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COMMENTARY

The multifaceted roles of nitric oxide in cancer

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The roles of nitric oxide (NO) in numerous disease states have generated considerable discussion over the past several years. NO has been labeled as the causative agent in different pathophysiological mechanisms, yet appears to protect against various chemical species such as those generated under oxidative stress. Similarly, NO appears to exert a dichotomy of effects within the multistage model of cancer. Chronic inflammation can lead to the production of chemical intermediates, among them NO, which in turn can mediate damage to DNA. Yet, NO also appears to be critical for the tumoricidal activity of the immune system. Furthermore, NO can also have a multitude of effects on other aspects of tumor biology, including angiogenesis and metastasis. This report will discuss how the chemistry of NO may impact the initiation and progression stages of cancer.

Introduction

Over the past decade, it has been realized that the diatomic radical, nitric oxide (NO*), plays a variety of regulatory functions *in vivo*. This molecule is produced by three isoforms of the enzyme nitric oxide synthase (NOS) which can regulate numerous physiological functions, as well as playing critical roles in the anti-pathogen and tumoricidal response of the immune system (1). Despite these beneficial effects, NO has also been implicated as a deleterious agent in various pathophysiological conditions including cancer. Some reports suggest that NO possesses anti-tumor properties, while others implicate NO in tumor promotion. This paper will discuss some of the salient chemical aspects of NO in an attempt to address these diametrically opposing roles in cancer biology.

The formation of tumors has been hypothesized to occur in several stages: initiation, promotion, and progression. Over the past several years, researchers have realized that the production of NO can impact at least some of the stages of cancer, with outcomes both positive and negative to the host. The earliest studies on NO indicated its complex role in cancer biology: NO derived from activated macrophages was shown to inhibit respiration in tumor cells (2,3), while other studies proposed that carcinogenic nitrosamines could be derived from NOS via the nitrosation chemistry of NO (4). Later, it was suggested that reactive nitrogen oxide species (RNOS) derived from NO may be carcinogenic by chemically altering DNA directly as well as enhancing the genotoxicity of other substances such as alkylating agents (5–7) and metals such as cadmium (8). Furthermore, while some studies suggested that expression of NOS correlated with reduced metastasis, other studies suggested that some tumors which express NOS are more aggressive *in vivo* than their counterparts which do not. To understand the multifaceted role of NO in cancer, it is important to consider spatial and temporal aspects of NO production, as well as the complex chemistry in which this seemingly simple molecule can participate.

***Abbreviations:** NO, nitric oxide; NOS, nitric oxide synthase; RNOS, reactive nitrogen oxide species; NO^+, nitrosonium cation; ROS, reactive oxygen species; Fpg protein, Formamidopyrimidine-DNA glycosylase; Fapy, 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine; IRB, iron-responsive binding protein; IRE, iron responsive elements; TfR, transferrin receptor; VEGF, vascular endothelial growth factor.

Chemistry and biochemistry of NO

There are several isoforms of NOS: nNOS (the first to be purified and cloned, hence also called NOS1), iNOS (NOS2), and ecNOS (NOS3). Each isoform is the product of a distinct gene (1). Historically, NOS have been classified into two distinct categories, constitutive (nNOS and ecNOS) and inducible (1). Generally, nNOS and ecNOS are present continuously, in neurons and endothelial cells, respectively, and require elevation in intracellular Ca^{2+} and attendant activation of calmodulin to produce NO. These isoforms are regulated primarily by calcium influx and generate low levels of NO for brief periods of time (9,10). On the other hand, iNOS needs to be induced by cytokines in essentially every cell type, and can generate locally high concentrations of NO for prolonged periods of time. The distinctions between ‘constitutive’ and ‘inducible’ NOS have blurred somewhat in recent years, with the finding of apparently basal expression of iNOS in kidney and lung which performs physiological regulatory roles (11), while conversely the expression of nNOS and ecNOS can be modulated following injury or treatment with cytokines (12–17). Furthermore, the initial suggestion that only nNOS and ecNOS are dependent on elevations of Ca^{2+}, while iNOS is not, has also been questioned, with the reports of iNOS enzymatic activity dependent on intracellular fluxes of Ca^{2+} and concomitant binding of calmodulin (18). However, in general cNOS is calcium dependent and continously present, while iNOS is calcium independent and is expressed only after cytokine exposure.

The driving hypothesis of our research, as well as that of several other groups, has been that the chemistry of NO is one of the primary determinants of its final effect on a given biological system. The potential chemical reactions of NO in biological systems is vastly more complex than that of NO in an oxygenated aqueous solution. However, the chemistry of NO, like the difference between the roles ascribed to ‘inducible’ and ‘constitutive’ NOS, can be separated based on

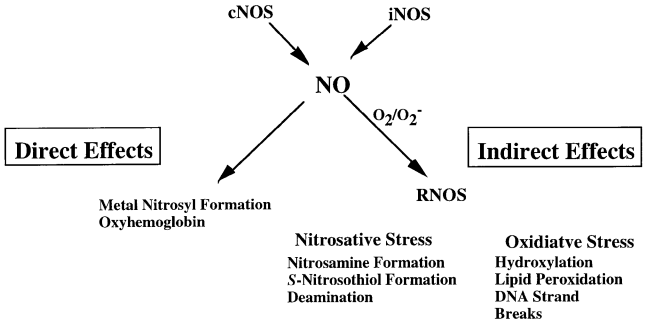

Fig. 1. The biochemistry/chemistry of NO.

concentrations of NO produced (19,20). We have used the phrase 'the chemical biology of NO' to describe its chemical reactions based on the chemistry of NO which occurs at different fluxes and concentrations (20,21). The chemical biology of NO can be divided into two types of effects, direct and indirect (Figure 1). Direct effects are those chemical reactions in which NO reacts directly with a given biological target. For instance, low levels of NO can react directly with heme-containing proteins such as guanylate cyclase, oxyhemoglobin, and cytochrome P450, and thereby may account for the neuromodulatory effects of nNOS and the vasodilatory effects of ecNOS. Indirect effects are those chemical reactions mediated by RNOS, formed through the reaction of NO either with oxygen or with superoxide. These reactions require high local concentrations of NO, of which iNOS may be the sole biological source.

The chemistry of the indirect effects of NO can be broken down further, to nitrosation and oxidation (Figure 1) (20). Nitrosation reactions are those in which RNOS donate nitrosonium cation (NO^+) to nucleophiles such as thiols and amines. The formation of these nitrosonium adducts in biological systems indicates the presence of conditions which can be termed nitrosative stress. Oxidative chemistry mediates the removal of electrons or hydroxylation reactions analogous to those described for reactive oxygen species (ROS). Formation of these oxidative lesions in biological systems is indicative of oxidative stress. Oxidative and nitrosative stresses produce different effects on intracellular processes and thus can be often associated with apparently opposite phenomena (20). While at first glance, it may appear that such a diverse range of chemical reactions may lead to uncontrollable outcomes, a finely-tuned balance appears to exist between nitrosative and oxidative stresses (22,23). With these basic principles in mind, we will describe some of the potential roles of NO in cancer.

NO and genotoxic mechanisms

Several studies have implied that over-expression of NOS in chronic inflammation can lead to genotoxicity. The DNA of macrophages expressing iNOS can be deaminated (24). Furthermore, lymphoma cells which induce NO production in macrophages can cause various DNA lesions in lacZ promoters (25), thus suggesting that NO generated *in vivo*, perhaps even during the immune response to tumors, may be genotoxic. Nitric oxide may mediate DNA lesions by any of three possible mechanisms: i) formation of carcinogenic nitrosamines; ii) direct modification of DNA, not by NO but by RNOS; and iii) inhibition of systems required to repair DNA lesions mediated by other genotoxic substances. In addition, some RNOS can mediate DNA strand breaks. Furthermore, as outlined below, nitrosative and oxidative stresses appear to yield different types of mutations in DNA.

Nitrosamines can be formed by chemical intermediates associated with nitrosative stress and are potentially carcinogenic. For example, RNOS generated from acidic nitrite form such carcinogenic nitrosamines in the stomach (26–31). The presence of NO in an aerobic environment can also result in the formation of nitrosamines (32). Though NO itself does not interact with bio-organic macromolecules such as DNA or proteins, high concentrations of NO can lead to the formation of RNOS such as N_2O_3 or in the presence of superoxide (O_2^-); peroxynitrite, which in turn can alter DNA and result in a variety of lesions (21). These chemical intermediates result in either nitrosative or oxidative stress to biological systems. The nitrosation reactions appear to be relevant *in vivo*, since stimulated macrophages and neutrophils express iNOS and generate high amounts of NO and hence RNOS, which can nitrosate amines (4,33). These observations led to the proposal that nitrosamines could be formed under conditions of chronic inflammation, which in turn can lead to cancer (34,35). Liu *et al*. demonstrated that nitrosamines could be generated from woodchuck liver chronically infected with hepatitis virus, and suggested that sufficient nitrosative stress exists under certain conditions *in vivo* to form carcinogenic nitrosamines (36,37).

Deamination of guanine, cytosine, and adenine is mediated *in vivo* primarily by the nitrosative chemistry of N_2O_3 (38,39). Both bacterial and mammalian cells exposed to NO exhibit lesions consistent with the chemistry of deamination (38,39). Nitrosation of an exocyclic amine group has been proposed to lead to the formation of a primary nitrosamine, followed by rapid deamination which culminates in the formation of an hydroxyl group.

$$NH_2\text{–}R + N_2O_3 \rightarrow R\text{–}NHNO + NO_2^- \quad (1)$$

$$R\text{–}NHNO \rightarrow R\text{–}NNOH \rightarrow R\text{–}OH + N_2 \quad (2)$$

This chemistry would lead to the conversion of cytosine to uracil, guanine to xanthine, methylcytosine to thymine, and adenine to hypoxanthine. Single stranded DNA is far more susceptible to nitrosative chemistry than double stranded DNA (40), which suggests that deamination should occur more prevalently during replication and transcription of DNA. In support of this hypothesis, Cooney and co-workers have shown that bacteria growing in log phase are more susceptible to mutations than bacteria in plateau phase (41). Such mechanisms involving nitrosation of nucleic acids (equations 1,2) may contribute to the spontaneous deamination which occurs *in vivo.*

Oxidative stress can also mediate damage to DNA. Oxidative chemistry induced by RNOS is thought to be mediated primarily by the formation of peroxynitrite (22,33), though it is important not to discount the involvement of reactive oxygen species created by Fenton-type oxidation. It has been suggested that oxidative damage to DNA in activated macrophages is due to the formation of peroxynitrite (24), and peroxynitrite generated synthetically at concentrations ranging from 0.05–8 mM can induce DNA strand breaks *in vitro* (42,43). SIN-1, an NO donor which generates both NO and superoxide, oxidizes guanine to form HOdG (44), though another study suggests that peroxynitrite does not increase the levels of HOdG in DNA (45). In addition to oxidation products, 8-nitroguanine has also been detected as a product of the

Table I. Summary of distribution of mutations of plasmid sp189 shuttled into Ad293 cells

Transversions	NO donors	NO (gas)	Nitrite (pH 5.4)	OONO-
GC→TA	14	4	29	65
GC→CG	8	1	9	28
Transitions				
GC→AT	55	29	46	11
AT→GC	7	60	12	–

reaction of peroxynitrite with guanine, suggesting that nitration of nucleotides could occur (45,46). Analogously to the treatment of a plasmid with NO depicted in Table I, Juedes and Wogan treated plasmids with peroxynitrite and transfected them into either *Escherichia coli* or mammalian AD293 cells (47). Treatment of plasmids with 2.5 mM peroxynitrite resulted primarily in GC→TA (65%) transversions, GC→CG (28%) transversions, and GC→AT transitions (11%), suggesting a different spectrum of mutations from that observed with agents which induce nitrosative stress (Table I). Agents such as ferrous ion, which induce oxidative damage to DNA, also primarily lead to the formation of transversions. Thus, this type of mutation may be indicative of oxidative stress while under conditions of nitrosative stress transitions appear to occur predominately.

The roles of NO and peroxynitrite in genotoxicity are complex, and experiments using these compounds to examine DNA mutations should be performed under conditions as close to those which actually occur *in vivo* as possible. Peroxynitrite is often administered as a large bolus of the synthetically generated compound. It should be realized that these preparations are contaminated with excessive nitrite and hydrogen peroxide, thereby possibly influencing the results obtained in these studies. Unlike the chemistry which occurs upon bolus delivery of peroxynitrite, the actions of peroxynitrite formed *in vivo* depend on the relative fluxes of NO and superoxide in a given microenvironment (48). The amount of peroxynitrite which reacts directly with the biological target is minimal if the flux of NO exceeds that of superoxide.

In fact, damage to DNA mediated by XO and hydrogen peroxide is abated by NO (49,50), presumably by attenuation of the Fenton chemistry which mediates DNA strand breaks. Furthermore, hydroxylation reactions are also quenched by NO (22,48). These results suggest that the presence of NO could abate the chemistry mediated by oxidants generated in classical Haber-Weiss chemistry. Taken together, these protective effects indicate that the direct modification of DNA by RNOS might be limited *in vivo*, while NO may actually protect against the chemistry of oxidative stress.

The induction of strand breaks, as well as oxidation and deamination of nucleic acids, requires the kinds of high concentrations of RNOS or NO which may occur only rarely. *In vivo*, antioxidants and RNOS scavengers such as ascorbate and GSH are abundant, decreasing the chance that RNOS or NO would accumulate in sufficient concentrations to modify DNA directly. Furthermore, cells treated with NO donors showed no appreciable mutations in the *p53* gene, as compared to high mutation frequencies in this gene after treatment with genotoxic substances such as ethylnitrosourea (51). These data suggest that NO and derived RNOS are not particularly potent mutagens, and raises the possibility of other roles for NO in genotoxic mechanisms.

In addition to the formation of nitrosamines and deamination of bases in DNA, recent studies indicate that NO may act indirectly by affecting the enzymatic activity of several DNA repair proteins. Initial studies were conducted to determine what types of mutations escaped repair in prokaryotic and eukaryotic systems. Plasmid shuttle vectors were treated with either NO gas under aerobic conditions (52), NO donor compounds (53), or acidic nitrite (54), and the rescued plasmids were assayed for mutations. The distribution of types of mutations varied, as indicated by Table I. Though many of the mutations were attributed to deamination, a recent study has suggested that this does not occur in human tumor lines (55). Such discrepancies, as well as the apparently protective effects of NO *in vitro* described above, led us to the hypothesis that NO and/or RNOS may affect DNA repair mechanisms and thus lead to mutations in DNA indirectly.

RNOS have a particular high affinity for amino acids containing thiol residues (56) suggesting that enzymes which have thiol residues critical to their function may be inhibited. We therefore examined the effect of NO on several purified DNA repair proteins, and found that the enzymatic activities of both purified alkyl transferase and alkyl transferase activity from whole cells exposed to NO were significantly inhibited (5). DNA alkyl transferase is involved in the repair of O^6-methylguanine and O^4-methylthymine residues, and contains a thiol group in its active site (57,58). We proposed that nitrosation of thiol residues in the active site, critical for repair of lesions induced by alkylation, was the primary mechanism of action of NO.

Another important class of DNA-binding proteins includes those containing zinc finger motifs (59). Zinc finger motifs contain two to four cysteine residues and up to two histidine residues. Formamidopyrimidine-DNA glycosylase (Fpg protein) is a zinc-finger protein which repairs oxidative damage to guanine, including such lesions as 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine (Fapy) and 8-oxoguanine, by glycosylase activity or by incision of DNA at abasic sites by α β δ elimination reactions. The zinc finger motif is required for the activities of the Fpg protein (60). Both activities of the Fpg protein were inhibited in the presence of NO under aerobic conditions, perhaps through the nitrosation of thiols by N_2O_3, subsequent ejection of the zinc, and consequent degradation of the structural integrity of the protein (61). Another study showed that the zinc finger protein LAC9 was degraded by NO, accompanied by the formation of *S*-nitrosothiol adducts as determined by Raman spectroscopy (62).

Another DNA repair enzyme which is inhibited by RNOS is DNA ligase. The chemistry of the NO/O_2 reaction suggests that at neutral pH nitrosation of cysteine should predominate over that of other amino acids, partly due to the fact that amine-containing amino acids are protonated at neutral pH. However, the enzymatic activity of T4 DNA ligase is inhibited by NO, despite having an active site which does not contain cysteine but instead contains a partially deprotonated lysine residue. This lysine is nitrosated and undergoes deamination analogously to the process described in equations 1 and 2, which results in inhibition of enzymatic activity (63). In addition to these *in vitro* findings, other experiments also showed that NO was capable of reducing the activity of ligase in mammalian cells (63).

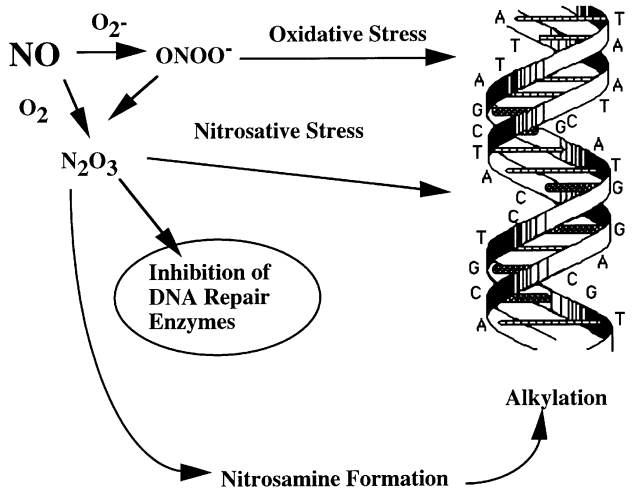

Fig. 2. Potential mechanisms for participation of NO in various genotoxic mechanisms.

Exposure of cells to NO results in an increased number of DNA single strand breaks (39). However, when purified DNA was exposed to NO, even at accumulated doses as high as 1 M (which can lead to the formation of RNOS), no single strand breaks were observed (52). This finding implies that direct chemical modification of DNA by NO or RNOS does not lead to DNA breaks, and thus DNA strand breakage *in vivo* must occur through other mechanism(s). One possibility is the inhibition of the activity of DNA ligase by NO, resulting in the accumulation of DNA strand breaks formed either during transcription or repair. The increase in DNA breaks due to NO-mediated inhibition of ligase could in turn activate the tumor suppressor gene, *p53* (64), or activate poly(ADP-ribose) synthetase (65).

Nitric oxide can affect the expression and activity of proteins critical to the cell cycle and apoptosis, which are in turn influenced by mutations in DNA (64,66,67). The regulatory effects of these oncogenes are critical for proper cellular growth and differentiation. The modulation of several oncogenes by NO has been examined, in particular those oncogenes associated with apoptosis. Exposure of cells to NO donors resulted in the upregulation of the tumor suppressor gene *p53* (66), possibly in response to DNA damage mediated by NO. Though deamination did not occur following exposure to similar amounts of NO delivered by an NO donor compound (51), the DNA damage could have resulted from inhibition of DNA repair proteins such as DNA ligase (63). The expression of *p53* has been associated with reduced expression of iNOS, which suggests that wild-type *p53* may be critical for the control of genotoxicity mediated by NO (66). It is not known whether mutated *p53* could bring about a similar reduction in iNOS.

The mechanisms by which NO participates in genotoxic events involve the indirect chemistry of NO (Figure 2). For such chemistry to occur *in vivo* requires high localized concentrations of NO such as that generated by iNOS. It would therefore be reasonable to expect that sites of potential carcinogenic risk are those which exhibit prolonged expression of iNOS, such as during episodes of chronic inflammation. Though RNOS can modify DNA, the yield and repair of these lesions do not make them powerful mutagens. Instead, in addition to the formation of carcinogenic nitrosamines, we are beginning to appreciate the indirect roles of NO in genotoxicity

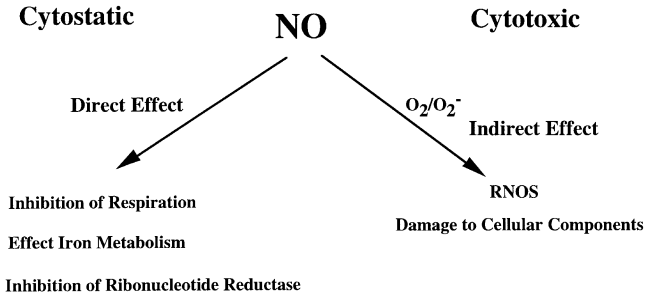

Fig. 3. Cytotoxic and cytostatic action of NO.

which may serve to increase the susceptibility of cells to other genotoxic agents.

NO and tumor biology

As discussed above, there exists a variety of mechanisms by which NO can mediate genotoxicity, and thus be considered a tumor initiating agent. However, NO may impact other stages of cancer development. These effects of NO are broad and often self-contradictory, spanning its involvement in cytostatic processes, cellular transformation, formation of neoplastic lesions, and regulation of various aspects of tumor biology. To this point we have focused on the intracellular events mediated by NO; we will now discuss the intercellular effects of NO.

Tumoricidal effects of NO

In seminal experiments involving cocultures of macrophages and lymphoma cells, NO generated from macrophages was shown to inhibit cellular respiration in the target cells (Figure 3) (2,3). Later reports demonstrated that NO derived from macrophages, Kupffer cells, natural killer cells, and endothelial cells participates in tumoricidal activity against many types of tumors (68–79). These studies suggest that NO has a cytostatic and/or cytotoxic effect on tumor cells.

Several molecular targets, such as aconitase and ribonucleotide reductase, have been implicated in the cytostasis/cytotoxicity mediated by NO. One of the first targets of NO associated with the tumoricidal activity of macrophages was mitochondrial aconitase (80). There are two enzymes which possess aconitase activity, mitochondrial aconitase and iron-responsive binding protein (IRB) which is found in the cytosol. Aconitase and iron-responsive binding protein contain an Fe_4S_4 center in which three iron molecules are bound via cysteine to the protein, while apical iron binds substrate. Several studies suggest that aconitase activity is modified by oxidation mediated by superoxide and peroxynitrite, and to a lesser extent by hydrogen peroxide and oxygen, but not by NO (81,82). Though these data suggest that indirect effects are primarily responsible for aconitase inhibition, the finding that anaerobic solutions of NO inactivate aconitase reversibly suggests that direct effects of NO are also involved (83).

The IRB is critical in regulating the transcription of iron responsive elements (IRE; RNA structures) which regulate the transferrin receptor (T_fR) or ferritin post-transcriptionally (84). The IRB exists in two forms: the holoprotein, which contains Fe_4S_4 and possesses aconitase activity but which cannot bind to the IRE; and the apoprotein, which has no aconitase activity but can bind to the IRE. Stimulation of iNOS activity increases cellular uptake of iron (83,85), while NO derived either from 100 μM SNAP or from nNOS following treatment of cortical

neurons with NMDA resulted in the binding of IRB to IRE (86). As is the case with the mitochondrial aconitase, superoxide and peroxynitrite inhibit the the aconitase activity of IRB, while NO does not (87). However, NO does stimulate the binding of IRB to the IRE, unlike superoxide or peroxynitrite (87). Superoxide and peroxynitrite may modify the IRB such that it cannot bind to the IRE effectively, probably by oxidation of a critical thiol group [see reviews in (88,89)]. Therefore, these reactive species may inhibit the protein irreversibly, abolishing both aconitase activity and IRE binding. If this were the case, the direct effects of NO would result in increased iron uptake; whereas, the indirect effects of NO or ROS would result in decreased iron uptake. These differential effects may be crucial in tumor growth versus the cytotoxicity/cytostasis mechanisms mediated by the immune system against tumor cells.

There are other iron metabolism proteins which are affected by NO, including the reaction of NO with ferritin to form Fe-NO complexes. As discussed above, cellular exposure to NO results in a detectable Fe-NO (90). An iron nitrosyl can be formed from ferritin, resulting in labilization of iron (90,91). Ferrochelatase, which is involved in the synthesis of heme proteins, is also inhibited by NO. Thus, NO appears not only to suppress cellular respiration but also to shift iron metabolism, which may contribute to cytostasis properties of NO.

Another important reaction implicated in cytostasis is that between NO and the tyrosyl radical species formed in ribonucleotide reductase (92–95). During normal catalytic turnover of this enzyme, a tyrosyl radical is formed. Ribonucleotide reductase is inhibited by NO, presumably by the reaction with this tyrosyl radical (96,97). Inhibition of this enzyme has been proposed as another factor in the cytostatic properties of NO, due to the suppression of DNA synthesis through the salvage pathway.

Nitric oxide donors appear to reduce the viability of several tumor lines (98) perhaps by deleting intracellular stores of GSH making the cell suspetible to other toxic mechanisms (56,98,99). Cells exposed to high fluxes of NO for short periods of time, through the use of chemical NO donors with short half-lives in solution, exhibited increased sensitivity to NO. However, when longer-lasting NO donors were used, there was little difference in proliferation in the presence or absence of intracellular GSH (98). Thus, high concentrations of NO may result in the formation of RNOS which mediates cell death, while lower fluxes of NO may interact with metal/tyrosyl radicals to mediate cytostasis. Macrophages in direct contact with tumor cells would be expected to generate 4–5 μM NO (100) and thus mediate indirect effects, whereas tumor cells farther away would experience lower fluxes of NO associated with direct effects.

Though most of the observations on the tumoricidal role of NO have been derived *in vitro*, additional evidence in tumor-bearing animals supports the contention that NO derived from leukocytes may have an antitumor role. Melanoma cells which were transfected with or stimulated to express iNOS show reduced cell growth *in vitro* and limited tumorigenesis and metastasis *in vivo* (101–104). These observations are supported by studies indicating that NO donors inhibit angiogenesis, tumor growth, and metastasis (105).

Some tumors have evolved mechanisms by which to suppress the expression of iNOS. Macrophages harvested from tumor-bearing animals exhibit a reduced ability to produce NO and diminished tumoricidal activity (106–108). Several studies demonstrate suppressed expression of iNOS in macrophages from tumor-bearing mice, which is thought to be due to systemic formation of tumor-derived suppressor agents such as IL-10, TGF-β1 and PGE_2 (109–111). These findings suggest a causal relationship between NO formation and tumoricidal activity.

One possible consequence of NO production is apoptosis, and this process has been implicated in the tumoricidal activity of NO (112). However, another mechanism by which the expression of iNOS may be reduced involves, paradoxically, apoptotic events within the growing tumor. Mastocytoma cells (113), sarcoma cells (102,114), L929 cells (114,115) and melanoma cells (103) all undergo extensive apoptosis upon exposure to NO, while other tumor cell lines such as A549 undergo limited apoptosis (5–20%) when exposed to chemical NO donors (116)(Y.Vodovotz, M.H.Barcellos-Hoff, and D.Wink, unpublished observations). Lymphocytes undergoing apoptosis present phosphotidylserine on their plasma membrane, and macrophages are then stimulated to phagocytose these lymphocytes (117). Phosphatidic acid suppresses the activity iNOS both *in vitro* (118,119) and in peritoneal macrophages from tumor bearing animals, at the transcriptional level (119). This leads to the possibility that apoptosis and subsequent presentation of phosphotidylserine may reduce NO generated from macrophages and result in reduction of antitumor activity within a given tumor.

The ability to treat metastatic disease effectively is a major clinical problem, and some reports suggest that NO may contribute to suppression of metastasis. Nitric oxide produced endogenously by tumor cells may reduce their metastatic potential, since transfection of iNOS into metastatic melanoma cells resulted in a dramatic decrease in metastasis (101). Additionally, NO produced by the host cells may also affect metastasis, by blocking the adhesion of tumor cells to the venular side of the microcirculation. Korthusis and co-workers have shown that NO inhibits tumor cell adhesion (120) in a manner similar to the inhibition of leukocyte adhesion described for NO in ischemia reperfusion injury (121–123). These data may suggest that low levels of NO produced by the endothelium will reduce metastasis to tissues such as the lung. However, the route of administration of NO may affect this outcome, since inhaled NO did not prevent the metastasis of melanoma cells to the lung (124). Other reports suggest that NO produced by the endothelium of the liver prevents metastasis of lymphoma cells (125) while NO produced in the vasculature of the brain limits the spread of colon cancer to that tissue (126). In addition, NO secreted by microglial cells might also suppress the spread of cancer to the brain (126).

Tumor-promoting effects of NO

In striking contrast to the antitumor roles of NO described above, NO has been proposed to be an important mediator of tumor growth. Within the paradigm of the multistage carcinogenesis model described above, NO has been reported to act in other stages of cancer growth in addition to initiation. For example, endogenously formed NO appeared to cause the neoplastic transformation of C3H 10T1/2 mouse fibroblasts (127). Another example of a role in tumor promotions is the NO-mediated secretion of mucin by colonic adenocarcinoma cells, which is thought to protect the tumor (128). Human adenocarcinoma (DLD-1) and murine mammary carcinoma (EMT-6) expressing iNOS show inhibited growth *in vitro*. However, contrary to melanomas which express NOS, these

cell lines are considerably more aggressive when transplanted into mice (129,130). This strongly suggests that NO production by these cells promotes tumor growth. Other studies suggest that 5-FUdR activity in colon cancer may be due in part to reduction in iNOS expression and may account for the activity of this chemotherapeutic drug (131). In order to address the issue of possible involvement of NO in tumor promotion, one must first examine the expression of isoforms of NOS by tumor cells or infiltrating leukocytes, and whether direct or indirect effects of NO might be expected to predominate based on the amount of NO produced by each isoform.

Both constitutive and inducible forms of NOS are present in tumors. Both isoforms of NOS have been detected in human breast tumors (132), cervical tumors (133), tumors associated with the central nervous system (134), colon (129,135), and head and neck (136) cancers. iNOS is expressed after cytokine stimulation in mammary carcinoma (Bani *et al*. 1995), melanoma (101) and human colon adenocarcinoma (129), and is expressed in breast cancer in human patients (132). Biopsies of human mammary tumors show that there is greater expression of iNOS in higher tumor grades which tend to be more invasive (129). These data support the hypothesis that NO may play a critical role in the growth and spread of tumors.

One way that NO could play an important role in tumor progression is in the regulation of angiogenesis. Enhanced angiogenesis can lead to accelerated growth of the primary tumor, as well as facilitating the process of metastasis (137,138). However, the data regarding how NO regulates angiogenesis are controversial. The process of angiogenesis will not be reviewed in detail here, but it is well established that the primary mediator of this process in tumors is the cytokine, vascular endothelial growth factor (VEGF). This cytokine is produced by many cell types in response to hypoxia and in some tumors its production may be independent of oxygenation status. VEGF binds to specific receptors on vascular endothelium, which in turn stimulates three processes that are essential for angiogenesis to proceed. First, enhanced vascular permeability leads to the formation of a provisional fibrin matrix, which provides a scaffolding for endothelial cell migration. Next, the cytokine stimulates endothelial cell proliferation and migration into the provisional matrix. The process is modulated by a number of other co-factors, including TNF_a, TGF_b, bFGF and the angiogenic activity of some of these factors may also be regulated by NO (139). It is also important to note that the hyperpermeability of vascular endothelium that is stimulated by VEGF occurs via stimulation of NO synthesis (140). Evidence for a pro-angiogenic effect of NO comes from the following observations: (i) exposure of glioblastoma and hepatocellular carcinoma cell lines to SNAP and NOR3 (both NO donor compounds) increased VEGF production, primarily by stabilizing mRNA levels (141); (ii) use of NO donors leads to increased angiogenesis in the cornea pocket assay, when angiogenesis is stimulated by substance P (142); (iii) use of NO donors stimulates proliferation of coronary post capillary endothelial cells, *in vitro* (143) and (iv) the human colon tumor line, DLD1, which was transfected with the NO synthase gene, grew more quickly and had better vascularized tumors than the parent line (129). There are also data which indicate that NO may actually downregulate angiogenesis. Examples of such evidence include: (i) VEGF production by arterial smooth muscle cells is down-regulated by NO. In this case, the inhibition occurs by inhibition of AP1 binding to the VEGF promoter (144); (ii) production of VEGF and its receptors are downregulated in *ex-vivo* perfused lungs and angiogenesis is inhibited in the chick corioallantoic membrane, when exposed to exogenous NO and upregulated when NO synthase inhibitors are used (145,146); (iii) primary tumor growth and metastasic frequency are lowered in the Lewis lung tumor model when animals are administered NO donor drugs (105) and (iv) proliferation and migration of endothelial cells is inhibited *in vitro* in the presence of NO donor drugs (147,148). It is clear from the data that are available thus far that NO has both positive and negative effects on angiogenesis.

However, two sets of data in tumor models suggest that it plays a positive role in stimulating angiogenesis. The results from the Lewis lung tumor study are difficult to interpret, since angiogenesis was not measured directly and it is likely that the tumors were made more hypoxic as a result of hypotension induced from the administration of the NO donor compounds (149). This would likely slow the growth of the primary tumor. Reasons for the discrepant results in other model systems may relate to the environment in which the cells were exposed to NO, the types of cells in which the assay was done or the presence or absence of other co-factors that are important in the angiogenic process. Clearly, the issue is very complicated and additional work is needed to further elucidate the role that it plays in tumor angiogenesis.

Another mechanism by which NO may promote tumor growth is by modulating the production of prostaglandins. NO increases the production of PGE_2, which may in turn increase the leakiness of tumor vasculature (106,150). Prostaglandin synthase activity is enhanced by NO (151), and several studies suggest that NO can shift the balance in arachidonic acid metabolism to favor prostaglandin synthases while limiting lipoxygenase products. The control of the production of NO and PGE_2 may affect the host's antitumor response adversely, since PGE_2 also suppresses NO-dependent macrophage tumoricidal activity. Additionally, permeability of the tumor vasculature is mediated by NO produced by the tumor cells themselves (152,153). Enhanced permeability may facilitiate angiogenesis, thus facilitating further tumor growth. It has also been speculated that higher levels of nutrients may be taken up by tumors in response to enhanced permeability, which supports further tumor growth (154). This latter hypothesis is an unlikely explanation, however, since transport of most critical nutrients (oxygen, glucose) is not dependent upon vascular permeability to any great extent (155). Therefore, it is possible that the effects are more indirect. One potential explanation is that activation of PGE_2 by NO may bring about the suppression of NO production and concomitant tumoricidal activity of macrophages, while facilitating angiogenesis.

The suppression of proliferation and infiltration of leukocytes is another systemic effect of NO of relevance in cancer biology. Several studies indicate that T cell proliferation is limited by NO, with adverse consequences for the antitumor response of the host (107,156). Furthermore, administration of NOS inhibitors results in increased activity of lymphocyte activated killer cells, thus limiting tumorigenesis (153). This may indicate that NO is essential in controlling the proliferation of tumor-infiltrating T-cells as well as at more distant sites.

Several reports have indicated that NO produced by tumor cells may prevent infiltration of leukocytes. One report suggests that inhibitor effect allows a greater infiltration of leukocytes in to the tumor (107). It was also shown that systemic LPS administration causes leukocyte adhesion in normal vascula-

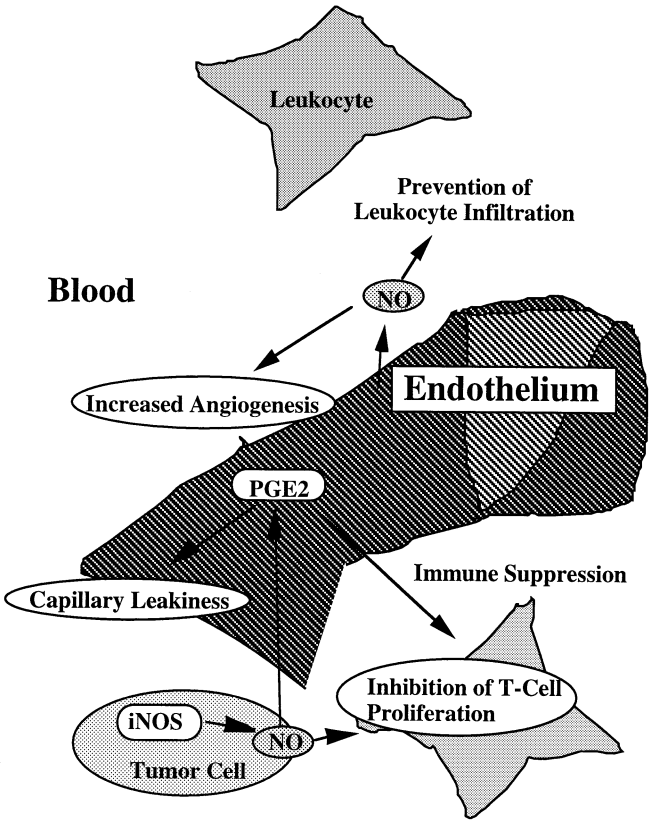

Fig. 4. Potential roles of NO in promoting tumor growth.

ture but not in tumor vascular and suggested that NO was secreted by the tumor cells to prevent adhesion (140). A study showed that ischemia reperfusion injury which stimulated more leukocyte infiltration was abated by NO donors (157). These studies would suggest that NO, in addition to increasing the vascular leakiness, downregulates expression of some adhesion molecules, such as VCAM, which are important for inflammatory and immune cell adhesion to vascular endothelium (158). In this context, radiation may tip the balance in favor of the immune system. We have found that tumor-infiltrating leukocytes derived from fibrosarcomas grown and irradiated *in vivo* (and subsequently regressing) produced 4-fold more NO *ex vivo* than did either leukocytes from unirradiated mice or tumor cells from either unirradiated or irradiated mice (Y.Vodovotz, D.Wink, and J.B.Mitchell, unpublished observations).

In summary, both direct and indirect effects appear to be involved in both the tumor-promoting and tumor-suppressing roles of NO. In the context of immune surveillance, direct interactions of NO with tyrosyl radicals and iron proteins can result in reversible cytostasis. In the context of tumor physiology, NO prevents binding of tumor cells to the endothelium probably through low fluxes of NO and hence direct effects. Additionally, other tumor promoting effects, such as the prevention of leukocyte infiltration, suppression of T-cell proliferation, increased vascular permeability, and angiogenesis may also be mediated through direct effects of NO. In contrast, we suggest that the potentially genotoxic effects of NO are mediated by indirect effects. Likewise, the cytotoxicity attributed to NO is probably instead mediated through RNOS.

Both the direct and indirect effects of NO are dictated by both amount and flux, and it is the former which may be an important determinant whether NO promotes or inhibits tumor growth (Figure 4). This may be illustrated best by comparing the transfection of NOS-containing tumor cells which produce different amounts of NO. Murine melanoma cells generate up to 10 times more NO than the human colon adenocarcinoma DLD-1. In the case of DLD-1, the tumor is far more aggressive *in vivo* than the NOS deficient parental cells. However, the melanoma cells expressing NOS are considerably less tumorigenic. This may be due in part to direct effects mediated by low concentrations of NO produced by DLD-1, whereas the melanoma cells generate copious amounts NO which cause apoptosis through indirect effects (103).

The role of NO in cancer is multi-dimensional. However, its effects can be put into perspective based on timing, location, and concentration. Tissues exposed to high concentrations of NO over long periods of time, such as during episodes of chronic inflammation or through environmental exposure, could accumulate mutations either because of NO itself or through the potentiation of other genotoxic agents. As the tumor begins to grow, macrophages and natural killer cells use NO derived from iNOS to kill tumor cells. However, as the tumor progresses, NO can mediate capillary leakiness, support angiogenesis, and limit leukocyte infiltration. Yet, NO could also limit metastasis and cause the apoptotic death of tumor cells. So it is important to consider timing and location as to what effect NO might have on a particular aspect of cancer biology. This is important in determining the proper use of systemic NOS inhibitors and NO donors, and requires a comprehensive knowledge of the chemistry, biochemistry, toxicological mechanisms, and physiological properties of NO.

References

1. Nathan,C. and Xie,Q. (1994) Regulation of biosynthesis of nitric oxide. *J. Biol. Chem.*, **269**, 13725–13728.
2. Hibbs,J.B., Vavrin,Z. and Taintor,R.R. (1987) L-arginine is required for the expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. *J. Immunol.*, **138**, 550–565.
3. Stuehr,D.J. and Nathan,C.F. (1989) A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *J. Exp. Med.*, **169**, 1543–1555.
4. Marletta,M.A. (1988) Mammalian synthesis of nitrite, nitrate, nitric oxide and N-nitrosating agents. *Chem. Res. Toxicol.*, **1**, 249–257.
5. Laval,F. and Wink,D.A. (1994) Inhibition by nitric oxide of the repair protein O^6-methylguanine-DNA-methyltransferase. *Carcinogenesis*, **15**, 443–447.
6. Cook,J.A., Krishna,M.C., Pacelli,R., DeGraff,W., Liebmann,J., Russo,A., Mitchell,J.B. and Wink,D.A. (1997) Nitric oxide enhancement of melphalan-induced cytotoxicity. *Br. J. Cancer*, **76**, 325–334.
7. Wink,D.A., Cook,J.A., Christodoulou,D., Krishna,M.C., Pacelli,R., Kim,S., DeGraff,W., Gamson,J., Vodovotz,Y., Russo,A. and Mitchell,J.B. (1997) Nitric oxide and some NO donor compounds enhance the cytotoxicity of cisplatin. *Nitric Oxide Biol. Chem.*, **1**, 3–17.
8. Misra,R.R., Hochadel,J.F., Smith,G.T., Waalkes,M.P. and Wink,D.A. (1996) Evidence that nitric oxide enhances cadmium toxicity by displacing the metals from metallothionein. *Chem. Res. Toxicol.*, **10**, 326–332.
9. Moncada,S., Palmer,R.M.J. and Higgs,E.A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.
10. Bredt,D.S. and Snyder,S.H. (1992) Nitric oxide, a novel neuronal messenger. *Neuron*, **8**, 3–11.
11. Guo,F.H., De Raeve,H.R., Rice,T.W., Stuehr,D.J., Thunnissen,F.B. and Erzurum,S.C. (1995) Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium *in vivo*. *Proc. Natl Acad. Sci. USA*, **92**, 7809–7813.
12. Lefer,A.M., Ma,X., Weyrich,A.S. and Scalia,R. (1993) Mechanism of the cardioprotective effects of transforming growth factor b1 in feline myocardial ischemia and reperfusion. *Proc. Natl Acad. Sci. USA*, **90**, 1018–1022.

13. Yu,W.A. (1994) Nitric oxide synthase in motor neurons after axotomy. *J. Histochem. Cytochem.*, **42**, 451–457.
14. Saxon,D.W. and Beitz,A.J. (1994) Cerebellar injury induces NOS in Purkinje cells and cerebellar afferent neurons. *NeuroReport*, **5**, 809–812.
15. Jia,Y., Wang,X. and Ju,G. (1994) Nitric oxide synthase expression in vagal complex following vagotomy in the rat. *NeuroReport*, **5**, 793–796.
16. Inoue,N., Benema,R.C., Sayegh,H.S., Ohara,Y., Murphy,T.J. and Harrison,D.G. (1995) Molecular regulation of the bovine endothelial cell nitric oxide synthase by transforming growth factor-b1. *Arterioscler. Thromb. Vasc. Biol.*, **15**, 1255–1261.
17. Lu,J., Schmiege,L.M., Kuo,L. and Liao,J.C. (1996) Downregulation of endothelial constitutive nitric oxide synthase expression by lipopolysaccharide. *Biochem. Biophys. Res. Commun.*, **225**, 1–5.
18. Iida,S., Ohshima,H., Oguchi,S., Hata,T., Suzuki,H., Kawasaki,H. and Esumi,H. (1992) Identification of inducible calmodulin-dependent nitric oxide synthase in the liver of rats. *J. Biol. Chem.*, **267**, 25385–25388.
19. Wink,D.A. and Ford,P.C. (1995) Nitric oxide reactions important to biological systems: A survey of some kinetics investigations. *Methods: A Companion to Methods in Enzymology*, **7**, 14–20.
20. Wink,D.A., Feelisch,M., Vodovotz,Y., Fukuto,J. and Grisham,M.B. (1998) The chemical biology of NO. An update. R*eactive Oxygen Species in Biological Systems* (in press).
21. Wink,D.A., Hanbauer,I., Grisham,M.B., Laval,F., Nims,R.W., Laval,J., Cook,J.C., Pacelli,R., Liebmann,J., Krishna,M.C., Ford,M.C. and Mitchell,J.B. (1996) The chemical biology of NO. Insights into regulation, protective and toxic mechanisms of nitric oxide. *Curr. Topics Cellular Reg.*, **34**, 159–187.
22. Wink,D.A., Cook,J.A., Kim,S., Vodovotz,Y., Pacelli,R., Kirshna,M.C., Russo,A., Mitchell,J.B., Jourd'heuil,D., Miles,A.M. and Grisham,M.B. (1997) Superoxide modulates the oxidation and nitrosation of thiols by nitric oxide derived reactive intermediates. *J. Biol. Chem.*, **272**, 11147–11151.
23. Billiar,T.R. (1995) The delicate balance of nitric oxide and superoxide in liver pathology. *Gastroenterology*, **108**, 603–605.
24. deRojas-Walker,T., Tamir,S., Ji,H., Wishnok,J.S. and Tannenbaum,S.R. (1995) Nitric oxide induces oxidative damage in addition to deamination in macrophage DNA. *Chem. Res. Toxicol.*, **8**, 473–477.
25. Gal,A. and Wogan,G.N. (1996) Mutagenesis associated with nitric oxide production in transgenic SJL mice. *Proc. Natl Acad. Sci. USA*, **93**, 15102–15107.
26. Correa,P., Haenszel,W., Cuello,C., Tannenbaum,S. and Archer,M. (1975) A model for gastric cancer epidemiology. *Lancet*, **2**, 58–60.
27. Marquardt,H., Rufino,F. and Weisburger,J.H. (1977) Mutagenic activity of nitrite-treated foods: human stomach cancer may be related to dietary factors. *Science*, **196**, 1000–1001.
28. Taylor,H.W. and Lijinsky,W. (1975) Tumor induction in rats by feeding heptamethyleneimine and nitrite in water. *Cancer Res.*, **35**, 812–815.
29. Lintas,C.L., Clark,A., Fox,J., Tannenbaum,S.R. and Newberne,P.M. (1982) *In vivo* stability of nitrite and nitrosamine formation in the dogstomach: effect of nitrite and amine concentration and of ascorbic acid. *Carcinogenesis*, **3**, 161–165.
30. Weisburger,J.H., Marquardt,H., Hirota,N., Mori,H. and Williams,G.M. (1980) Induction of cancer of the glandular stomach in rats by an extract of nitrite-treated fish. *J. Natl Cancer Inst.*, **64**, 163–167.
31. Mirvish,S.S., Salmasi,S., Cohen,S.M., Patil,K. and Mahboubi,E. (1983) Liver and forestomach tumors and other forestomach lesions in rats treated with morpholine and sodium nitrite, with and without sodium ascorbate. *J. Natl Cancer Inst.*, **71**, 81–85.
32. Williams,D.L.H. (1988) Reagents involved in nitrosation. In Williams,D.L.H. (ed), *Nitrosation*. Cambridge Press, New York, p. 5.
33. Miles,A.M., Gibson,M., Krishna,M., Cook,J.C., Pacelli,R., Wink,D.A. and Grisham,M.B. (1995) Effects of superoxide on nitric oxide-dependent N-nitrosation reactions. *Free Radical Res.*, **233**, 379–390.
34. Liu,R.H. and Hotchkiss,J.H. (1995) Potential genotoxicity of chronically elevated nitric oxide: a review. *Mutat. Res.*, **339**, 73–89.
35. Ohshima,H. and Bartsch,H. (1994) Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat. Res.*, **305**, 253–264.
36. Liu,R.H., Baldwin,B., Tennant,B.C. and Hotchkiss,J.H. (1991) Elevated formation of nitrate and N-nitrosodimethylamine in woodchucks (*Marmota monax*) associated with chronic woodchuck hepatitis virus infection. *Cancer Res.*, **51**, 3925–3929.
37. Liu,R.H., Jacob,J.R., Tennant,B.D. and Hotchkiss,J.H. (1992) Nitrite and nitrosamine synthesis by hepatocytes isloated from normal woodchucks (*Marmota monax*) and woodchucks chronically infected with woochuck hepatitis virus. *Cancer Res.*, **52**, 4139–4143.
38. Wink,D.A., Kasprzak,K.S., Maragos,C.M., Elespuru,R.K., Misra,M., Dunams,T.M., Cebula,T.A., Koch,W. H. Andrews,A.W., Allen,J.S. and Keefer,L.K. (1991) DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science*, **254**, 1001–1003.
39. Nguyen,T., Brunson,D., Crespi,C.L., Penman,B.W., Wishnok,J.S. and Tannenbaum,S.R. (1992) DNA damage and mutation in human cells exposed to nitric oxide. *Proc. Natl Acad. Sci. USA*, **89**, 3030–3034.
40. Merchant,K., Chen,H., Gonzalez,T.C., Keefer,L.K. and Shaw,B.R. (1996) Deamination of single-stranded DNA cytosine residues in aerobic nitric oxide solution at micromolar total NO exposures. *Chem. Res. Toxicol.*, **9**, 891–896.
41. Arroyo,P.L., Hatch-Pigott,V., Mower,H.F. and Cooney,R.V. (1992) Mutagenicity of nitric oxide and its inhibition by antioxidants. *Mutat. Res.*, **281**, 193–202.
42. King,P.A., Anderson,V.E., Edwards,J.O., Gustafson,G., Plumb,R.C. and Suggs,J.W. (1992) A stable solid that generates hydroxyl radical upon dissolution in aqueous solution: reactions with proteins and nucleic acids. *J. Am. Chem. Soc.*, **114**, 5430.
43. Salgo,M.G., Stone,K., Squadrito,G.L., Battista,J.R. and Pryor,W.A. (1995) Peroxynitrite causes DNA nicks in plasmid pBR322. *Biochem. Biophys. Res. Commun.*, **210**, 1025–1030.
44. Inoue,S. and Kawanishi,S. (1995) Oxidative DNA damage induced by simultaneous generation of nitric oxide and superoxide. *FEBS Lett.*, **371**, 86–88.
45. Yermilov,V., Rubio,J. and Ohshima,H. (1995) Formation of 8-nitroguanine in DNA treated with peroxynitrite *in vitro* and its rapid removal from DNA by depurination. *FEBS Lett.*, **376**, 207–210.
46. Yermilov,V., Rubio,J., Becchi,M., Friesen,M.D., Pignatelli,B. and Ohshima,H. (1995) Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite *in vitro*. *Carcinogenesis*, **16**, 2045–2050.
47. Juedes,M.J. and Wogan,G.N. (1996) Peroxynitrite-induced mutation spectra of pSP189 following replication in bacteria and in human cells. *Mutat. Res.*, **349**, 51–61.
48. Miles,A.M., Bohle,D.S., Glassbrenner,P.A., Hansert,B., Wink,D.A. and Grisham,M.B. (1996) Modulation of superoxide-dependent oxidation and hydroxylation reactions by nitric oxide. *J. Biol. Chem.*, **271**, 40–47.
49. Pacelli,R., Krishna,M.C., Wink,D.A. and Mitchell,J.B. (1994) Nitric oxide protects DNA from hydrogen peroxide-induced double strand cleavage. *Proc. Am. Assoc. Cancer Res.*, **35**, 540.
50. Yoshie,Y. and Ohshima,H. (1997) Nitric oxide synergistically enhances DNA strand breakage induced by polyhydroxyaromatic compounds, but inhibits that induced by the Fenton reaction. *Arch. Biochem. Biophys.*, **342**, 13–21.
51. Felley-Bosco,E., Mirkovitch,J., Ambs,S., Mace,K., Pfeifer,A., Keefer,L.K. and Harris,C.C. (1995) Nitric oxide and ethylnitrosourea: relative mutagenicity in the *p53* tumor suppressor and hypoxanthine-phosphoribosyltransferase genes. *Carcinogenesis*, **16**, 2069–2074.
52. Routledge,M.N., Wink,D.A., Keefer,L.K. and Dipple,A. (1993) Mutations induced by saturated aqueous nitric oxide in the pSP189 supF gene in human Ad293 and E. coli MBM7070 cells. *Carcinogenesis*, **14**, 1251–1254.
53. Routledge,M.N., Wink,D.A., Keefer,L.K. and Dipple,A. (1994) DNA sequence changes induced by two nitric oxide donor drugs in the supF assay. *Chem. Res. Toxicol.*, **7**, 628–632.
54. Routledge,M.N., Mirsky,F.J., Wink,D.A., Keefer,L.K. and Dipple,A. (1994) Nitrite-induced mutations in a forward mutation assay: influence of nitrite concentration and pH. *Mutat. Res.*, **322**, 341–346.
55. Schmutte,C., Rideout,W.M., Shen,J.C. and Jones,P.A. (1994) Mutagenicity of nitric oxide is not caused by deamination of cytosine or 5-methylcytosine in double-stranded DNA. *Carcinogenesis*, **15**, 2899–2903.
56. Wink,D.A., Nims,R.W., Darbyshire,J.F., Christodoulou,D., Hanbauer,I., Cox,G.W., Laval,F., Laval,J., Cook,J.A., Krishna,M.C., DeGraff,W. and Mitchell,J.B. (1994) Reaction kinetics for nitrosation of cysteine and glutathione in aerobic nitric oxide solutions at neutral pH. Insights into the fate and physiological effects of intermediates generated in the NO/O_2 reaction. *Chem. Res. Toxicol.*, **7**, 519–525.
57. Ling-Ling,C., Nakamura,T., Nakatsu,Y., Sakumi,K. and Hayakawa,H. (1992) Specific amino acid sequences required for O^6-methylguanine-DNA-methyltransferase activity: analysis of three residues at or near the methyl acceptor site. *Carcinogenesis*, **13**, 837–843.
58. Zak,P., Kliebl,K. and Laval,F. (1994) Repair of O^6-methylguanine and O^4-methylthymine by human and rat O^6-methylguanine-DNA-methyltransferase. *J. Biol. Chem.*, **269**, 730–733.
59. Rhodes,D. and Klug,A. (1993) Zinc fingers. *Sci. Am.*, **268**, 56–65.
60. O'Connor,T., Graves,R.V., Murcia,G., Castaning,B. and Laval,J. (1993) Fpg protein of *Escherichia coli* is a zinc finger protein whose cystiene residues have a structural and/or function. *J. Biol. Chem.*, **268**, 9063–9070.
61. Wink,D.A. and Laval,J. (1994) The Fpg protein, a DNA repair enzyme,

is inhibited by the biomediator nitric oxide *in vitro* and *in vivo*. *Carcinogenesis*, **15**, 2125–2129.
62. Kroncke,K.-D., Fechsel,K., Schmidt,T., Zenke,F.T., Dasting,I., Wesener,J.R., Bettermann,H., Breunig,K.D. and Kolb-Bachofen,V. (1994) Nitric oxide destoys zinc-finger clusters inducing zinc release from metallothionein and inhibition of the zinc finger-type yeast transcription activator LAC9. *Biochem. Biophys. Res. Commun*., **200**, 1105–1110.
63. Graziewicz,M., Wink,D.A. and Laval,F. (1996) Nitric oxide inhibits DNA ligase activity. Potential mechanisms for NO mediated DNA damage. *Carcinogenesis*, **17**, 2501–2505.
64. Messmer,U.K., Ankarcrona,M., Nicotera,P. and Brune,B. (1994) *p53* expression in nitric oxide-induced apoptosis. *FEBS Lett.*, **355**, 23–26.
65. Zhang,J., Dawson,V.L., Dawson,T.M. and Synder,S.H. (1994) Nitric oxide activation of poly(ADP-ribose)synthetase in neurotoxicity. *Science*, **263**, 687–689.
66. Forrester,K., Ambs,S., Lupold,S.E., Kapust,R.B., Spillare,E.A., Weinberg,W.C., Felley-Bosco,E., Wang,X.W., Geller,D.A., Tzeng,E., Billiar,T. and Harris,C.C. (1996) Nitric oxide-induced *p53* accumulation and regulation of inducible nitric oxide synthase expression by wild-type *p53*. *Proc. Natl Acad. Sci. USA*, **93**, 2442–2447.
67. Sandau,K., Pfeilschifter,J. and Brune,B. (1997) The balance between nitric-oxide and superoxide determines apoptotic and necrotic death of rat mesangial cells. *J. Immunology*, **158**, 4938–4946.
68. Li,L.M., Kilbourn,R.G., Adams,J. and Fidler,I.J. (1991) Role of nitric oxide in lysis of tumor cells by cytokine-activated endothelial cells. *Cancer Res*., **51**, 2531–2535.
69. Yim,C.-Y., Bastian,N.R., Smith,J.C., Hibbs,J.B. and Samlowski,W. (1993) Macrophage nitric oxide synthesis delays progression of ultraviolet light-induced murine skin cancers. *Cancer Res*., **53**, 5507–5511.
70. Yim,C.Y., McGregor,J.R., Kwon,O.D., Bastian,N.R., Rees,M., Mori,M., Hibbs,J.B.J. and Samlowski,W.E. (1995) Nitric oxide synthesis contributes to IL-2-induced antitumor responses against intraperitoneal Meth A tumor. *J. Immunol*., **155**, 4382–4390.
71. Kurose,I., Miura,S., Fukumura,D., Yonei,Y., Saito,H., Tada,S., Suematsu,M. and Tsuchiya,M. (1993) Nitric oxide mediates Kupffer cell-induced reduction of mitochondrial energization in hepatoma cells: a comparison with oxidative burst. *Cancer Res*., **53**, 2676–2682.
72. Fukumura,D., Yonei,Y., Kurose,I., Saito,H., Ohishi,T., Higuchi,H., Miura,S., Kato,S., Kimura,H., Ebinuma,H. and Ishi,H. (1996) Role in nitric oxide in Kupffer cell-mediated hepatoma cell cytotoxicity *in vitro* and *ex vivo*. *Hepatology*, **24**, 141–149.
73. Curley,S.A., Roh,M.S., Feig,B., Oyedeji,C., Kleinerman,E.S. and Klostergaard,J. (1993) Mechanisms of Kupffer cell cytotoxicity *in vitro* against the syngeneic murine colon adenocarcinoma line MCA26. *J. Leukoc. Biol*., **53**, 715–721.
74. Klostergaard,J., Leroux,M.E. and Hung,M.C. (1991) Cellular models of macrophage tumoricidal effector mechanisms *in vitro*. Characterization of cytolytic responses to tumor necrosis factor and nitric oxide pathways *in vitro*. *J. Immunol*., **147**, 2802–2808.
75. Jiang H., Stewart,C.A., Fast,D.J. and Leu,R.W. (1992) Tumor target-derived soluble factor synergizes with IFNg and IL-2 to activate macrophages for tumor necrosis factor and nitric oxide production to mediate cytotoxicity of the same target. *J. Immunol*., **149**, 2137–2146.
76. Leu,R.W., Leu,N.R., Shannon,B.J. and Fast,D.J. (1991) IFN-g differentially modulates the susceptibility of L1210 and P815 tumor targets for macrophage-mediated cytotoxicity. Role of macrophage-target interaction coupled to nitric oxide generation, but independent of tumor necrosis factor production. *J. Immunol*., **147**, 1816–1822.
77. Cipone,G.M., Festuccia,C., Cironi,L., Cavallo,G., Chessa,M.A., Pensa,V., Tubaro,E. and Santoni,A. (1994) Induction of the nitric oxide-synthesizing pathway in fresh and interleukin 2-cultured rat natural killer cells. *Cell. Immunol*., **157**, 181–194.
78. Xiao,L., Eneroth,P.H.E. and Qureshi,G.A. (1995) Nitric oxide synthase pathway may mediate human natural killer cell cytotoxicty. *Scand. J. Immunol*., **42**, 505–511.
79. Lepoivre,M., Boudbid,H. and Petit,J.-F. (1994) Antiproliferative activity of interferon combined with lipopolysaccharide on murine adeno-carcinoma: Dependence on an L-arginine metabolism with production of nitrite and nitrate. *Cancer Res*., **49**, 1970–1976.
80. Lancaster,J.R. and Hibbs,J.B. (1990) EPR demonstration of iron-nitrosyl complex formation by cytotoxic activated macrophages. *Proc. Natl Acad. Sci. USA*, **87**, 1223–1227.
81. Castro,L., Rodrigue,M. and Radi,R. (1994) Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. *J. Biol. Chem*., **269**, 29409–29415.
82. Hausladen,A. and Fridovich,I. (1994) Superoxide and peroxynitrite inactivate aconitases, but nitric oxide does not. *J. Biol. Chem*., **269**, 29405–29408.
83. Drapiers,J.C., Hirling,H., Wietzerbin,J., Kaildy,P. and Kuhn,L.C. (1993) Biosynthesis of nitric oxide activates iron regulatory factor in macrophages. *EMBO J*., **12**, 3643–3649.
84. Klausner,R.D., Rouault,T.A. and Harford,J.B. (1993) Regulating the fate of mRNA: the control of cellular iron metabolism. *Cell*, **72**, 19–28.
85. Weiss,G., Goossen,B., Doppler,W., Pantopoulos,K., Werner-Felmayer,G. and Hentze,M.W. (1993) Translational regulation via iron-responsive elements by the nitric oxide/NO synthase pathway. *EMBO J*., **12**, 3651–3657.
86. Jaffrey,S.R., Cohen,N.A., Rouault,T.A., Klausner,R.D. and Synder,S.H. (1994) The iron-responsive elements binding protein: a target for syn aptic actions of nitric oxide. *Proc. Natl Acad. Sci*., **91**, 12994–12998.
87. Bouton,C., Raveau,M. and Drapier,J.C. (1996) Modulation of iron regulatory protein function. Further roles of nitrogen and oxygen-derived reactive species. *J. Biol. Chem*., **271**, 2300–2306.
88. Drapier,J.-C. and Bouton,C. (1996) Modulation by nitric oxide of metalloprotein regulatory activities. *BioEssays*, **18**, 1–8.
89. Hentze,M.W. and Kuhn,L.C. (1996) Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide and oxidative stress. *Proc. Natl Acad. Sci. USA*, **93**, 8175–8182.
90. Lee,M., Arosio,P., Cozzi,A. and Chasteen,N. D. (1994) Identification of the EPR-active iron-nitrosyl complexes in mammalian ferritins. *Biochemistry*, **33**, 3679–3687.
91. Bastian,N., Yim,C.Y., Hibbs,J.B. and Samlowski,W.E. (1994) Induction of iron-derived ESR signals in murine cancers by nitric oxide: evidence for multiple intracellular targets. *J. Biol. Chem*., **269**, 5127–5131.
92. Lepoivre,M., Chenais,B., Yapo,A., Lemaire,G., Thelander,L. and Tenu,J.P. (1990) Alterations of ribonucleotide reductase activity following induction of the nitrite-generating pathway in adenocarcinoma cells. *J. Biol. Chem*., **265**, 14143–14149.
93. Kwon,N.S., Stuehr,D.J. and Nathan,C.F. (1991) Inhibition of tumor cell ribonucleotide reductase by macrophage-derived nitric oxide. *J. Exp. Med*., **174**, 761–767.
94. Lepoivre,M., Fieschi,F., Coves,J., Thelander,L. and Fontecave,M. (1991) Inactivation of ribonucleotide reductase by nitric oxide. *Biochem. Biophys. Res. Commun*., **179**, 442–448.
95. Lepoivre,M., Flaman,J.M. and Henry,Y. (1992) Early loss of the tyrosyl radical in ribonucleotide reductase of adenocarcinoma cells producing nitric oxide. *J. Biol. Chem*., **267**, 22994–23000.
96. Lepoivre,M., Flaman,J.M., Bobe,P., Lemaire,G. and Henry,Y. (1994) Quenching of the tyrosyl free radical of ribonucleotide reductase by nitric oxide. Relationship to cytostasis induced in tumor cells by cytotoxic macrophages. *J. Biol. Chem*., **269**, 21891–21897.
97. Roy,B., Lepoivre,M., Henry,Y. and Fontecave,M. (1995) Inhibition of ribonucleotide reductase by nitric oxide derived from thionitrites: reversible modifications of both subunits. *Biochemistry*, **34**, 5411–5418.
98. Petit,J.F., Nicaise,M., Lepoivre,M., Guissani,A. and Lemaire,G. (1996) Protection by glutathione against the antiproliferative effects of nitric oxide. Dependence on kinetics of NO release. *Biochem. Pharmacol*., **52**, 205–212.
99. Luperchio,S., Tamir,S. and Tannenbaum,S.R. (1996) NO-induced oxidative stress and glutathione metabolism in rodent and human cells. *Free Radic. Biol. Med.*, **21**, 513–519.
100. Laurent,M., Lepoivre,M. and Tenu,J.P. (1996) Kinetic modelling of the nitric oxide gradient generated *in vitro* by adherent cells expressing inducible nitric oxide synthase. *Biochem. J*., **314**, 109–113.
101. Dong,Z., Staroselsky,A.H., Qi,X., Xie,K. and Fidler,I.J. (1994) Inverse correlation between expression of inducible nitric oxide synthase activity and production of metastasis in K-1735 murine melanoma cells. *Cancer Res*., **54**, 789–793.
102. Xie,K., Huang,S., Dong,Z., Gutman,M. and Fidler,I.J. (1995) Direct correlation between expression of endogenous inducible nitric oxide synthase and regression of M5076 reticulum cell sarcoma hepatic metastases in mice treated with liposome containing lipopeptide CGP 31362. *Cancer Res*., **55**, 3123–3131.
103. Xie,K., Huang,S., Dong,Z., Juang,S.H., Gutman,M., Xi,E.Q.W., Nathan,C. and Fidler,I.J. (1995) Transfection with the inducible nitric oxide synthase gene suppresses tumorigenicity and abrogates metastasis by K-1735 murine melanoma cells. *J. Exp. Med.*, **181**, 1333–1343.
104. Xie,K., Dong,Z. and Fidler,I.J. (1996) Activation of nitric oxide synthase gene for inhibition of cancer metastasis. *J. Leukoc. Biol*., **59**, 797–803.
105. Pipili-Synetos,E., Papageorgious,A., Sakkoula,E., Sotiropoulou,G., Fotsis,T., Karakiulakis,G. and Maragoudakis,M. E. (1995) Inhibition of angiogenesis, tumour growth and metastasis by the NO-releasing

vasodilators, isosorbide mononitrate and dinitrate. *Br. J. Pharmacol*., **116**, 1829–1834.
106. Gardner,T.E., Naama,H. and Daly,J.M. (1995) Peritoneal and splenic macrophage functions in the tumor-bearing host. *J Surg. Res*., **59**, 305–310.
107. Lejeune,P., Lagade,C.P., Onier,N., Pinard,D., Ohshima,H. and Jeannin,J.F. (1994) Nitric oxide involvement in tumor-induced immunosuppression. *J. Immunol*., **152**, 5077–5083.
108. Dinapoli,M.R., Calderon,C.L. and Lopez,D.M. (1996) he altered tumoricidal capacity of macrophages isolated from tumor-bearing mice is related to reduce expression of the inducible nitric oxide synthase gene. *J. Exp. Med*., **183**, 1323–1329.
109. Alleva,D.G., Burger,C.J. and Elgert,K.D. (1994) Tumor-induced regulation of suppressor macrophage nitric oxide and TNF-α production. Role of tumor-derived IL-10, TGF-β and prostaglandin E2. *J. Immunol*., **153**, 1674–1686.
110. Vodovotz,Y. (1997) Control of nitric oxide production by transforming growth factor-β1: mechanistic insight and potential relevance to human disease. *Nitric Oxide: Biol. Chem*., **1**, 3–17.
111. Maeda,H., Tsuru,S. and Shiraishi,A. (1994) Improvement of macrophage dysfunction by administration of anti-transforming growth factor-b antibody in EL4-bearing hosts. *Jpn. J. Cancer Res*., **85**, 1137–1143.
112. Nicotera,P., Bonfoco,E. and Brüne,B. (1995) Mechanisms for nitric oxide-induced cell death: Involvement of apoptosis. *Adv. Neuroimmunol*., **5**, 411–420.
113. Kitajima,I., Kawahara,K., Nakajima,T., Soejima,Y., Matsuyama,T. and Maruyama,I. (1994) Nitric oxide-mediated apoptosis in murine mastocytoma. *Biochem. Biophys. Res. Commun*., **204**, 244–251.
114. O'Donnell,V.B., Spycher,S. and Azzi,A. (1995) Involvement of oxidants and oxidant-generating enzyme(s) in tumour-necrosis-factor-alpha-mediated apoptosis: role for lipoxygenase pathway but not mitochondrial respiratory chain. *Biochem. J*., **310**, 133–141.
115. Cui,S., Reichner,J.S., Mateo,R.B. and Albina,J.E. (1994) Activated murine macrophages induce apoptosis in tumor cells through nitric oxide-dependent or -independent mechanisms. *Cancer Res.*, **54**, 2462–2467.
116. Vodovotz,Y., Hsing,A., Cook,J.A., Miller,R.W., Wink,D., Ritt,D.M., Mitchell,J.B. and Danielpour,D. (1997) Qualitative and quantitative analysis of DNA fragmentation using digital imaging. *Anal. Biochem*., **250**, 147–152.
117. Fadok,V.A., Voelker,D.R., Campbell,P.A., Cohen,J.J., Bratton,D.L. and Henson,P.M. (1992) Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol*., **148**, 2207–2216.
118. Calderon,C., Huang,Z.H., Gage,D.A., Sotomayor,E.M. and Lopez,D.M. (1994) Isolation of a nitric oxide inhibitor from mammary tumor cells and its characterization as phosphatidyl serine. *J. Exp. Med*., **180**, 945–958.
119. DiNapoli,M.R., Calderon,C.L. and Lopez,D.M. (1997) Phosphatidyl serine is involved in the reduced rate of transcription of the inducible nitric oxide synthase gene in macrophages from tumor-bearing mice. *J. Immunol*., **158**, 1810–1817.
120. Kong,L., Dunn,G.D., Keefer,L.K. and Korthuis,R.J. (1996) Nitric oxide reduces tumor cell adhesion to isolated rat postcapillary venules. *Clin. Exp. Metastasis*, **14**, 335–343.
121. Kubes,P., Suzuki,M. and Granger,D.N. (1991) Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc. Natl Acad. Sci USA*, **88**, 4651–4655.
122. Kubes,P., Kanwar,S., Niu,X.F. and Gaboury,J.P. (1993) Nitric oxide inhibition induces leukocyte adhesion via superoxide and mast cells. *FASEB J*., **264**, G143–G149.
123. Ma,X.-L., Weyrich,A.S., Lefer,D.J. and Lefer,A.M. (1993) Diminished basal nitric oxide release after mycardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium. *Circ. Res*., **72**, 403–412.
124. Hirano,S. (1997) In vitro and *in vivo* cytotoxic effects of nitric oxide on metastatic cells. *Cancer Lett*., **115**, 57–62.
125. Rocha,M., Kruger,A., Van Rooijen,N., Schirrmacher,V. and Umansky,V. (1995) Liver endothelial cells participate in T-cell-dependent host resistance to lymphoma metastasis by production of nitric oxide *in vivo*. *Int. J. Cancer*, **63**, 405–411.
126. Murata,J., Ricciardi-Castagnoli,P., Dessous L'Eglise Mange,P., Martin,F. and Juillerat-Jeanneret,L. (1997) Microglial cells induce cytotoxic effects toward colon carcinoma cells: measurement of tumor cytotoxicity with a gamma-glutamyl transpeptidase assay. *Int. J. Cancer*, **70**, 169–174.
127. Mordan,L.J., Burnett,T.S., Zhang,L.X., Tom,J. and Cooney,R.V. (1993) Inhibitors of endogenous nitrogen oxide formation block the promotion of neoplastic transformation in C3H 10T1/2 fibroblasts. *Carcinogenesis*, **14**, 1555–1559.
128. Gottke,M. and Chadee,K. (1996) Exogenous nitric oxide stimulates mucin secretion from LS174T colonic adenocarcinoma cells. *Inflamm. Res*., **45**, 209–212.
129. Jenkins,D.C., Charles,I.G., Thomsen,L.L., Moss,D.W., Holmes,L.S., Baylis,S.A., Rhodes,P., Westmore,K., Emson,P.C. and Moncada,S. (1995) Roles of nitric oxide in tumor growth. *Proc. Natl Acad. Sci. USA*, **92**, 4392–4396.
130. Edward,S.P., Cendan,J.C., Topping,D.B., Moldawer,L.L., MacKay,S., Copeland,E.M.I.I.I. and Lind,D.S. (1996) Tumor cell nitric oxide inhibits cell growth *in vitro*, but stimulates tumorigenesis and experimental lung metastasis *in vivo*. *J. Surg. Res*., **63**, 49–52.
131. Jin,Y., Heck,D.E., DeGeorge,G., Tian,Y. and Laskin,J.D. (1996) 5-Fluorouracil suppresses nitric oxide biosynthesis in colon carcinoma cells. *Cancer Res*., **56**, 1978–1982.
132. Thomsen,L.L., Miles,D.W., Happerfield,L., Bobrow,L.G., Knowles,R.G. and Moncada,S. (1995) Nitric oxide synthase activity in human breast cancer. *Br. J. Cancer*, **72**, 41–44.
133. Thomsen,L.L., Lawton,F.G., Knowles,R.G., Beesley,J.E., Riveros-Moreno,V. and Moncada,S. (1994) Nitric oxide synthase activity in human gynecological cancer. *Cancer Res*., **54**, 1352–1354.
134. Cobbs,C.S., Brenman,J.E., Aldape,K.D., Bredt,D.S. and Israel,M.A. (1995) Expression of nitric oxide synthase in human central nervous system tumors. *Cancer Res*., **55**, 727–730.
135. Radomski,W., Jenkins,D.C., Holmes,L. and Moncada,S. (1991) Human colorectal adenocarcinoma cells: differential nitric oxide synthesis determines their ability to aggregate platelets. *Cancer Res*., **51**, 6073–6078.
136. Prazma,J., Petrusz,P., Mims,W., Ball,S.S. and Weissler,M.C. (1995) Immunohistochemical characterization of nitric oxide synthase activity in squamous cell carcinoma of the head and neck. *Otolaryngol Head Neck Surg*., **113**, 541–549.
137. Folkman,J. (1997) Angiogenesis and angiogenesis inhibition: an overview. *EXS*, **79**, 1–8.
138. Weidner,N., Semple,J.P., Welch,W.R. and Folkman,J. (1991) Tumor angiogenesis and metastasis–correlation in invasive breast carcinoma. *N. Engl. J. Med*., **324**, 1–8.
139. Montrucchio,G., Lupia,E., de Martino,A., Battaglia,E., Arese,M., Tizzani,A., Bussolino,F. and Camussi,G. (1997) Nitric oxide mediates angiogenesis induced *in vivo* by platelet-activating factor and tumor necrosis factor-α. *Am. J. Pathol*., **151**, 557–563.
140. Wu,N.Z., Klitzman,B., Dodge,R. and Dewhirst,M.W. (1992) Diminished leukocyte-endothelium interaction in tumor microvessels. *Cancer Res*., **52**, 4265–4268.
141. Chin,K., Kurashima,Y., Ogura,T., Tajiri,H., Yoshida,S. and Esumi,H. (1997) Induction of vascular endothelial growth factor by nitric oxide in human glioblastoma and hepatocellular carcinoma cells. *Oncogene*, **15**, 437–442.
142. Ziche,M., Morbidelli,L., Masini,E., Amerini,S., Granger,H.J., Maggi,C.A., Geppetti,P. and Ledda,F. (1994) Nitric oxide mediates angiogenesis *in vivo* and endothelial cell growth and migration *in vitro* promoted by substance P. *J. Clin. Invest*., **94**, 2036–2044.
143. Morbidelli,L., Chang,C.H., Douglas,J.G., Granger,H.J., Ledda,F. and Ziche,M. (1996) Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. *Am. J. Physiol*., **270**, H411–H415.
144. Tsurumi,Y., Murohara,T., Krasinski,K., Chen,D., Witzenbichler,B., Kearney,M., Couffinhal,T. and Isner,J.M. (1997) Reciprocal relation between VEGF and NO in the regulation of endothelial integrity. *Natl Med*., **3**, 879–886.
145. Tuder,R.M., Flook,B.E. and Voelkel,N.F. (1995) Increased gene expression for VEGF and the VEGF receptors KDR/Flk and Flt in lungs exposed to acute or to chronic hypoxia. Modulation of gene expression by nitric oxide. *J. Clin. Invest*., **95**, 1798–1807.
146. Pipili-Synetos,E., Sakkoula,E., Haralabopoulos,G. Andriopoulou,P., Peristeris,P. and Maragoudakis,M.E. (1994) Evidence that nitric oxide is an endogenous antiangiogenic mediator. *Br. J. Pharmacol*., **111**, 894–902.
147. Yang,W., Ando,J., Korenaga,R., Toyo-oka,T. and Kamiya,A. (1994) Exogenous nitric oxide inhibits proliferation of cultured vascular endothelial cells. *Biochem. Biophys. Res. Commun*., **203**, 1160–1167.
148. Lau,Y.T. and Ma,W.C. (1996) Nitric oxide inhibits migration of cultured endothelial cells. *Biochem. Biophys. Res. Commun*., **221**, 670–674.
149. Shan,S.Q., Rosner,G.L., Braun,R.D., Hahn,J., Pearce,C. and Dewhirst,M.W. (1997) Effects of diethylamine/nitric oxide on blood perfusion and oxygenation in the R3230Ac mammary carcinoma. *Br. J. Cancer*, **76**, 429–437.
150. Maeda,H., Noguchi,Y., Sato,K. and Akaike,T. (1994) Enhanced vascular permeability in solid tumor is mediated by nitric oxide and inhibited by both new nitric oxide scavenger and nitric oxide synthase inhibitor. *Jpn. J. Cancer Res*., **85**, 331–334.
151. Hubbard,N.E. and Erickson,K.L. (1995) Role of 5′-lipoxygenase

metabolites in the activation of peritoneal macrophages for tumoricidal function. *Cell. Immunol*., **160**, 115–122.
152. Nakano,S., Matsukado,K. and Black,K.L. (1996) Increased brain tumor microvessel permeability after intracarotid bradykinin infusion is mediated by nitric oxide. *Cancer Res*., **56**, 4027–4031.
153. Orucevic,A. and Lala,P.K. (1996) Effects of N(g)-methyl-L-arginine, an inhibitor of nitric oxide synthesis, on interleukin-2-induced capillary leakage and antitumor responses in healthy and tumor-bearing mice. *Cancer Immunol. Immunother.*, **42**, 38–46.
154. Noguchi,Y., Fujii,S., Beppu,T., Ogawa,M. and Maeda,H. (1996) Excessive production of nitric oxide in rat solid tumor and its implication in rapid tumor growth. *Cancer*, **77**, 1598–1604.
155. Curry,F.R. (1985) Effect of albumin on the structure of the molecular filter at the capillary wall. *Fed. Proc*., **44**, 2610–2613.
156. Mills,C.D. (1991) Molecular basis of 'suppressor' macrophages. Arginine metabolism via the nitric oxide synthetase pathway. *J. Immunol*, **146**, 2719–2723.
157. Parkins,C.S., Dennis,M.F., Stratford,M.R., Hill,S.A. and Chaplin,D.J. (1995) Ischemia reperfusion injury in tumors: the role of oxygen radicals and nitric oxide. *Cancer Res*., **55**, 6026–6029.
158. Khan,B.V., Harrison,D.G., Olbrych,M.T., Alexander,R.W. and Medford,R.M. (1996) Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc. Natl Acad. Sci. USA*, **93**, 9114–9119.

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