

An Emerging Role for Endothelial Nitric Oxide Synthase in Chronic Inflammation and Cancer

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Abstract

Nitric oxide (NO) is a free radical that is involved in carcinogenesis. Recent literature indicates that endothelial NO synthase (eNOS) can modulate cancer-related events (angiogenesis, apoptosis, cell cycle, invasion, and metastasis). We review the literature linking eNOS to carcinogenesis to encourage future research assessing the role of eNOS in cancer prevention and treatment. [Cancer Res 2007;67(4):1407–10]

Introduction

Chronic inflammation is a risk factor for cancer. During inflammation, activated inflammatory cells can release reactive nitrogen species (RNS), reactive oxygen species (ROS), lytic enzymes, and cytokines to exacerbate the inflammatory reaction. Although nitric oxide (NO) and ROS scavenge each other, there is often excessive reactive species that can attack nearby epithelial and stromal cells by damaging the DNA or post-translationally modifying proteins, altering function.

NO is synthesized from L-arginine and oxygen by four major isoforms of NO synthase (NOS): neuronal NOS, endothelial NOS (eNOS), inducible NOS (iNOS), and, more recently, mitochondrial NOS. They share the same EC number of 1.14.13.39 according to the enzyme commission nomenclature. NO is a small pleiotropic free radical that was first discovered in cardiovascular and neural systems as a vasodilator and neurotransmitter, respectively (1). NO is a highly diffusive hydrophobic molecule (2) and is therefore a key signaling molecule in inflammation-driven diseases, including cancer. NO can also react with superoxide and form potent secondary intermediates, such as peroxynitrite (ONOO⁻) and nitrogen dioxide (NO₂), which have cytotoxic effects through lipid and DNA damage, and post-translational modification of proteins (e.g., nitration and nitrosylation). Although there are many physiologic benefits for RNS (e.g., in the cardiovascular system), they have been implicated both as anti-inflammatory/anticancer agents and proinflammatory/procancer agents. Ultimate carcinogenic effects depend on the genetic makeup of its target, the surrounding microenvironment, the activity and localization of NOS isoforms, and the overall levels of NO/RNS (2–5). In the latter case, it is becoming increasingly clear that RNS at low but pathologic levels can have precancerous consequences (3). This is relevant to eNOS because this NOS isoform produces low levels of NO. Although there is some evidence that eNOS can have antitumor properties under specific circumstances, it seems

that the majority of the literature supports a protumorigenic role of eNOS (Fig. 1). The following sections outline a role for eNOS in carcinogenesis.

eNOS and Inflammation

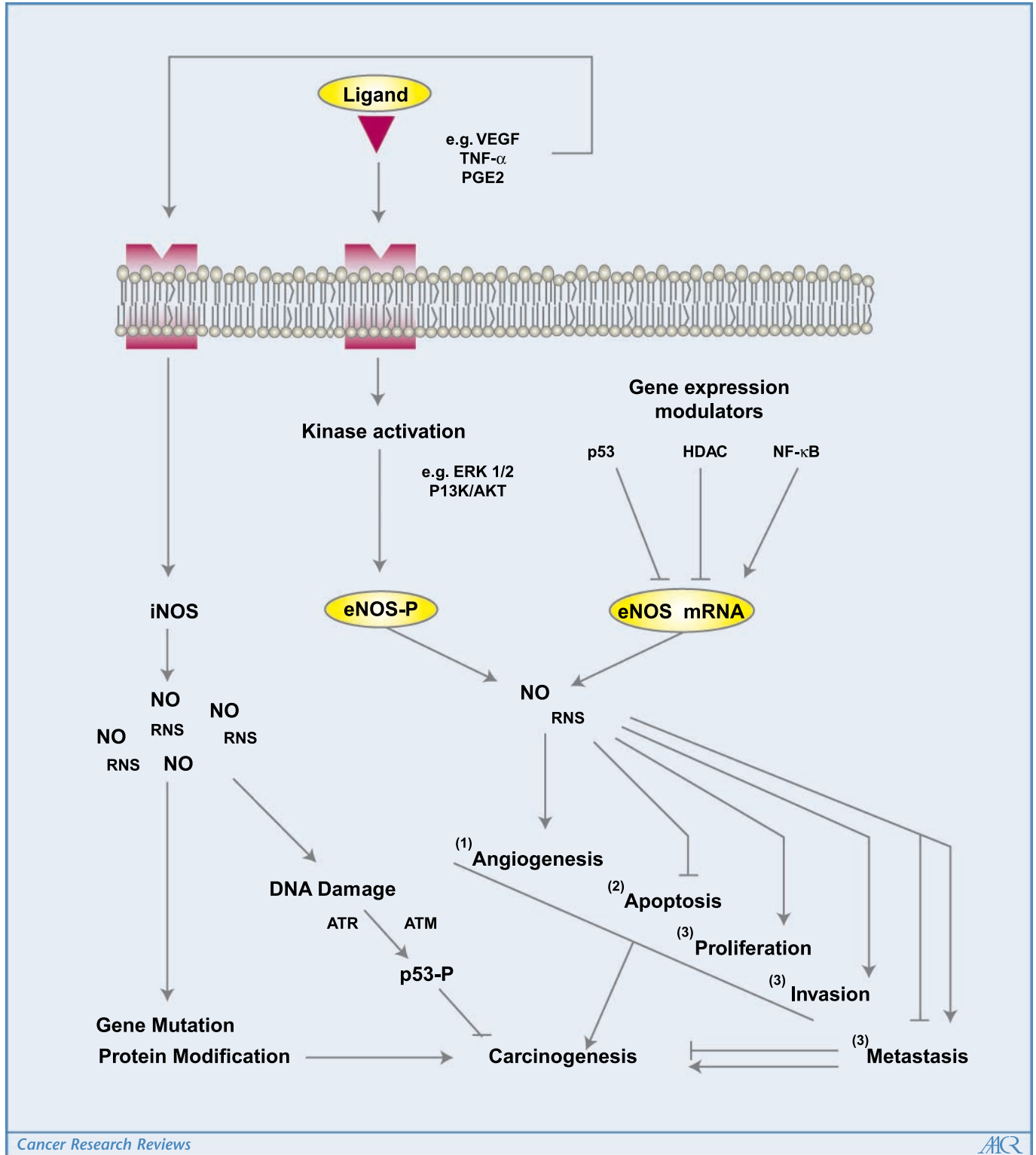
Chronic inflammation can drive cancer. NO is closely related to inflammatory status and regarded as a key inflammation mediator. Until recently, iNOS has received most of the attention because it can be induced by a variety of inflammatory cytokines and can produce micromolar levels of NO, which damage DNA and modify protein structure/function (2–5). However, eNOS, which produces nanomolar levels of NO, also plays a role in inflammation. eNOS, for example, can regulate the expression of the proinflammatory molecules nuclear factor- κ B (NF- κ B) and cyclooxygenase-2 (6–8). Alternatively, proinflammatory cytokines can modulate the expression of eNOS. Based on different model systems and different cell types/tissues examined, eNOS is both proinflammatory and anti-inflammatory. eNOS, for example, seems to be protective against acute pancreatitis (9). Although the role of eNOS has not been carefully examined in other high cancer risk, chronic inflammatory diseases, a good example of divergent results in the same tissue examined (colon) is that of studies using mouse models of colitis in eNOS^{+/+} versus eNOS^{-/-} mice (10–12). Reviews on the chemical and biological heterogeneity of NO have recently been published (4, 5). However, the discrepancies in results from the above studies (10–12) with eNOS^{+/+} versus eNOS^{-/-} mice may, in part, be due to the particular animal and model being used. We prefer using multiple models to test one hypothesis. One model developed by Yang et al. (13) is particularly suited to the study of colon cancer associated with ulcerative colitis. This model uses 1% dextran sodium sulfate in drinking water, given in cycles (7 days on, 10 days off) over 15 cycles. Because patients with ulcerative colitis are often encouraged to eat a high iron diet, the chow contains excess iron (2-fold). This closely resembles human ulcerative colitis both histologically and symptomatically. Alarming, the excess iron stimulates colon cancer associated with colitis. The role of eNOS in colon carcinogenesis with this specific model has not been studied.

eNOS Inhibits Apoptosis and Promotes Angiogenesis, Tumor Cell Proliferation, Mobility, and Invasiveness

Targeting iNOS for cancer prevention and treatment has been extensively studied and reviewed, with paradoxical outcomes for the antitumor and protumor consequences of iNOS expression or inhibition (5). Although iNOS remains a viable candidate for purposes of cancer prevention and treatment, we suggest that targeting eNOS may also be a viable strategy or at least deserves consideration. Multiple clinical observational studies have shown a dysregulation of eNOS expression in both mucosal and vascular cells of tumors. Protumorigenic agents, such as estrogen, have been

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Figure 1. Linking eNOS to carcinogenesis. eNOS can be activated by post-translational modification (e.g., phosphorylation by ERK1/2 or PI3K/Akt pathways) or by transcription (e.g., NF- κ B). Depending on the cellular milieu, release of low concentrations of NO can stimulate angiogenesis, inhibit apoptosis, or stimulate proliferation and invasion. eNOS may also play a role in metastases. In comparison, iNOS gene transfer and the release of NO have been shown to inhibit cancer cell growth and metastases (39). Alternatively, gene mutation (41, 42) and potentially deleterious protein modification (43, 44) may occur from exposure to bolus RNS, indicating that this strategy would be beneficial to cancer treatment but may have dangerous side effects if used as in long-term chemopreventive strategies. 1, extensive experimental data; 2, moderate experimental data; 3, little experimental data. TNF- α , tumor necrosis factor- α ; HDAC, histone deacetylase.

shown to induce eNOS expression in tumor cells (14). Genetic comparison studies on healthy people and cancer victims have shown that gene polymorphisms in eNOS are associated with the development of multiple cancers (15, 16). Although the functional effect of eNOS polymorphisms has yet to be determined, these data support the hypothesis that an abnormal *eNOS* gene might drive tumorigenesis in humans.

eNOS and Angiogenesis Cascade

Angiogenesis is a key step in solid tumor progression. To this end, in addition to tumor cell expression, eNOS is particularly expressed by vascular endothelial cells or surrounding stromal cells and therefore has been a focus of attention in angiogenesis. Several cancer treatment methods influence eNOS expression and activity. Low-dose irradiation-induced angiogenesis is believed to be mediated by NO from eNOS (17). *In vivo* studies have shown that caveolin-1 (a molecule that binds to and inhibits eNOS) vector transfection impairs NO-dependent tumor blood flow and results in a dramatic tumor growth delay (18). Cavtratin, which is a cell-permeable peptide derived from caveolin and can reduce microvascular endothelium hyperpermeability, can inhibit eNOS function and tumor progression in mice. The effects of cavtratin are largely attenuated in eNOS^{-/-} mice (19). More directly, also using eNOS^{-/-} mice, others have found that NO mediates branching and longitudinal extension of blood vessels in B16 melanomas and that this process is predominantly mediated by eNOS (20). Finally, somatostatin inhibits tumor angiogenesis through inhibition of eNOS and mitogen-activated protein kinase pathways (21).

In cell culture models, eNOS plays an essential role in endothelial cell proliferation and is a central mediator of several endothelium growth stimulators, such as vascular endothelial growth factor (VEGF) and prostaglandin E₂ (PGE₂; refs. 22, 23). Both molecules activate the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the former (VEGF) increases eNOS activity by enhancing eNOS phosphorylation. As reviewed by Duda et al. (22), VEGF can also activate eNOS by the induction of calcium flux and the recruitment of heat shock protein 90. Further, VEGF increases angiogenesis in both iNOS^{+/+} and iNOS^{-/-} mice but not in eNOS^{-/-} mice, supporting a predominant role of eNOS in VEGF-induced angiogenesis and vascular permeability (22). PGE₂ increases endothelial cell sprouting (the first step in neoangiogenesis) through the NO/cyclic guanosine 3',5'-monophosphate (cGMP) pathway (23). Thus, selective modulation of eNOS activity independently, or in association with PGE₂ or VEGF inhibition, may be a promising strategy for altering angiogenesis and slowing tumor growth and spread.

eNOS and Apoptosis

NO can be either antiapoptotic or proapoptotic (2, 4, 5). In our experience, lower concentrations of NO have antiapoptotic effects, although this is dictated by the specific model and experimental conditions used. More specific to this review, published studies indicate that eNOS is antiapoptotic in tumor epithelial cells. For example, eNOS inhibits tumor necrosis factor-related apoptosis-inducing ligand-induced and ROS-induced apoptosis in prostate tumor cells (24, 25). Others have found that eNOS can decrease apoptosis and increase survival through the Bcl-2 (26) and soluble guanylate cyclase (sGC)/cGMP pathways (27). In addition, eNOS has a p53-binding site. Wild-type p53 down-regulates the transcription of the eNOS promoter (28), which may be a mechanism for the proapoptotic and anticancer nature of wild-type p53. Finally, eNOS may be a molecular node in growth factor-mediated inhibition of

apoptosis (29). Thus, eNOS inhibition specific to tumor cells may be a viable option for the stimulation of apoptosis and treatment of cancer alone or in combination with chemotherapeutic agents.

eNOS and Proliferation

eNOS has been identified as a key molecule for endothelial cell activation and proliferation. This is particularly important in association with angiogenesis as discussed above. The influence of eNOS on epithelial cell proliferation is less studied. Consistent with the nature of NO, effects depend on the specific cell type, genetic background of the target, and the NO concentration. The importance of NO concentration is shown by the observation that *S*-nitroso-*N*-acetylpencillamine (a NO donor) stimulates proliferation of myoblast cells at low concentrations (1–10 μmol/L) but inhibits proliferation of the same cells at higher concentrations (50 μmol/L; ref. 30). Several groups, including ourselves,¹ have observed that low concentrations of NO, consistent with levels released by eNOS, can stimulate cancer cell cycle progression and proliferation. More specific to eNOS, studies have shown that the eNOS/NO pathway plays a role in oral squamous cancer cell DNA/RNA synthesis and proliferation apart from promoting angiogenesis (31). eNOS enzymatic activity most likely contributes to epithelial regeneration, as eNOS^{-/-} animals show reduced wound margin epithelia associated with reduced keratinocyte proliferation (32). Although this provides some evidence of eNOS driving proliferation, other studies implicate eNOS in inhibiting vascular smooth muscle cell cycle through p21^{WAF1/CIP-1} and p27^{KIP1}, which proves beneficial in cardiovascular disease (33) but offers little insight into carcinogenesis. Clearly, much more research is necessary to further elucidate its specific role and mechanisms in epithelial cell cycle control.

eNOS in Invasion and Metastasis

eNOS and associated NO can increase the permeability of the tumor-blood barrier and therefore may play a role in tumor invasion. Clinical studies on human colon cancer samples suggest that high eNOS expression can be positively correlated with tumor cell vascular invasion (34). High eNOS expression is also correlated to trophoblast cancer cell vascular invasion (35). The pathway may involve sGC/cGMP/cGMP-dependent protein kinase (PKG) and other signaling molecules. sGC on the cell membrane is a key NO receptor. Once NO binds to sGC, its intracellular catalytic segment is activated and catalyzes GTP to cGMP and then activates downstream PKG and extracellular signal-regulated kinase 1/2 (ERK1/2). The activation of this signaling pathway acts as a signal for cancer cell migration, which is an essential step in tumor cell invasion and metastasis (36). eNOS may also promote cell invasiveness by inhibiting tissue inhibitor of metalloproteinase (TIMP)-2 and TIMP-3 (37). Our understanding of eNOS in tumor metastasis is poor. Using eNOS^{-/-} mice, one study found a key role of NO from eNOS in inhibiting melanoma metastasis to the lung (38).

Conclusions

As has been reviewed many times before, the protumorigenic versus antitumorigenic effect of NO depends on the cellular makeup, surrounding microenvironment (e.g., other inflammatory species and free radicals), the activity and localization of NOS isoforms, and overall levels. Relative to iNOS, studies on eNOS in carcinogenesis are in their infancy. Evidence is accumulating that

¹ Unpublished data.

low (but above basal/pathologic) levels of NO from eNOS can be procarcinogenic. This can be from its stimulatory effects on angiogenesis, cell proliferation, and invasion and from its inhibitory effects on apoptosis. Therefore, selective targeting of eNOS may prove a useful therapeutic or chemopreventive measure. Because of the critical role of eNOS in cardiovascular function, to circumvent potential adverse effects when entering translational stages of eNOS inhibition in tumorigenesis, it would be advantageous to target the tumor locally and directly rather than systemically. To directly address whether eNOS is procarcinogenic (or anticarcinogenic), we encourage more studies that focus on eNOS^{-/-} mice and tumorigenesis and eNOS gene transfer, or small interfering RNA (siRNA) to eNOS into epithelial, stromal, or tumor cells, and assessment of tumorigenesis. Recent studies have shown direct iNOS transduction to be cytotoxic, resulting in abrogated tumor growth (39). Further, iNOS^{-/-} mice seem to have increased

tumorigenesis (40), indicating that iNOS suppresses tumorigenesis. We put forward the need for evaluation of eNOS on carcinogenesis in a similar manner that iNOS has been evaluated over the last decade. Given the procarcinogenic role of noncytotoxic levels of NO, and the importance of multiple targets in chemoprevention, it may be interesting to examine dual targeting of iNOS (e.g., iNOS transduction) and eNOS (e.g., eNOS siRNA) as a useful cancer chemotherapeutic or chemopreventive strategy.

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