of NO, thrombospondin-1 is converted from a weak stimulator to a potent inhibitor of VSMC responses. Both exogenous and endogenous thrombospondin-1 inhibit NO signaling in VSMC. This activity is mediated by the type 1 repeats and utilizes the same CD36-dependent cGMP signaling pathway in endothelial and VSMC.

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1. Introduction

VSMC play central roles in the physiological regulation of blood flow and the pathogenesis of cardiovascular disease and tumor angiogenesis. Vascular smooth muscle tone is regulated by innervation, circulating endocrine factors, and paracrine factors released by the adjacent vascular endothelium including the vaso-

dilator NO [1,2]. In addition to this acute regulation, changes in the composition of the extracellular matrix surrounding VSMC have been observed in chronic cardiovascular disease [3,4]. Although such changes in matrix composition are generally recognized to influence the biomechanical properties of the vessel wall, recent studies have extended the functions of matrix proteins to regulation of VSMC growth, gene expression, and function [3].

Thrombospondin-1 (TSP1) is an extracellular modulator of angiogenesis, inflammatory responses, tumorigenesis, wound healing, and platelet function [5,6]. TSP1 is normally present at low levels in vessel extracellular matrix, but its expression is elevated in the extracellular matrix associated with atherosclerotic lesions [7] and in adventitia of blood vessels from diabetic rats [8]. A polymorphism

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in TSP1 that influences its conformation has also been associated with increased risk of premature coronary artery disease [9]. TSP1 interacts with at least eight recognized cell surface receptors and with several extracellular matrix components, proteases, and growth factors [5,10]. The biological activities of TSP1 depend on the specific molecular context in which it is presented in the matrix and the relative expression and activity of each TSP1 receptor expressed on target cells [11]. Interactions of TSP1 with different receptors on endothelial and VSMC can either stimulate or inhibit their survival, function, and growth [10,12–14]. Under most conditions and at physiological concentrations, TSP1 is a net inhibitor of endothelial cell migration and proliferation [12,15] but stimulates the same responses in VSMC [13,14,16–18].

TSP1 was initially described to be a required positive regulator for VSMC growth based on the ability of specific TSP1 antibodies to inhibit proliferation [16]. Although others have replicated this result and shown specific synergism with platelet derived growth factor (PDGF) [18], we reported that VSMC isolated from TSP1 null mice proliferate normally in medium lacking TSP1 [14]. This and the relatively normal vasculature of TSP1 null mice argued that TSP1 is not essential for VSMC growth. However, we did confirm specific defects in proliferative and chemotaxis responses to PDGF in TSP1 null cells. Activation of metalloproteinases and latent TGFβ by TSP1 can also modulate VSMC responses [19,20]. Additional studies have implicated α3β1 integrin [13,14], αvβ3 [21], CD36 [14], and CD47 [22] as SMC TSP1 receptors mediating its proliferative

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**Fig. 1.** NO is a sGC-dependent stimulator of VSMC adhesion, chemotaxis and proliferation. Attachment of HAVSMC (2 × 10^5/mL) was determined on dishes coated with type I collagen (3 μg/mL) after incubating for 1 h in the presence of the indicated concentrations of DEA/NO (A) or DEA/NO ± the sGC inhibitor ODQ (10 μmol/L) (B), cells were fixed, stained, and counted microscopically. Chemotaxis was assessed in modified Boyden chambers (C–D). VSMC (1 × 10^4/well) were added to the upper chamber and allowed to migrate for 5.5 h at 37 °C to DEA/NO at the indicated concentrations of serum (control) in the lower chamber (C) or 10 μmol/L DETA/NO ± ODQ (10 μmol/L) (D). Proliferation was quantified using a tetrazolium reduction assay (E). VSMC (5 × 10^4/well) were plated in 96-well culture plates with medium containing 2% FCS and the indicated concentrations of NO donor ± ODQ (10 μmol/L). Net proliferation was determined by subtracting the initial cell signal and normalized to the net 72 h signal without NO donor (100%). Under similar conditions, the effect of DETA/NO on VSMC DNA synthesis was measured by immunoassay for bromodeoxyuridine incorporation (F). *P<0.05 vs. control, one-way ANOVA.
activity. TSP1 also promotes chemotaxis of SMC, and αvβ3 integrin [13], α3β1[14], α2β1 integrin and CD47 [23] may mediate this activity of TSP1.

Recently, we reported that exogenous and endogenous TSP1 potently inhibit NO-stimulated endothelial cell proliferation, adhesion, and migration [24]. The inhibitory effects of TSP1 were localized to its CD36-binding type 1 repeats and mediated by blocking NO-stimulated accumulation of intracellular cGMP at the level of soluble guanylyl cyclase (sGC).

NO signaling is an important physiological regulator of vascular smooth muscle tone [2] as well as VSMC migration [25,26], adhesion [27], and proliferation [28]. Although TSP1 generally elicits opposing responses in endothelial and smooth muscle cells, we now report that TSP1 inhibits NO-stimulated responses in VSMC in a manner identical to that we described previously in endothelial cells. Furthermore, NO dramatically increases the sensitivity of VSMC to the inhibitory effects of TSP1.

2. Materials and methods

2.1. Cells and reagents

Aortic-derived VSMCs were isolated from wild type (WT) and TSP1 null mice as previously described and cultured in smooth muscle growth medium [14]. Human aortic VSMC

Fig. 2. TSP1 inhibits NO stimulated VSMC responses. HAVSMC (5×10^3/well) were plated in 96-well culture plates with medium containing 2% FCS. Proliferation was determined in the absence (●) or presence of 10 μmol/L DETA/NO (○) and the indicated concentrations of TSP1 (A). Stimulation of proliferation by 10 μmol/L DETA/NO and inhibition by 2.2 nmol/L TSP1 were determined on wells pre-coated with 3 μg/mL of type I collagen, 0.1% gelatin, and on uncoated wells (B). The effect of NO ± TSP1 on VSMC DNA synthesis was measured by immunoassay for bromodeoxyuridine incorporation (C). Chemotaxis was assessed in modified Boyden chambers (D). VSMC (1×10^6/well) were added to the upper chamber and allowed to migrate for 5.5 h. Motility was induced by 100 μmol/L DETA/NO in the lower chamber and assessed at the indicated concentrations of TSP1 added in the upper chamber. The effect of TSP1 at the indicated concentrations on basal (○) or DETA/NO (10 μmol/L, ○) stimulated adhesion of HAVSMC (1×10^6/well) was determined on wells pre-coated with collagen (3 μg/mL, E), fibronectin (3 μg/mL, F), or vitronectin (2 μg/mL, G). *P<0.05 vs. control, **P<0.05 vs. NO alone, two-way ANOVA. **P<0.05 vs. control + NO, one-way ANOVA.
HAVSMC (Cambrex, Walkersville, MD) were maintained in smooth muscle cell growth medium supplemented with the manufacturer's additives (SM-GM, Cambrex) and 2% fetal calf serum (FCS) in 5% CO₂ at 37 °C. Cells were utilized within passages 4–9. Purity of cultures was monitored by lack of immunochemical detection of CD31 and expression of α smooth muscle actin (Sigma, St. Louis, MO). CD36 expression was verified by staining with monoclonal CD36 antibody (clone CRF D-2712, BD Biosciences) [14].

Monomeric type I collagen was obtained from Inamed (Fremont, CA). Oxadiazole [4,3-a]quinoxalin-1-one (ODQ) was obtained from Sigma (St. Louis, MO). Diethyline NONOate (DEA/NO) and diethyltriamine NONOate (DETA/NO) were provided by Joseph Saavedra and Larry Keefer (NIH, Frederick, MD). Recombinant proteins expressed in insect cells containing the N-terminal (NoC1) and type 1 repeat regions of TSP1 (3TSR) were provided by Deane Mosher (University of Wisconsin) and Jack Lawler (Harvard Medical School). Inhibitors of phosphodiesterase-1 (PDE1, 8-methoxymethyl-3-isobutyl-1-methylxanthine), PDE5 (4-(3,4-methylenedioxy)benzylamino-6-methoxyquinazoline), and PDE3 (trequinsin) were obtained from Calbiochem (San Diego, CA). TSP1 was prepared from human platelets obtained from the NIH blood bank as previously described [29]. The CD36 agonist antibody SMΦ was from Chemicon International (Temecula, CA).

2.2. Animals

C57B16 WT and TSP1 null mice [30] were housed in a pathogen free environment. Handling and care of animals were in compliance with the guidelines established by the Animal Care and Use Committee of the National Institutes of Health.

**Fig. 3.** NO-stimulated cGMP accumulation in VSMC is blocked by TSP1. HAVSMC were treated with 10 μmol/L DEA/NO for 5 min after pretreatment for 15 min with TSP1 (0–22 nmol/L), and intracellular cGMP levels were determined by immunoassay (A). **P<0.05 vs. NO alone, one-way ANOVA.** cGMP was quantified in cells pretreated with specific inhibitors of PDE1 (25 μmol/L), PDE3 (2 μmol/L), or PDE5 (1 μmol/L) ± TSP1 (2.2 nM) followed by 5 min treatment with 10 μmol/L DEA/NO (B). **P<0.05 vs. NO alone, **P<0.05 vs. PDE + NO, two-way ANOVA.

**Fig. 4.** Type 1 repeats of TSP1 inhibit VSMC responses to NO. Adhesion of HAVSMC (1 × 10⁴/well) on wells coated with type I collagen (3 μg/mL) was determined after 1 h in the absence (●,▲) or presence of 10 μmol/L DEA/NO (○,△) and the indicated concentrations of recombinant type 1 repeats (3TSR) or amino-terminal domain of TSP1 (NoC1) (A). Results (mean±SD, n=3) are presented as a percent of the NO-stimulated control. Intracellular cGMP levels were determined from cells preincubated 15 min with 1 μmol/L 3TSR and treated for 5 min with 10 μmol/L DEA/NO (B). Mean cGMP levels from duplicate determinations are presented from at least three independent experiments. **P<0.05 vs. control with NO, two-way ANOVA. **P<0.05 vs. NO alone, one-way ANOVA.
2.3. Cell proliferation

Proliferation of VSMCs was measured with a non-radioactive colorimetric assay (CellTiter 96, Promega, Madison, WI) using uncoated 96-well culture plates (Nunc, Denmark) or Nunc Maxisorp wells pre-coated with monomeric type I collagen (3 μg/mL), or 0.1% gelatin. Cells (5 x 10^3/well) in 100 μl of medium containing 2% FCS were incubated at 37 °C in a 5% CO2 for 72 h. Proliferation was assessed per the manufacturer’s instructions. Appropriate zero time controls were run for all assays and subtracted from the signal at 72 h. DNA synthesis was quantified by enzyme immunoassay measurement of bromodeoxyuridine incorporation (Calbiochem, San Diego, CA).

2.4. Cell adhesion assay

Cell adhesion was measured in 96-well Nunc Maxisorp plates pre-coated with 3 μg/mL of monomeric type I collagen or fibronectin or 2 μg/ml vitronectin. Harvested cells were plated at 1 x 10^4/well in medium plus 0.1% BSA and treatment agents and incubated in 5% CO2 for 1 h. Wells were washed with PBS, and the adherent cells were fixed with 1% glutaraldehyde for 10 min, washed and stained with 1% crystal violet for 20 min. After washing, the dye was solubilized with 10% acetic acid and quantified at 570 nm. Alternatively, cell attachment and spreading on type I collagen (3 μg/mL) adsorbed onto bacteriological dishes after 1 h at 37 °C (Falcon, Becton-Dickinson, Franklin Lakes, NJ) were determined microscopically as described previously [31].

2.5. Chemotaxis

Modified Boyden chambers and 8-μm pore polycarbonate membranes (Neuro Probe, Gaithersburg, MD) coated with 100 μg/mL gelatin in 0.1% aqueous acetic acid were used. VSMCs were suspended and allowed to recover for 30 min in complete medium. After centrifugation, the cells were resuspended in assay medium (SM-GM containing 0.1% BSA) and added to the upper chambers (± treatment agents). Chemotactants were added to the lower chambers (± treatment agents). Chemotactic agents were added to the lower chambers in assay medium. Migrated cells after 5.5 h were fixed, stained, and counted microscopically.

2.6. Intracellular cGMP measurement

VSMC (10^4 cells/well) were plated in complete SM-GM containing 2% FCS for 24 h, weaned in 1% FCS in SM-GM without additives for 24 h, and treated with NO donors and other agents in serum free medium (SM-GM+0.1% BSA). Intracellular cGMP levels were determined using an enzyme immunoassay (Amersham Biosciences).

Fig. 5. CD36 ligation is sufficient to inhibit NO signaling in VSMC. Adhesion of HAVSMC was determined in the absence or presence of 10 μmol/L DETA/NO ± a CD36 agonist antibody (clone Smφ) (A). Proliferation of VSMC (5 x 10^3/well) was determined by MTS assay after 72 h incubation ± DETA/NO (10 μmol/L) and CD36 antibody Smφ (B). Results are normalized to initial cell numbers (0%) and net proliferation without NO donor (100%). VSMC chemotaxis was assessed after 5.5 h with medium ± 100 μmol/L DETA/NO added in the lower chambers and ODQ (10 μmol/L) or CD36 antibody Smφ (0.1 μg/mL) (D). Mean cGMP levels from duplicate determinations are presented from at least three experiments. *P<0.05 vs. NO alone (control), two-way ANOVA. **P<0.05 vs. NO alone, one-way ANOVA.
2.7. Statistics

All experiments were replicated at least three times. Results are presented as the mean±SD with analysis of significance done using Student’s t test or one-way or two-way ANOVA with Tukey post hoc test where indicated using Origin software (version 7, OriginLabs Corp., Northampton, MA), with significance taken at P values <0.05.

3. Results

3.1. NO stimulates VSMC responses in a biphasic manner and requires sGC activity

DEA/NO was used to assess acute responses to NO due to its rapid release kinetics (t1/2 = 2–5 min), whereas the slow release donor DETA/NO (t1/2 = 22 h) was used to sustain NO levels for the longer term proliferation and migration assays. The donor concentrations employed produce physiological levels of NO (<1 to 1000 nmol/L) under the conditions used here [32,33]. As in endothelial cells [24], low doses of NO donors (0.1–10 μmol/L) stimulated whereas high doses inhibited HAVSMC adhesion to type I collagen, chemotaxis, and proliferation (Fig. 1A, C, E). The stimulatory effect of low dose NO donors on each response was mediated by sGC, based on the ability of ODQ to abrogate the respective NO-driven responses (Fig. 1B, D, E).

Because NO/cGMP signaling in some cell types induces mitochondrial biogenesis independent of cell proliferation [34], the increased tetrazolium reduction in Fig. 1E could be independent of proliferation. However, bromodeoxyuridine incorporation showed that exogenous NO also exerted a biphasic effect upon HAVSMC DNA synthesis that paralleled the tetrazolium response (Fig. 1F). DETA/NO at 1000 μmol/L completely inhibited DNA synthesis, consistent with the return to basal cell number as assessed by the MTS assay at 1000 μmol/L DETA/NO (Fig. 1E).

3.2. NO-stimulated VSMC responses are potently inhibited by TSP1

As previously reported [14], low doses of exogenous TSP1 in the absence of NO enhanced HAVSMC proliferation but higher doses inhibited it (Fig. 2A). In the presence of low dose NO, however, TSP1 only inhibited proliferation. At or above 0.22 nmol/L TSP1, the net proliferative effect of NO was abolished. Stimulation of HAVSMC proliferation by NO was somewhat substrate dependent. NO increased cell proliferation to a greater degree in cells incubated on native type I collagen or on uncoated plates relative to cells incubated on gelatin (denatured collagen, Fig. 2B). Addition of 2.2 nmol/L TSP1 moderately inhibited basal proliferation on the uncoated substrate but did not significantly alter proliferation on the other substrates. However, NO-stimulated cell proliferation was...
strongly inhibited by the same concentration of TSP1 on all substrates. The anti-proliferative activity of TSP1 was confirmed by examining the effect of TSP1 on NO-stimulated bromodeoxyuridine incorporation (Fig. 2C). TSP1 at ≥22 pmol/L inhibited DNA synthesis induced by NO to below basal levels.

Similarly, TSP1 potently inhibited low dose NO stimulation of VSMC chemotaxis and adhesion (Fig. 2D–G). The anti-adhesive activity was substrate independent, as sub-nanomolar TSP1 comparably inhibited NO-stimulated but not basal cell adhesion on plates coated with native type I collagen, fibronectin or vitronectin (Fig. 2E–G). Adhesion on these substrates is mediated by different integrins, indicating global effects of NO and TSP1 on VSMC adhesion.

3.3. NO-stimulated cGMP accumulation in VSMC is blocked by TSP1

As in endothelial cells, NO stimulated accumulation of intracellular cGMP in HAVSMMC, and TSP1 potently inhibited cGMP levels stimulated by NO (Fig. 3A). Inhibiting each of the known cGMP phosphodiesterases in VSMC (PDE1, PDE3, and PDE5) moderately increased basal VSMC adhesion but did not prevent the activity of exogenous TSP1 to completely inhibit NO-stimulated VSMC adhesion to type I collagen (Fig. 3B). Thus, TSP1 probably regulates synthesis rather than degradation of cGMP.

3.4. Type 1 repeats of TSP1 inhibit NO-stimulated responses in VSMC

Two recombinant regions of TSP1 were used to localize its inhibitory activity for VSMC responses to NO (Fig. 4). As previously shown for endothelial cells [24], NO-stimulated HAVSMMC adhesion to collagen was inhibited significantly by recombinant type 1 repeats of TSP1 (3TSR, Fig. 4A), which contain its CD36 binding site [12]. 3TSR at 100 pmol/L significantly inhibited NO-driven cell adhesion, which is comparable to the activity of native TSP1. In contrast, a recombinant trimeric N-terminal region of TSP1 (NoC1), which contains heparin and several β1 integrin binding sites [10] and a sequence that inhibits endothelial cell adhesion to collagen [35], did not inhibit NO-stimulated adhesion and modestly increased basal adhesion (Fig. 4A). Likewise, NO-stimulated intracellular cGMP accumulation was significantly inhibited by 3TSR (Fig. 4B), whereas NoC1 had no effect (data not shown).

3.5. Ligation of the TSP1 receptor CD36 is sufficient to inhibit NO-stimulated responses in VSMC

CD36 is an important functional receptor for the type 1 repeats of TSP1 and is expressed in the VSMC cultures used here [14]. Ligation of CD36 by an antibody with reported agonist activity (SMΦ [36]) potently inhibited NO-stimulated HAVSMMC adhesion on type I collagen (Fig. 5A), proliferation (Fig. 5B), and cell migration (Fig. 5C). O2D (10 μmol/L) similarly abrogated NO-stimulated cell migration (Fig. 5C). The CD36 antibody SMΦ also inhibited NO-induced cGMP accumulation, indicating that CD36, as well as the domain of TSP1 recognized by this receptor, regulate NO-stimulated cGMP signaling (Fig. 5D). Therefore, ligation of CD36 by the CD36-binding domain of TSP1 or an agonist antibody is sufficient to inhibit NO signaling in VSMC.

3.6. Endogenous TSP1 inhibits VSMC responses to NO and cGMP signaling

To determine whether endogenous TSP1 regulates NO-stimulated responses, we compared aortic VSMC from WT and TSP1−/− mice (Fig. 6). TSP1 null aortic VSMC proliferation was similar to that of WT under basal conditions, but addition of NO significantly stimulated TSP1 null cell proliferation relative to WT (Fig. 6A). The differential proliferative response to NO, though modest, was consistently seen with six independent isolates. The enhanced proliferation of TSP1 null VSMC is TSP1-dependent, because addition of exogenous TSP1 in a dose-dependent manner reversed the enhanced NO-stimulated proliferation of TSP1 null versus WT VSMC cells (Fig. 6B).

TSP1−/− aortic VSMC also exhibited approximately 2-fold higher basal adhesion to type I collagen, and their adhesion was further stimulated by NO (Fig. 6C) relative to WT cells. The lack of endogenous TSP1 also resulted in 2-fold higher basal levels of cGMP in the absence of NO (Fig. 6D) and a significantly greater accumulation of cGMP in the presence of exogenous NO, demonstrating that endogenous TSP1 markedly limits NO-stimulated cGMP signaling in VSMC. Inhibiting all three known cGMP phosphodiesterases using combined PDE1, PDE3, and PDE5 inhibitors somewhat elevated basal cGMP but did not eliminate the differential between TSP1 null and WT aortic VSMC nor their differential responses to exogenous NO (Fig. 6D). These results further indicate that inhibition of NO-stimulated cGMP signaling by endogenous or exogenous TSP1 involves regulation of sGC rather than a cGMP phosphodiesterase.

4. Discussion

The present data show that TSP1 inhibits NO-stimulated VSMC responses with a comparable potency as reported previously for endothelial cells [24]. In both vascular cell types, this inhibition is replicated by the type 1 repeats of TSP1 or ligation of its receptor CD36 [12,36]. Although nanomolar TSP1 inhibits VSMC proliferation in the absence of NO [14], inhibition of NO-stimulated VSMC proliferation, chemotaxis and adhesion by 20–200 pmol/L TSP1 is novel. This high potency suggests that TSP1 physiologically limits NO signaling in VSMC. Indeed, endogenous TSP1 produced by VSMC significantly perturbs both their basal and NO-induced cGMP signaling, and exogenous TSP1 complements this TSP1 null phenotype.

TSP1 can stimulate or inhibit VSMC responses by engaging different TSP1 receptors. TSP1 stimulates VSMC proliferation and chemotaxis via α3β1 integrin [13,14], which recognizes the NoC1 region of TSP1 and moderately enhanced adhesion of unstimulated VSMC in our experiments. The C-terminus of TSP1 stimulates VSMC chemotaxis through α5β3 integrin and CD47 [13,20,37]. Generally, these positive activities of TSP1 require nanomolar concentrations of the protein. Conversely,
recombinant C-terminal domain of TSP1 and a synthetic peptide derived from this region of TSP1 that binds to CD47 inhibit VSMC proliferation at nanomolar to micromolar concentrations [14]. The CD36-dependent activity of the central type 1 repeats of TSP1 to inhibit NO-driven VSMC responses occurs at picomolar concentrations. CD36 is a well known functional TSP1 receptor in endothelial cells [12,36], but previously no function for CD36 could be identified in VSMC [13]. CD36 signaling activates p38 MAP kinase [38], and both sGC and the associated Arf-GTPase activator protein AGAP1 are targets of Src kinase activity [39]. The role of these or other mediators in TSP1 regulation of cGMP remains to be determined.

TSP1 exhibits opposing effects on some endothelial and VSMC responses [12–18], but our results show that NO via cGMP signaling modifies VSMC responses to TSP1 to parallel those of endothelial cells. The stimulatory concentrations of the NO donors utilized in this study generate NO concentrations representative of physiological endothelial NO synthase activity [32,33]. cGMP signaling is a major regulator of VSMC physiology, and endogenous TSP1 signaling may be an internal rheostat upon sGC responses to NO in VSMC. Consistent with this hypothesis, cGMP levels in VSMC from TSP1 null mice were consistently greater under basal conditions. Furthermore, cGMP levels stimulated by exogenous NO were significantly greater in TSP1 null VSMC than in WT cells.

The endogenous levels of TSP1 to which VSMC are exposed are unknown. Reported plasma levels of TSP1 (100–200 pM) are sufficient to inhibit the NO responses reported here and to reverse the enhanced NO-stimulated proliferation of TSP1 null VSMC [40]. TSP1 levels in the extracellular matrix are elevated in atherosclerotic lesions [7] and in adventitia of blood vessels from diabetic rats [8], suggesting that such diseased arteries could be impaired in their pharmacological responses to NO-generating drugs as well as responses to physiological NO signals. Although atherosclerosis initially involves dysfunction in endothelial NO production [41], our results suggest that TSP1-mediated desensitization of VSMC responsiveness to NO could also play a role in this disease.

Some of the TSP1 in vessel matrix may be produced by the VSMC [19]. Furthermore, NO and cGMP signaling can suppress expression of TSP1 in VSMC [42,43]. This parallels the suppression of TSP1 by low dose NO and cGMP signaling in endothelial cells [44] and mesangial cells [45]. Therefore, low dose NO may enhance its own responses in VSMC by limiting endogenous TSP1 expression.

In addition to the loss of TSP1 expression associated with tumor progression, alterations in TSP1 expression have been implicated in a number of vascular diseases. Several of the phenotypes reported are consistent with our results. Under conditions of chronic and progressive soft tissue ischemia as found in diabetic limbs, TSP1 expression is increased and correlates with limb amputation rates [46]. TSP1 expression was also increased in a diabetic rat model, a finding with clinical relevance given the significant association of diabetes with ischemic vascular disease [8,47]. In a model of kidney ischemia/reperfusion injury, the absence of endogenous TSP1 resulted in greater tissue survival [48]. TSP1 is over-expressed in areas of atherosclerotic narrowing of blood vessels [7]. Furthermore, a structural polymorphism in TSP1 increases the risk of premature, familial myocardial infarction [49], but the activity of this TSP1 variant to inhibit NO signaling remains to be determined.

In summary, our results suggest that increased TSP1 expression in certain disease states could limit the sensitivity of the affected vessels to physiological NO signaling and that TSP1 may either stimulate or inhibit proliferation of smooth muscle, depending on NO levels. This should serve as an incentive to further investigate the pathophysiological role of TSP1 in VSMC function. Based on the well known function of NO to regulate vascular smooth muscle tone [50], future studies should also consider the role of TSP1 in regulating NO-dependent vasodilation and blood flow.

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