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Forum Review

The Biphasic Nature of Nitric Oxide Responses in Tumor Biology

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ABSTRACT

The dual or biphasic responses of cancer to nitric oxide (NO) arise from its concentration dependent ability to regulate tumor growth, migration, invasion, survival, angiogenesis, and metastasis. The outcome of these various NO-dependent processes is dictated by several factors including NO flux, the chemical redox environment, and the duration of NO exposure. Further, it was recently discovered that an NO-induced redox flux in vascular endothelial cells hypersensitizes these cells to the antiangiogenic effects of thrombospondin-1. This suggests a novel treatment paradigm for targeting tumor-driven angiogenesis that combines redox modulation with mimetic derivatives of thrombospondin-1. This article discusses the biphasic nature of NO in cancer biology and the implications of NO-driven redox flux for modulation of tumor-stimulated angiogenesis, growth, and metastasis. *Antioxid. Redox Signal.* 8, 1329–1337.

INTRODUCTION

The field of nitric oxide study has emerged as one of the more prolific areas in biomedical science, as it affects most every aspect of physiology and the medical sciences. Unlike those of bioeffectors whose actions are often based on specific structure–function relations, the biologic properties of NO are ultimately determined by redox chemistry and the location of NO as well as derived reactive nitrogen oxide species (RNOS), which are key determinants of the cellular response to NO. Over the course of the last two decades, studies have demonstrated how these basic chemical reactions regulate normal physiology and pathology (54, 72–74). Although studies have provided new insight into many conditions such as ischemia–reperfusion injury, heart disease, and immunology, this review focuses on the role of NO in cancer biology.

THE DICHOTOMOUS BEHAVIOR OF NO IN CANCER

Nitric oxide (NO) is released intracellularly during the oxidation of L-arginine by the nitric oxide synthase (NOS) enzymes. Three isoforms of NOS have been identified; neuronal (nNOS, NOS1) and endothelial (eNOS, NOS3) are constitutive Ca^{+}-dependent forms of the enzyme that are regulated by negative feedback and release low fluxes of NO over a short period to regulate neural and vascular function, respectively (60). In addition, the Ca^{+}-independent inducible (iNOS, NOS2) form produces large amounts of NO throughout the life of the enzyme and is involved in immune surveillance. The distinct timing and control of these three isoforms dictate the high–low range of NO flux required for specific physiologic processes as well as the generation of other RNOS that facilitate numerous redox-regulated pathways.

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The role of NO in cancer has been quite perplexing, as both protective and cytotoxic responses have been demonstrated (24, 42). NO was first shown as an essential component of the macrophage antitumor activity against leukemia cells (24). Recently, tumoricidal activity of NO was further examined by overexpression of iNOS in various tumor cell lines (41). In this study, the transfection of human tumor cells with an adenoviral vector containing iNOS resulted in enhanced iNOS activity 100-fold beyond control. The complete inhibition of tumor growth as well as regional lymph node metastases in nude mice occurred in an iNOS/NO-dependent manner as tumor cells transfected with mutated forms of iNOS cDNA demonstrated reduced tumor-suppressive activity, whereas wild-type (wt) iNOS transfectants yielded complete growth inhibition *in vivo*. Inducible NOS overexpression was also associated with dramatic upregulation of vascular endothelial growth factor (VEGF) and interleukin (IL)-8 angiogenic cytokines, which were associated with enhanced *in vitro* endothelial cell tube formation. Moreover, the ablated tumor growth was associated with NO-mediated cytostasis and enhanced apoptosis (34%) of the transfected cells as well as bystander cells (41, 75–77). Similar NO-induced tumor-suppressive effects were observed in nude mice implanted with malignant rat cells previously exposed to activated Kupffer cells or cell-culture supernatants of activated Kupffer cells (6). In this study, the tumoricidal effects involved upregulation of tumor-necrosis factor (TNF)-α and interferon (IFN)-γ, with concomitant increases in NO production; NO-induced tumor apoptosis was associated with increased DNA damage, elevated caspase-8, and Bcl2 suppression (6).

These effects of acute, high concentrations of NO on tumor growth and metastasis are provocative and warrant a comparison of iNOS and NO levels in normal tissue and human cancer as they relate to conditions of chronic inflammation (20, 26, 73). Toward this end, clinical and experimental studies have demonstrated inverse correlations between iNOS expression and patient survival (12, 38, 65). Another report has demonstrated increased iNOS levels in tumor tissue of colorectal cancer patients (CRCs) when compared with the surrounding normal tissue; beyond this elevated level, iNOS expression declined as a function of tumor progression toward a more metastatic state (1, 20). This observation suggests the occurrence of an NO biphasic response as a function of the levels of iNOS expression and NO concentration over the course of tumor promotion and progression (20). Interestingly, the involvement of an oxidant biphasic response in tumor progression has also been demonstrated (52).

The idea of an NO biphasic response in tumor biology suggests that low NO levels are required for tumor promotion and growth. As the levels of NO increase beyond an "optimal" concentration for tumor growth and survival, growth arrest and/or cell-death pathways are initiated. Cell death may be circumvented by modulation or mutation of oncogenes and/or tumor suppressors (*i.e.*, P53 and Bcl2), resulting in the selective survival and clonal expansion of cell populations that are NO resistant (12, 20, 27). The circumvention of cell death in response to intolerable levels of NO may be supported by the *in vivo* growth results of two human tumor cell lines (253J BV bladder and SKOV3 ipl ovarian cancer cell lines) transfected with adenoviral vector containing iNOS (41). In these transfectants, the *in vivo* growth of cells expressing high levels of iNOS was suppressed for ~30 days before the observation of small but measurable tumors. Although it is possible that the latent tumor development was associated with loss of the iNOS expression vector, it is also plausible that the resultant tumors represented a population of NO-resistant cells. The elucidation of growth and metastatic potential of these or similarly derived cells would be invaluable to this debate. Toward this end, enhanced *in vivo* growth and metastatic potential has been demonstrated by DLD-1 human colon adenocarcinoma cells that were stably transfected with iNOS (32).

Endothelial NOS is also relevant in tumor progression, as it has been shown to mediate tumor growth and metastasis by stimulation of tumor cell invasion, migration, and angiogenesis in clonal derivatives of spontaneous mammary tumors (38). Further studies demonstrated that NO-induced migration of these tumor cells was cGMP and pERK dependent, suggesting that low NO levels mediated the response (31). Mural cell recruitment is a process required for vascular morphogenesis and maturation. An elegant study by Kashiwagi *et al*. (34) demonstrated that NO produced primarily by eNOS in vascular endothelial cells mediates mural cell recruitment, which occurred during tumor angiogenesis of highly metastatic B16F10 murine melanomas. Moreover, the implantation of B16F10 cells in $eNOS^{-/-}$ and $iNOS^{-/-}$ animals showed a requirement of eNOS in NO-mediated tumor vessel branching and longitudinal extension, but not circumferential growth of blood vessels in B16 melanomas. The results of these studies have demonstrated NO concentration dependencies suggestive of a biphasic role of NO in tumor biology; whereas low levels of NO promote tumor growth, high levels are cytostatic, cytotoxic, and can generate NO-resistant cell populations as well (53).

NO CONCENTRATION DEPENDENCE AND SIGNAL TRANSDUCTION

A key affect of NO is its ability to modulate protein and enzyme activities by direct interaction of NO with Fe^{2+} centers or through protein modification by other RNOS (54). The transcription factors P53 and HIF-1 are important signaling molecules in cancer biology that are modulated by NO and regulate cellular growth, apoptosis, and adaptation to stress through altered gene expression (80). The Brune laboratory has performed extensive studies identifying both concentration- and time-dependent effects associated with NO-induced apoptotic death of macrophages in response to NO donors of differing half-lives (5). These studies have demonstrated NO-mediated apoptosis through the upregulation of P53, activation of caspase enzymes, and modulation of the Bcl2 family of proteins, which occurs independent of cyclic guanosine monophosphate (cGMP) (5). Moreover, NO regulation of these pathways is exquisitely sensitive and dependent on the environmental milieu, as the introduction of other reactive oxygen species (ROS) such as superoxide (O_2^-) can redirect signaling, resulting in a shift from cell-death to cell-survival pathways, which is also cell specific and glutathione depen-

dent (3, 5, 62, 71). Mechanisms associated with redox suppression of apoptosis involve S-nitrosation of active-site cysteine residues, resulting in the inactivation of key apoptotic enzymes such as caspases (4, 37, 43, 48, 78, 79).

While P53 regulates cell cycle, apoptosis, and DNA repair in relation to stress, HIF-1 responds to subtle changes in oxygen tension resulting from mild hypoxia and is therefore associated with mediation of cell-survival pathways. Interestingly, the intracellular levels of these transcription factors are similarly regulated by posttranslational modifications culminating in proteasomal degradation (56, 81), which are influenced by NO. In an unstressed cell, HIF-1α is targeted for proteasomal degradation by hydroxylation at Pro 402 and 564 via prolyl hydroxylases and subsequent conjugation with von Hippel–Lindau/E-3 ubiquitin ligase complex. Both NO and mild hypoxia inhibit prolyl hydroxylase function and proteasomal degradation, resulting in HIF-1α stabilization and accumulation (47, 56, 81). Prolyl hydroxylases are nonheme Fe^{2+}-containing proteins; the inhibitory affects of NO are proposed to involve competitive inhibition at the Fe^{2+}-active site of the enzyme (47). In addition, cGMP-independent NO stabilization of HIF-1α and subsequent DNA binding activity by the NO donor DETA/NO (100 and 500 μ*M*) was mediated by S-nitrosation involving NO^+ equivalents (49), suggesting the involvement of both NO and RNS in HIF-1 regulation.

Under normoxic conditions, P53 is also maintained at low or undetectable levels via MDM2 conjugation and cytoplasmic proteasomal degradation. Nitric oxide can interfere with the degradation process by causing posttranslational modifications, which stabilize P53 through phosphorylation and acetylation at several sites of the protein (26, 35, 46, 64, 82). Furthermore, cell fractionation and heterokaryon analysis have demonstrated nuclear accumulation of phosphorylated P53 protein after stabilization by NO, suggesting that impaired nuclear export contributes to P53 stabilization in response to NO (57). Therefore, these studies have demonstrated the involvement of several mechanisms in association with NO-mediated stabilization of P53 and HIF-1α, which are key regulators of cancer progression.

Other signaling cascades important to cell death and survival are the mitogen-activated protein kinases (MAPK), which include extracellular regulated kinase ($ERK_{1/2}$), c-Jun NH_2-terminal kinase (JNK) and p38; in addition, Akt is a downstream target of phosphatidylinositol 3'-kinase that is important in the transmission of cell-survival signals of growth factors. Chaudhuri and co-workers (50) demonstrated that high concentrations of NO (1 m*M* DETA/NO) lead to enhanced apoptosis by inactivation of the prosurvival $ERK_{1/2}$ and Akt signaling cascades through enhanced levels of MKP-1 phosphatase in breast cancer cells.

Signal-transduction mechanisms associated with NO concentration, duration of exposure, and NO response as it relates to chronic inflammation and the development of colon cancer also have been reported (26). Nitric oxide from various donors (>100 μ*M* Sper/NO, DETA/NO, or GSNO) induced both ataxia-telangiectasia mutated (ATM) and ATM-Rad3-related (ATR) kinase-dependent p-ser-15 P53 posttranslational modifications, leading to an increase in $p21^{waf-1}$ and a G_2/M cell-cycle arrest (26). Similar levels of p-ser-15 P53 were identified in MCF7 breast carcinoma cells co-cultured with stimulated ANA-1 murine macrophage expressing iNOS. Therefore, the fluxes of NO produced by the donor at specific concentrations were representative of the microenvironment surrounding iNOS-expressing leukocytes. Moreover, noncancerous colon tissues from patients with ulcerative colitis (a cancer-prone chronic inflammatory disease) demonstrated a positive correlation between the levels of iNOS protein and p-ser-15 P53, as immunostaining of HDM-2 and $p21^{WAF1}$ was consistent with transcriptionally active P53 in these tissues. This study highlights not only a pivotal role of NO in the induction of cellular stress and the activation of a P53 response pathway during chronic inflammation, but also the exploitation of the same pathway by cancer cells as a protective mechanism against inflammatory stress (26).

The effect of NO concentration and duration on posttranslational regulation of signaling proteins under normoxic conditions has also been examined. This work demonstrated discrete NO-threshold profiles associated with pERK, p-ser15-P53, and HIF-1α expression in MCF-7 breast cancer cells (64). A 30-min exposure to low steady-state concentrations of NO (<50 n*M*), which corresponded to 1–10 μ*M* Sper/NO, induced transient $ERK_{1/2}$ phosphorylation that was cGMP dependent. Intermediate Sper/NO concentrations (50 μ*M*) yielding >100 n*M* steady-state levels of NO caused HIF-1a stabilization, which required the persistence of intermediate fluxes of NO. High steady-state fluxes of NO (>300 n*M*), corresponding to 100 μ*M* Sper/NO concentration, led to the accumulation of p-ser15-P53. Where HIF-1α responded to acute increases and decreases of NO, P53 phosphorylation occurred after 2 h of exposure to the NO donor and persisted well after the NO flux disappeared. In contrast, ERK phosphorylation was transient and decreased despite the continued presence of NO. These observations are indicative of the unique temporal and concentration dependence of signaling profiles in response to NO and provide insight into the dichotomous behavior of NO. For example, HIF-1 and pERK are progrowth, whereas P53 phosphorylation is associated with cell-cycle delay, DNA repair, and apoptosis. The ramifications *in vivo* suggest that, despite the toxic effects of high fluxes of NO, these discrete signaling profiles could facilitate the selection and clonal expansion of a mutated genotype(s) under chronic inflammation and iNOS expression (20, 26, 64).

Another important variable of these distinct concentration gradients relative to their biologic response is the enzymatic activity of specific NOS isoforms. Two of the isoforms, nNOS and eNOS, are controlled by calcium influx, which generates a burst of 10–50 n*M* NO lasting only minutes (61). However, phosphorylation of eNOS removes the calcium dependence and prolongs the production of NO (16, 45). The inducible form iNOS has always been assumed to generate only high concentrations of NO for prolonged periods. As discussed earlier, the local microenvironment of activated murine macrophages can be mimicked using millimolar concentrations of NO donors. In addition, NO flux in iNOS-expressing cells can range from high (300 n*M*) to low (<100 n*M*) levels, depending on the cytokine combination used for induction (13). These observations suggest that *in vivo* NO fluxes are controlled specifically and correlate with the three

distinct thresholds previously described (13, 64). In addition to its rate of synthesis and NO concentration, the biologic outcome also depends on cellular consumption and the redox environment. These discrete NO-signaling profiles suggest that NO concentration can be finely tuned to elicit different biologic responses, which has recently been extended by studies identifying an NO biphasic angiogenic response.

REGULATION OF ANGIOGENESIS THROUGH NO/TSP1 CROSS-TALK

Angiogenesis has emerged as a major target for the treatment of cancer (15, 19). Whereas numerous reports have shown both positive and negative effects of NO on angiogenesis (10), other studies have identified eNOS as a central regulator of the angiogenic process (8, 18, 58). Toward this end, it was recently shown that the regulatory event critical to the promotion of angiogenesis involves the phosphorylation of eNOS, which alters the enzyme to a calcium-independent state, resulting in the continuous flux of NO (18). Interestingly, many effects of various growth factors that regulate the induction of angiogenesis, such as VEGF, angiopoieten-2, and estrogen are all mediated through the phosphorylation of eNOS at serine 1179 (7, 14, 16, 28, 63), which activates eNOS for the production of NO. In contrast, the antiangiogenic agents, endostatin and somatostatin, both activate PP2A, an enzyme that dephosphorylates eNOS at serine 1179 (21, 67). Taken together, these observations are supportive of a central role of eNOS and NO generation during angiogenic response.

The regulation of angiogenesis involves a tight balance between pro- and antiangiogenic factors. Although NO has been shown to mediate the response of proangiogenic molecules, little is known of its role in the response(s) of antiangiogenic factors. Thrombospondins 1 (TSP1) and TSP2 are potent antiangiogenic matricellular proteins that exert diverse effects on several angiogenic cell responses including cell proliferation, adhesion, migration, and survival. TSP1 and TSP2 have been found to contribute to immune response, and they also function as tumor suppressors (37, 39, 44, 55). All of these effects arise from interaction of the whole protein or specific domains with various cell-surface receptors. Recently a unique cross-talk relation between NO and TSP1 has been demonstrated, which modulates angiogenic responses of vascular cells to NO, as shown in Fig. 1 (30). The identification of this important reciprocal relation between NO and TSP1 stems from the observation of enhanced NO-mediated endothelial cell migration in tissue samples taken from TSP1 null mice when compared with wild-type controls by using an explant model (30). This model used muscle biopsies implanted in type 1 collagen gel and incubated in growth medium for 10 days. Vascular cell (endothelial and smooth muscle) migration through the extracellular matrix was quantified by microscopically measuring the farthest distance of cell migration from the biopsy sample border. This method provides a rapid and reproducible assessment of vascular cell migration and matrix invasion, as hallmarks of an angiogenic response, as well as a means to test multiple pharmacologic agents in either a wound-healing or tumor-driven model of angiogenesis.

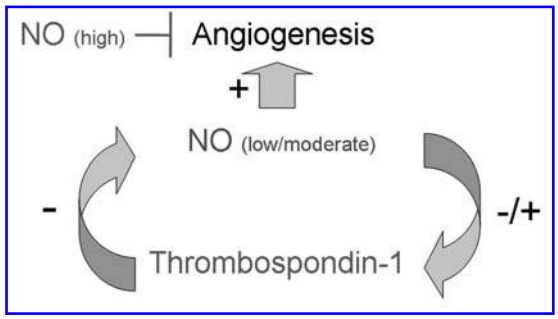

FIG. 1. Regulation of angiogenic response through NO/TSP1 crosstalk. Low levels of NO induce angiogenesis and downregulate TSP1. NO-induced angiogenic response is suppressed by increases in TSP1.

The explant model has shown that 100 μ*M* DETA/NO ($t_{1/2}$ = 24 h) modestly enhanced endothelial cell migration in tissue from wild-type animals, whereas it dramatically accelerated vascular cell migration in tissue from TSP1-null animals. These results indicate that TSP1 is involved in the control of NO-stimulated angiogenic responses. In addition, vascular cell migration was found to respond to the NO donor DETA/NO in a biphasic manner; low doses (0.1–10 μ*M*) enhanced cell migration, and high doses (>100–1,000 μ*M*) attenuated the response. Similarly, supplemental L-arginine, a substrate of eNOS, increased vascular cell migration. However, in the presence of the NOS inhibitor L-NAME, the explant angiogenic response was completely abrogated. These results demonstrate that endogenous NOS activity and low doses of the NO donor stimulate an angiogenic response characterized by vascular cell invasion of and migration through extracellular collagen matrix. In contrast, higher concentrations of NO, consistent with that produced in the vicinity of activated macrophages, attenuated the angiogenic response (30).

TSP1 LIGATION OF CD36 INHIBITS THE NO ANGIOGENIC RESPONSE

An angiogenic response involves mobilization of an otherwise quiescent vascular network and changes in vascular cell adhesion, proliferation, and migration. *In vitro* analysis of each of these specific cellular responses demonstrated that both exogenous and endogenous TSP1 potently inhibited the stimulatory effects of low-dose NO (30). More important, low-dose exogenous NO dramatically increased the sensitivity of endothelial cells to the inhibitory effects of exogenous TSP1. Although TSP1 at high doses (≥10 μg/ml) directly inhibits endothelial cell proliferation, adhesion, and migration, low-dose NO treatment (≤10 μ*M*) increased the inhibitory potency of TSP1 by ≥1,000-fold. Further, these inhibitory effects were mimicked by a recombinant fragment of TSP1 containing its type 1 repeats. The major antiangiogenic effects of TSP1 have been mapped to this domain (66) and involve, in part, ligation of CD36 (9, 29). Interestingly, antibody ligation of CD36 also inhibited NO-driven endothelial cell responses in every angiogenic assay tested. These results

are particularly relevant because derivatives of TSP1 peptide sequences that act through the CD36 receptor have completed phase I clinical trials (23, 25) and are currently in phase II clinical trials for patients with renal cell carcinoma and soft tissue sarcomas (11).

On a molecular level, NO stimulates soluble guanylate cyclase, resulting in increased synthesis of intracellular of cGMP. Significantly, Isenberg *et al*. (30) demonstrated that exogenous TSP1 prevented the NO-driven flux of intracellular cGMP, summarized in Fig. 2. CD36 antibody ligation and recombinant type 1 repeats of TSP1 also blocked NO-induced cGMP accumulation, implicating CD36 in this signaling response. Comparison of wild-type and TSP1-null mouse endothelial cell responses to NO showed that endogenous TSP1 also inhibits cGMP accumulation. This differential response to NO was not sensitive to inhibiting cGMP phosphodiesterases, indicating that TSP1 and CD36 signaling regulates synthesis rather than degradation of cGMP. This result supports the idea that sGC is a primary, although not the only, target for TSP1. It was also found that vascular cell responses, driven by 8-Br-cGMP, were inhibited by exogenous TSP1, indicating that at least one target for TSP1 exists downstream from sGC. These results suggest a new and important therapeutic paradigm for altering tumor-driven angiogenic responses based on dose dependence of NO flux and treatment with peptides or mimetics of TSP1 that act through CD36.

SIGNALING CASCADES AND NO PROLIFERATIVE RESPONSE IN ENDOTHELIAL CELLS

CD36-dependent signaling by TSP1 mediates inhibition of endothelial cell responses to NO and involves sGC as well as undefined targets downstream of cGMP. The identification of such targets may lead to new therapeutic strategies to control pathologic angiogenesis. Further understanding of the significance of this CD36 pathway in regulating NO-dependent angiogenic responses was achieved by examining the signaling profiles of key regulatory proteins in endothelial cells (51). In contrast to the response of MCF-7 cells, NO released by DETA/NO did not stabilize HIF-1α in human umbilical vein endothelial cells (HUVECs), which could have been an effect of timing and donor specificity. However, the levels of p-ser15-P53 increased in a dose-dependent manner with the highest induction (approximately sixfold above control) occurring at 1,000 μ*M* DETA/NO. Interestingly, an inverse relation between the levels of pERK and the MAPK phosphatase MKP-1 was found. Compared with control, increases in pERK followed a bell-shaped response, as low DETA/NO concentration (10 μ*M*) increased pERK levels whereas higher doses decreased phosphorylation. In contrast, MKP-1 levels decreased to a minimum in response to low-dose NO (10 μ*M*) and then rebounded to levels greater than twofold beyond control at high doses. These alterations in MAPK proteins and pP53 indicate that low-dose NO favors proliferation, whereas high doses are inhibitory, and in this respect are similar to the NO signaling profiles reported in MCF7 tumor cells (51, 64).

Endothelial cells, although known to synthesize and respond to TSP1, were found to respond to low-dose NO with a 50% decrease in expression of TSP1 (51), consistent with previous observations in kidney mesangial cells (68). As donor concentration increased, a modest reaccumulation of secreted TSP1 was observed (51). These findings suggest that NO-mediated proangiogenic responses involve the downregulation of TSP1. The presence of ODQ (guanylate cyclase inhibitor) or U0126 (MEK inhibitor) abated the NO-induced suppression of TSP1, as well as the proliferative response of HUVECs, indicating that the proangiogenic effects of NO are both cGMP and pERK dependent. Interestingly, exogenous TSP1 also inhibited NO-induced ERK phosphorylation, suggesting that in addition to cGMP dependence, the NO/TSP1 cross-talk is mediated, at least in part, through ERK phosphorylation; this is summarized in Fig. 3 (51).

RELEVANCE OF NO/TSP CROSSTALK

The role of NO in cancer depends on the temporal and spatial aspects of NO flux and changing cellular responses to NO during disease progression, which combine to yield either positive or negative outcomes for the tumor and host (74). Despite this complexity, a rationale for the duality of NO is beginning to emerge from the demonstration of the NO dose de-

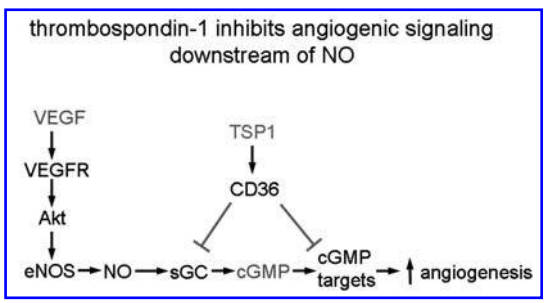

FIG. 2. Thrombospondin-1 suppression of NO-induced angiogenic response involves CD36 inhibition of sGC.

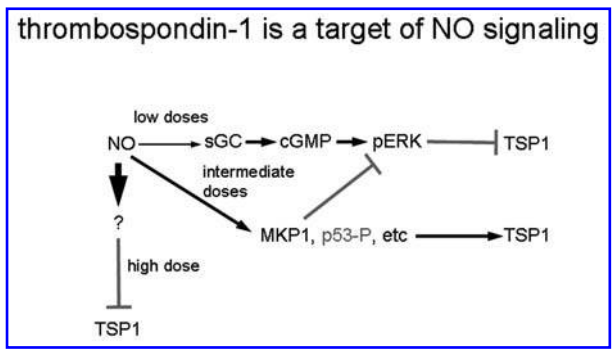

FIG. 3. Low fluxes of NO downregulate TSP1 in a sGC- and pERK-dependent manner, whereas moderate fluxes of NO are associated with P53 phosphorylation, MKP1 phosphatase induction, and modest reaccumulation of TSP1.

pendence of specific cellular and molecular targets in tumors (64). Further understanding has been derived from recent reports of the importance of redox flux in hypersensitizing vascular cells to the antiangiogenic effects of TSP1, a process involving cGMP and pERK (30, 51). An NO flux of as little as 2 n*M* was sufficient to induce vascular cell responses to picomolar amounts of exogenous TSP1, as manifested by inhibition of cellular adhesion, proliferation, and migration. A similar biphasic angiogenic response to the NO donor SNAP has been reported in microvascular endothelial cells, as low doses (0.1–0.3 m*M*) significantly enhanced cell migration, adhesion, and ERK phosphorylation, whereas higher doses (0.5–4 m*M*) attenuated these responses (33). Estradiol, a known mediator of tumor angiogenesis as well as eNOS activation, induced both proliferative and migration responses of HUVECs, which required ERK phosphorylation and TSP1 suppression (69, 59). These reports identify TSP1, cGMP, and ERK as regulators of NO-induced angiogenic response. Multiple approaches are currently being developed for the application of both TSP1 and TSP2 in cancer therapy (40, 70). Low-dose chemotherapy, also known as antiangiogenic chemotherapy or metronomic dosing, involves the optimization of the effects of cytotoxic drugs by administering them continuously at low, nontoxic doses (22, 40). Low-dose chemotherapy appears to provide a promising new approach because the targeted endothelial cells within the tumor bed are genetically stable and are therefore at a reduced risk of developing drug resistance, and low dosage produces significantly fewer side effects because of selectivity of endothelial cells (40). Toward this end, a recent report has shown that TSP1 secreted from the tumor microenvironment mediated the antiangiogenic and tumor growth–suppressive effects of low-dose cyclophosphamide (22). Evidence of endothelial cell selectivity was demonstrated by a reduction in CD31-positive vasculature (endothelial cell marker) in the tumors of cyclophosphamide-treated animals. Moreover, these antiangiogenic and tumor-suppressive responses did not occur with tumor cells that did not express TSP1 (22). These observations and the reports previously discussed, which demonstrated the involvement of NO generated by eNOS in tumor progression and metastasis (31, 34, 38), strengthen the potential relevance of the NO/TSP1 cross-talk relation (30, 51) in tumor biology. Taken together, these reports suggest that the elucidation of temporal and concentration-dependent redox pathways that drive tumor progression will facilitate the identification of novel therapeutic approaches for the treatment of cancer.

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ABBREVIATIONS

cGMP, cyclic guanosine monophosphate; DETA/NO, diethyltriamine NONOate; HIF, hypoxia inducible factor; IL-8, interleukin-8; MAPK, mitogen-activated protein kinase; NO, nitric oxide; pERK, phosphorylated extracellular regulated kinase; sGC, soluble guanylyl cyclase; NOS, nitric oxide synthase; RNOS, reactive nitrogen oxide species; Sper/NO, spermine NONOate; TNF-α, tumor necrosis factor-α; TSP1, thrombospondin-1; VEGF, vascular endothelial growth factor.

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