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Review Article

# Targeting nitric oxide for cancer therapy

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## Abstract

A blueprint for the ideal anticancer molecule would include most of the properties of nitric oxide ($NO^{\bullet}$), but the ability to exploit these characteristics in a therapeutic setting requires a detailed understanding of the biology and biochemistry of the molecule. These properties include the ability of $NO^{\bullet}$ to affect tumour angiogenesis, metastasis, blood flow and immuno surveillance. Furthermore $NO^{\bullet}$ also has the potential to enhance both radio- and chemotherapy. However, all of these strategies are dependent on achieving appropriate levels of $NO^{\bullet}$, since endogenous levels of $NO^{\bullet}$ appear to have a clear role in tumour progression. This review aims to summarize the role of $NO^{\bullet}$ in cancer with particular emphasis on how the properties of $NO^{\bullet}$ can be exploited for therapy.

## Introduction

Nitric oxide is an inorganic gas that previous to 1987 was regarded as a toxic pollutant produced by internal combustion engines and power stations (Brennan 2000). It was then shown to be responsible for the actions of endothelium-derived relaxing factor (EDRF) (Palmer et al 1987) and it was this finding that led to an explosion of interest with thousands of papers having been published to date. The molecular arrangement of $NO^{\bullet}$ leaves an unpaired electron, making the molecule a highly reactive free radical. Its small size and lipophilicity mean that $NO^{\bullet}$ can easily diffuse through cell membranes (Kroncke 2001) and depending on the conditions, it is able to diffuse distances of several hundred microns through tissue, making $NO^{\bullet}$ a perfect signalling molecule.

The ubiquitous role of nitric oxide as a key regulator of cellular processes is now very well established (Moncada 1999; Osorio & Recchia 2000) as is its role in the cytotoxic response of leucocytes to invading pathogens (Albina & Reichner 1998). These two classes of action may, at first sight, appear incompatible; the regulation of smooth muscle tone or control of platelet aggregation requires the local generation of precisely controlled quantities of $NO^{\bullet}$ to maintain vascular parameters within physiological bounds (Somers & Harrison 1999), whereas cytotoxicity (mainly indirectly through the interaction with superoxide (Li et al 2000)) requires the rapid generation of concentrations that are several orders of magnitude higher (Leiro et al 2001). Not surprisingly, these disparate functions require the involvement of different enzymes in the $NO^{\bullet}$ synthetic reaction. It is now generally accepted that there are three main isoforms of nitric oxide synthase (NOS): eNOS, a calcium-dependent isoform, the activation of which regulates cell signalling outside the CNS; nNOS, also calcium dependent, which is involved in signalling generally within the CNS; and iNOS, the inducible isoform whose expression is regulated by a variety of factors including bacterial lipopolysaccharides and which is capable of generating high concentrations of $NO^{\bullet}$. In all cases the substrate for the reaction is the amino acid L-arginine.

All three of these isoforms have been detected in tumours (Tozer & Everett 1997). Although the influence of each on tumour pathophysiology is not fully understood, it is clear that endogenous $NO^{\bullet}$ production in tumours has profound effects on tumour blood flow, angiogenesis and metastatic potential (Wink et al 1998) and it is not surprising that its manipulation has been identified by many investigators as an exciting target for cancer therapy (see Brennan 2000; Lala & Chakraborty 2001 for reviews; Figure 1 for summary). One issue that has not yet been resolved is whether there is more potential for therapeutic benefit in inhibiting $NO^{\bullet}$ production in tumours or by enhancing it. We will first examine the available data relating to the role of $NO^{\bullet}$ in malignancy. Additional information can be

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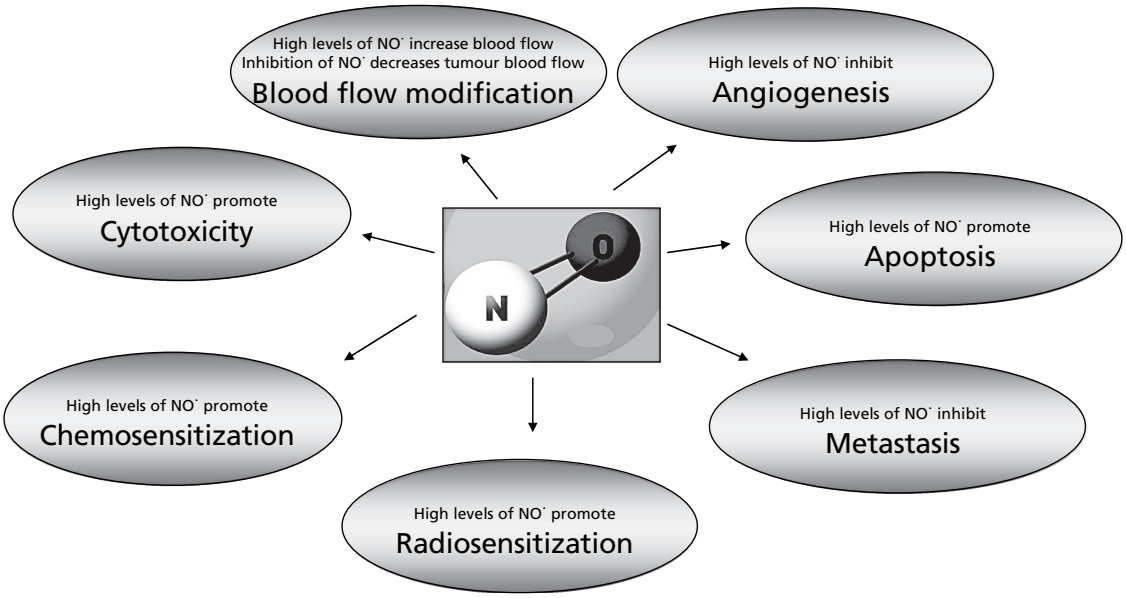


**Figure 1** $NO^{\bullet}$ has many of the ideal characteristics of an anticancer molecule.

found in recent reviews (Ekmekcioglu et al 2005; Lechner et al 2005).

## The role of $NO^{\bullet}$ in tumour biology

*Angiogenesis*

Without vascular proliferation, tumour expansion would be impossible and solid tumours would not be a significant clinical problem (Folkman 1990). It has been recognized for some time that $NO^{\bullet}$ is an important mediator of angiogenesis in a variety of in-vitro and in-vivo model systems (Guo et al 1995; Murohara et al 1998; Jadeski & Lala 1999; Ziche & Morbidelli 2000; Kashiwagi et al 2005). There are also numerous reports that higher concentrations of $NO^{\bullet}$ can be anti-angiogenic (Pipili-Synetos 1994; Lau & Ma 1996; Ray-Chaudhury et al 1996; Powell et al 2000). Generally these studies did not describe full [$NO^{\bullet}$] response curves so that pro and anti-angiogenic action of $NO^{\bullet}$ could not be fully characterized. One recent study does however give a clear picture of the concentration/response profile in an in-vitro tube formation model using gastric endothelial cells (Jones et al 2004). Tube formation was enhanced (< 1.5-fold) at $NO^{\bullet}$ concentrations up to 18.5 $\mu$M then declined progressively so that at 36 $\mu$M there was no effect and by 280 $\mu$M tube formation was dramatically (4-fold) inhibited. This kind of information is needed for other endpoints of angiogenesis in a wide variety of tissues so that therapeutic interventions involving $NO^{\bullet}$ can be designed effectively.

Little is known about the anti-angiogenic signalling pathways activated by $NO^{\bullet}$ (though anti-apoptotic mechanisms may play a role), but the pro-angiogenic mechanisms have been widely studied. As tumours outgrow their blood supply or are deprived of oxygen, a signalling response to hypoxia is initiated, which activates the transcription factor HIF-1$\alpha$, initiating transcription of a host of genes, many of which are involved in angiogenesis (Harris 2002). Stimulators of angiogenesis include growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF). Increased expression of VEGF is now well established as a major mechanism in the pro-angiogenic activity of $NO^{\bullet}$ in normal tissues (Frank et al 1999a, b) and there is evidence that this may involve inhibition of protein kinase C$\delta$ (Shizukuda et al 1999). There is also considerable evidence that similar mechanisms exist in tumours. VEGF expression has been shown to be induced in human glioblastoma and liver cancer cells by exposure to a $NO^{\bullet}$ donor drug (Chin et al 1997); conversely, exposure to inhibitors of constitutive and inducible isoforms of $NO^{\bullet}$ synthase reduced microvessel density and VEGF expression in human tumour xenografts of gastric cancer (Wang et al 2005) and inhibited development of the vascular architecture in a murine melanoma model (Kashiwagi et al 2005). $NO^{\bullet}$ also upregulates IL-8 (Andrew et al 1995; Xiong et al 2001; Hellmuth et al 2004), which has a well established role as a pro-angiogenic factor (Wakabayashi et al 1995; Charalambous et al 2005). Finally, irradiation has been shown to dose-dependently induce the activation of the pro-angiogenic $NO^{\bullet}$ pathway in endothelial cells through increases in endothelial nitric oxide synthase abundance and phosphorylation (Sonveaux et al 2003).

$NO^{\bullet}$ has also been shown to down-regulate inhibitors of angiogenesis; it acts as an inhibitor of angiostatin in a heart muscle model (Matsunaga et al 2002). Recent evidence implicates thrombospondin-1 (TSP-1) as a functional angiogenic switch that is inhibited by low concentrations (100 nM) of $NO^{\bullet}$ donor drug (Ridnour et al 2005; Isenberg et al 2005). This effect was reversed at higher concentrations (100 $\mu$M). Conversely TSP-1 inhibited $NO^{\bullet}$ mediated, pro-angiogenic signalling via extracellular signal-related kinase (pERK) (Ridnour et al 2005).

*Blood flow*

There is clear evidence that NO• production is upregulated in most tumours compared with their normal tissue counterpart (Brennan 2000). This is consistent with the concept that tumour blood vessels are maintained in a dilated condition to maximize perfusion through the inadequate vascular architecture of the tumour. The concept that endogenous NO• generation is supportive of tumour growth was first demonstrated in 1994: the growth rates of a rat carcinosarcoma and a mouse carcinoma were inhibited by ~50% when the animals were administered the non-specific NOS inhibitor $N^G$-nitro-L-arginine methyl ester (L-NAME, 70–400 mg $kg^{-1}$/day) in their drinking water (Kennovin et al 1994). The effect of NOS inhibition could be induced at any time during the growth of the tumour and was very rapidly reversible on cessation of drug administration (Kennovin et al 1994). A similar antitumour effect was seen after daily intraperitoneal administration of L-NAME (80 mg $kg^{-1}$) in a renal subcapsular CC531 adenocarcinoma model in rats (de Wilt et al 2000).

Several mechanisms may contribute to the anti-tumour effect of NOS inhibition including loss of a pro-angiogenic stimulus from low level NO• production in the tumour (see above), but other mechanisms are more likely. The rapid return to normal tumour growth rates when NOS inhibition was withdrawn suggests a physiological mechanism such as blood flow reduction. There is now overwhelming evidence that tumour blood flow modification is a major consequence of NOS inhibition in tumours. A rapid decrease in blood flow (by 55%) was observed in a rat carcinosarcoma after administration of the non-specific NOS inhibitor $N^{\omega}$ -nitro-L-arginine (L-NA, 1 mg $kg^{-1}$, i.p.), whereas there was no effect in most normal tissues (Tozer & Everett 1997; Tozer et al 1997, 1998). Reduced tumour vessel perfusion was also reported in a rat mammary carcinoma growing in a window chamber, but in this case there were effects in surrounding normal vasculature, although these were reversible with excess arginine substrate (Meyer et al 1995). In another window chamber study, L-NAME (10 mg $kg^{-1}$, i.v.) reduced vessel diameter and local perfusion in a human tumour xenograft, with the major effects occurring at the level of the arterioles (Fukumura et al 1997). A similar effect of intravenous L-NAME was seen in a rat glioma model in which blood flow was reduced by 44% compared with a 15% reduction in flow to normal brain (Swaroop et al 1998). Blood flow reductions after both L-NA and L-NAME were observed in RIF-1 and SCCVII, but not EMT6 or FSaR tumours in mice; this difference was attributed to low constitutive expression of NO• in EMT6 and FSaR (Korbelik et al 2000). Thus, in most tumours studied inhibition of NO• production results in reduced tumour blood flow. There is evidence that the reverse can also apply at least in rat glioma (Swaroop et al 1998) and mouse liver tumour (Jordan et al 2000) models, in which administration of NO• donor drugs significantly enhanced blood flow.

*Metastasis*

NO• has been shown to have potent influences on many of the key characteristics that define the metastatic phenotype of cancer cells including motility, adhesion and invasion (recently reviewed by Williams & Djamgoz 2005). The effect of NO• on motility of leucocytes has been widely studied and both stimulatory and antagonistic actions have been reported (Williams & Djamgoz 2005). There is also information on tumour cell migration. One group showed that expression of eNOS in mouse mammary carcinomas correlated with growth rate and metastasis in-vivo, and migration and invasiveness in-vitro (Jadeski et al 2000), and went on to show that guanylate cyclase and mitogen activated protein (MAP) kinase are important downstream mediators (Jadeski et al 2003). iNOS has also been implicated in the metastatic process. iNOS expression in human primary melanomas has been shown to correlate inversely with the incidence of distant metastases in liver, lung and brain (Tschugguel et al 1999), whereas the opposite correlation had been found previously in breast cancer (Duenas-Gonzales et al 1997). In an elegant experiment using iNOS–/– mice, Wei et al (2003) showed that iNOS expression in the stroma supplying tumours dramatically slowed their growth and reduced metastasis. Furthermore, overexpression of this isoform using gene transfer techniques suppressed the tumorigenicity and metastatic potential of oral cancer cells in-vivo (Harada et al 2005). Similarly, adenoviral delivery of the iNOS gene (AdiNOS) dramatically reduced the tumorigenicity and metastasis of human pancreatic cancer cells in-vivo (Le et al 2005). Conversely, spontaneous lymphomas and sarcomas in iNOS double knockout mice developed much more rapidly than in iNOS +/+ mice. This was attributed to the higher level of apoptosis, decreased proliferation and higher expression of death receptor signals (Hussain et al 2004).

One key event in the process of tumour invasion is vascular basement membrane degradation involving activation of matrix metalloproteinases (MMPs). NO• generated from the donor spermine-NONOate or peroxynitrite from SIN-1 has been shown to inhibit MMP-9 expression and activation by mechanisms involving caveolin-1 at the interface between human NSCLC cells and endothelial cells in co-culture (Phillips & Birnby 2004). However, there was a positive correlation between endogenous iNOS activity and MMP-9 expression, but not with MMP-1 and MMP-2 in human head and neck squamous cell carcinomas (HNSCC) (Franchi et al 2002). Expression of MMPs-1, 3, 10 and 13 was transcriptionally enhanced in human melanoma cell lines by exposure to the NO• donor *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) (Ishii et al 2003). Treatment with lipopolysaccharide increased lung metastases in a murine lung model and correlated with elevated levels of both iNOS and MMP-2 in the tumour nodules (Harmey et al 2002). There is also some indirect evidence that peroxynitrite ($ONOO^-$), generated from NO• and superoxide ($O_2^-$) under pathological conditions, may activate proMMPs to MMPs in tumours (Wu et al 2001). It has also been suggested that platelet aggregation facilitates the formation of metastatic foci. NO• donors such as *S*-nitroso-*N*-acetylpenicillamine and *S*-nitrosoglutathione have been shown to inhibit both platelet aggregation and MMP-2 release from platelets and tumour cells (Jurasz et al 2001). Clearly the data demonstrate that levels of NO• generated dictate whether pro- or antimetastatic mechanisms will predominate.

*Necrosis and apoptosis*

There is evidence that NO• is poorly reactive with most biomolecules, but is highly reactive with other free radicals (Halliwell et al 1999). A significant component of the cytotoxic effect of NO• is probably mediated through its

reaction with $O_2^-$ to form the apoptosis inducer $ONOO^-$ (Szabo & Ohshima 1997; Schimmel & Bauer 2001). It has been identified as one of the toxic species generated during immune surveillance (Moncada & Higgs 1991) and is responsible for a component of the damage occurring during reperfusion injury (Gursoy-Ozdemir et al 2000). This relatively simple picture is confounded, however, by several reports of a protective role for $NO^\bullet$ through interaction with other, more damaging reactive oxygen species (Wink et al 1993, 1998); thus, it has been suggested that $NO^\bullet$ can act in a pro- or antioxidant manner depending on the precise cellular targets, the levels of $O_2^-$ (Laskin et al 2001) and oxygen (Dedon & Tannenbaum 2004) and the rate of $NO^\bullet$ production in a given tissue (Jourd'heuil et al 2001). There is evidence that the balance between apoptotic and necrotic cell death is critically dependent on glucose concentrations and the glycolytic capacity of the cell (Bal-Price & Brown 2000), which could account for the extensive apoptosis seen in many tumours exposed to high levels of $NO^\bullet$.

There is now a large body of evidence, mainly from studies using either iNOS gene transfer or nitric oxide donor drugs including NO donating non-steroidal anti-inflammatory drugs (NO-NSAIDs), that concentrations of NO in the micromolar range lead to apoptosis in a wide variety of human cancer cells including prostate (PC3 and LNCaP) (Royle et al 2004), pancreas (Gansauge et al 1998; Kashfi & Rigas 2005), breast (Mortensen et al 1999), bladder (Huguenin et al 2004, 2005; Fabbri et al 2005) and colon (Kwak et al 2000; Chung et al 2003; Gao et al 2005). Extensive apoptosis has been demonstrated in murine and human tumour models in-vivo after iNOS gene therapy using direct injection of liposomal (Worthington et al 2002), adenoviral (Cook et al 2004) or targeted retroviral (Kuroki 2004) vectors. The mechanisms are not fully understood, but inhibition of survivin expression has been implicated in one study (Chao et al 2004).

*Escape from immune surveillance*

$NO^\bullet$ generation is a major constituent of the immune response to pathogens including cancer cells, so enhancement of this response has been proposed as a possible therapeutic approach (Hino et al 2005). An $NO^\bullet$-donating aspirin compound has been shown to promote eradication of mouse mammary and colon carcinomas in-vivo by enhancing immune function (De Santo, et al 2005), and inhibition of NOS with isoform-specific and non-specific agents caused a delay in the immune rejection of an immunogenic lymphoma in the mouse (Hu et al 2004). However, Yamaguchi et al (2005) have reported a negative correlation between dendritic cell infiltration into gastric cancers and the level of iNOS expression. Furthermore, the suppression of the immune response that accompanied the growth of intra-hepatic colon tumours in rats could be inhibited by administering L-NAME, implicating endogenous $NO^\bullet$ production as an immune-suppressant in this system (Hegardt et al 2001). Immune response to metastatic melanoma cells was also inhibited by NO expression (Zhang & Xu 2001).

*NOS expression and tumour characteristics*

Given the role of $NO^\bullet$ in most of the angiogenesis signalling pathways, it is perhaps not surprising that correlations have been established between the expression of NOS isoforms and tumour characteristics. A positive association was demonstrated between the level of expression of endothelial NOS (eNOS) (Pan et al 2005) and iNOS (Hara & Okayasu 2004) and the grade of malignancy in brain tumours. In breast cancer, iNOS expression was found to be significantly higher than in benign lesions, suggesting a role of iNOS in breast carcinogenesis; furthermore, disease free survival was significantly worse in cases showing strong iNOS expression (Bulut et al 2005; Loibl et al 2005) and lymph node metastasis was more likely (Duenas-Gonzalez et al 1997). Recent observations in mammary phyllodes tumours suggested that it may be NOS expression in the stromal component of the tumour that reflects malignancy and potential for progression rather than expression in the tumour parenchyma (Tse et al 2005). iNOS expression also correlated positively with tumour grade in endometrial cancer (Li et al 2005) and with metastatic potential in pancreatic cancer (Kasper 2004). Similar association was seen in ovarian cancer (Raspollini et al 2004) and in cervical cancer (Chen et al 2005), in which iNOS protein levels were a significant independent predictor of disease relapse as well as survival. The relationships in non-small cell lung cancer patients were much less clear: intense expression of all three NOS isoforms was a favourable prognostic sign in one study (Puhakka et al 2003), while in others, iNOS expression was not a significant independent predictor of survival (Marrogi et al 2000; Rubio 2005). Finally, there is evidence for at least one tumour site (head and neck squamous cell carcinoma) in which iNOS activity may promote lymphangiogenesis (Franchi et al 2005), pointing to a potential influence on dissemination.

While some inconsistency in the data remains, the available evidence suggests broadly that low endogenous levels of $NO^\bullet$, whether generated by eNOS or iNOS, contribute to the malignant phenotype, whereas high levels of $NO^\bullet$ generated exogenously by donor drugs or by intervention to enhance iNOS expression result in inhibition of malignancy. However, the concentration of $NO^\bullet$ at which a transition from pro- to anti-malignant activity occurs has not been defined and indeed may vary from one tumour type to another, depending on the levels of endogenous NOS amongst other factors. Furthermore, the role of oxidizing/nitrosating species, formed as a consequence of $NO^\bullet$ generation, needs to be considered especially in studies using non-physiological (high) levels of $NO^\bullet$ at non-physiological oxygen tensions, where the formation of these species might not be physiologically relevant in-vivo. In addition, few papers describe the role of the $NO^\bullet$ oxidation product, nitrogen dioxide, which is eight orders of magnitude more reactive towards key biological targets such as thiol moieties than is nitric oxide (Ford et al 2002; Folkes & Wardman 2004) and thus may play a role in the observed biological responses along with peroxynitrite and dinitrogen dioxide, especially in non-physiological studies, which may be translated in-vivo. Experimental efforts should be directed at defining these relationships.

## Therapeutic potential of NO targeting

There is already clear evidence that therapeutic benefit could arise from two diametrically opposed $NO^\bullet$-targeted strategies: the depletion of endogenous production on the one hand and massive overproduction on the other. Due to the ubiquitous

nature of $NO^{\bullet}$ in cellular signalling, any modification to $NO^{\bullet}$ levels will have numerous and variable consequences for different tumour and normal cells. It is, therefore, not possible to predict the consequences of intervention in a given system. A considerable body of evidence has now accumulated, however, that allows some broad conclusions to be drawn.

*Inhibition of $NO^{\bullet}$ generation*

We were the first to show that the inhibition of NOS in-vivo by chronic administration of a non-isoform-specific L-arginine analogue led to retardation of tumour growth in mice and rats (Kennovin et al 1994). This has since been confirmed in several other studies (Orucevic & Lala 1996; Chinje & Stratford 1997; de Wilt et al 2000). When a specific iNOS inhibitor (1400W) was administered to mice bearing different tumour types, tumour growth inhibition was dependent on the constitutive level of iNOS expression. Tumours expressing iNOS constitutively (EMT6) or genetically engineered to express iNOS constitutively (colon adenocarcinoma DLD-1) showed marked growth inhibition whereas growth of the parental, non-iNOS-expressing line was not affected by the drug (Thomsen et al 1997). These studies confirm that the dominant consequence of endogenous $NO^{\bullet}$ production in tumours, regardless of the iNOS isoform involved, is to promote growth, rather than to enhance host defence mechanisms that inhibit growth.

So does NOS inhibition have a potential role in cancer therapy? It is clear that long-term administration of NOS inhibitors would be required for tumour growth suppression because withdrawal results in a rapid resumption of the normal growth rate (Kennovin et al 1994). However, the use of non-specific NOS inhibitors is problematical. Chronic administration of L-NAME has been shown to cause a variety of undesirable cardiovascular changes including myocardial fibrosis (Babal et al 1997; Pechanova et al 2004) in addition to serious hypertension (210 mmHg systolic after 21 days compared with the normal value of ~140 mmHg) in a rat model after doses lower than the optimum for tumour growth inhibition (Kanagy 1997). Furthermore, L-NAME administration caused attachment of leucocytes to arterial endothelium, with obvious consequences for development of atherosclerosis (Nabah et al 2005). Thus, it seems unlikely that chronic administration of non-specific NOS inhibitors is a viable treatment option in an elderly population of cancer patients. The use of inhibitors specific for iNOS should largely avoid these cardiovascular effects as iNOS does not contribute significantly to $NO^{\bullet}$ generation in the normal vasculature. However, this approach would be viable only against tumours that express high levels of iNOS (Thomsen et al 1997) and even then may not suppress growth of all regions within the tumour (Franchi et al 2005).

*Nitric oxide overexpression*

The evidence we have reviewed points strongly to a potential therapeutic role for strategies that generate high levels of $NO^{\bullet}$ via pro-apoptotic, antimetastatic, radiosensitizing and chemosensitizing activities.

*Single agent activity* $NO^{\bullet}$ can be directly cytotoxic to cancer cells, predominantly by the generation of pro-apoptotic intermediates such as peroxynitrite and $N_2O_3$ (see Lechner et al 2005 for recent review) and it can inhibit DNA repair enzymes including poly (ADP-ribose) polymerase (Sidorkina et al 2003). Perhaps the most effective way of generating high concentrations of $NO^{\bullet}$ in cancer cells is to use iNOS gene transfer techniques. Viral mediated gene transfer of iNOS was used to infect a murine melanoma cell line in-vitro, and when these cells were implanted in mice tumours grew more slowly and were less likely to metastasize than uninfected cells (Juang et al 1997, 1998). The first in-vivo transfection study simply used naked iNOS DNA injection in a mouse thyroid cancer model and demonstrated significant growth inhibition (Soler et al 2000). Viral vectors have also been used successfully for iNOS gene therapy (Kuroki et al 2000). Our own studies have focused on the use of liposomal vectors to deliver a plasmid containing iNOS driven by constitutive or inducible promoters and have shown inhibition of tumour growth in syngeneic mouse tumour and xenograft models (Worthington et al 2002, 2004, 2005). The most impressive growth delay was achieved using the pE9 promoter which incorporates radioresponsive elements from the EGR1 gene: a single intra-tumoral injection of 25 $\mu$g of an EGR1iNOS-containing plasmid alone induced a growth delay greater than that achieved with a single dose of 20 Gy X-irradiation (300 kVp). Our data suggested also that generation of $NO^{\bullet}$ was capable of further activating the E9 promoter, through a positive feedback loop, yielding higher and sustained levels of $NO^{\bullet}$ (Worthington et al 2002, 2004, 2005). Growth inhibition by iNOS gene therapy was less dramatic, however, in human colon cancer xenograft models; it was equivalent to less that 10 Gy X-rays in our own recent study using HT-29 xenografts (Worthington et al 2004) and equivalent to 3 fractions of 2 Gy in HT-116 xenografts (Cook et al 2004).

However, to apply $NO^{\bullet}$ overexpression as a single modality fails to exploit its radio- and chemosensitizing characteristics. Furthermore, there is evidence that the pro-apoptotic activity of $NO^{\bullet}$ can be enhanced in a tumour-specific manner by other classes of anticancer agent. A recent study in a breast cancer cell line showed enhanced $NO^{\bullet}$-induced apoptosis (by a factor of 10 on a concentration basis) following the addition of an inhibitor of farnesyltransferase, with no effect in normal breast epithelial cells (Pervin et al 2001).

*Hypoxic radiosensitization* The characterization of the oxygen effect in radiation biology over 50 years ago (Gray et al 1953) is well established in the history of radiation science, but it is much less well known that $NO^{\bullet}$ was also shown to be a potent radiosensitizer in bacteria and mammalian cells soon after (Howard-Flanders 1957; Gray et al 1958; Dewey 1960). However, it was not until the last decade that the potential importance of this property was revisited (Mitchell et al 1993, 1996, 1998; Janssens et al 1999). These authors generated $NO^{\bullet}$ by several mechanisms (authentic $NO^{\bullet}$ gas, $NO^{\bullet}$-releasing agents or a nitroxyl releasing agent in combination with an electron acceptor) and showed that concentrations in the high micromolar range gave sensitizer enhancement ratios in-vitro of 2.1–2.5, almost as efficient as oxygen and much more efficient than any radiosensitizing drugs that have been tested in-vivo. Very similar results have been obtained using other $NO^{\bullet}$ donors (Griffin et al 1996). An alternative approach,

involving induction of iNOS with interferon gamma, achieved an enhancement ratio of 2.4 in-vitro (Janssens et al 1998). More recently gene therapy strategies have been used successfully to radiosensitize tumour cells in-vitro and in-vivo. We have used liposomal delivery of the iNOS gene construct driven by constitutive (CMV) or radiation-inducible (WAF-1) promoters and achieved sensitizer enhancement ratios of 1.6–2.4 in mouse and human colon cancer cells in-vitro and in-vivo (Worthington et al 2002, 2004). Very similar results were obtained using adenoviral delivery of iNOS to human colon cancer xenografts in combination with single dose and fractionated (2 Gy) irradiation (Wang et al 2004). In addition, this group showed that AdiNOS infection increased tumour vascularity, which could enhance oxygen delivery and provide an additional mechanism of radiosensitization via the oxygen effect.

The evidence for radiosensitization by $NO^{\bullet}$ at high concentrations is clear, but it may, at much lower levels, play a role in cell signalling by irradiated cells leading to a radioprotective bystander effect (Matsumoto et al 2001). Thus, if high concentrations can be achieved in tumours whilst maintaining lower levels in normal tissue, there is the potential for enhanced therapeutic gain.

In addition to radiosensitization, $NO^{\bullet}$ has been shown to play a major role in radiation-induced bystander mechanisms including those of relevance to therapy. It was shown that a nitric oxide-specific scavenger, present in the culture medium, reduced cellular damage in the surrounding cell population, indicating that radiation-induced $NO^{\bullet}$ generation contributed to the bystander effect (Shao et al 2003; Sokolov et al 2005).

*Chemosensitization* $NO^{\bullet}$ is involved in many fundamental aspects of cancer cell biology (see Gatti et al 2004; Ekmekcioglu et al 2005; Lechner et al 2005; for comprehensive reviews). It is also capable of nitrosating or oxidizing zinc finger-containing proteins (Kroncke 2001; Kroncke et al 2002) leading to their denaturation. Previous studies have shown that zinc finger-containing DNA repair proteins including Fpg (Wink & Laval 1994), DNA ligase (Graziewicz et al 1996) and O6–methylguanine-DNA-methyltransferase (Laval & Wink 1997) can be inhibited by $NO^{\bullet}$ donor compounds in-vitro and in-vivo.

It is not surprising, therefore, that levels of $NO^{\bullet}$ have a profound influence on cellular responses to cytotoxic agents. Furthermore, there is evidence that $NO^{\bullet}$ is involved directly in mediating the effects of some of these agents including cisplatin (Son & Hall 2000) and 5-fluorouracil (Oshima et al 2001). Intervention to inhibit NOS markedly reduced cisplatin toxicity in rat gut and kidney (Srivastava et al 1996) and in the cochlea (Watanabe et al 2000). Generation of high concentrations of $NO^{\bullet}$ however, has been shown to chemosensitize in most systems. The interaction with cisplatin has been most widely studied. Pretreatment of V79 lung fibroblasts with either $NO^{\bullet}$-saturated medium for 30 min or the $NO^{\bullet}$ donor drugs DEA/NO or PAP/NO for 60 min resulted in dramatic sensitization to subsequent cisplatin exposure (Wink et al 1997). Similar results were later obtained in head and neck squamous carcinoma cells using different $NO^{\bullet}$ donors (Azizzadeh et al 2001), though in this study only the long-acting donor (DETA/NO) was effective as a chemosensitizer. A different class of $NO^{\bullet}$ releasing agent (diazeniumdiolates) was also used to enhance cisplatin cytotoxicity in a rat liver epithelial cell line (Liu et al 2004). This group showed that the $NO^{\bullet}$ releasing agents increased intracellular concentrations of cisplatin, possibly via activation of MAP kinase pathways.

Exposure to $NO^{\bullet}$ gas, $NO^{\bullet}$ donor or iNOS gene transfer have been used in-vitro to sensitize MCF-7 human breast cancer cells to the cytotoxic effect of clinically relevant concentrations of doxorubicin (Evig et al 2004); $NO^{\bullet}$ was effective as a sensitizer only when given before doxorubicin, not after. Furthermore, no chemosensitizing effect was seen in cardiac myocytes. Another use of $NO^{\bullet}$ as a chemosensitizer is the combination with Taxol. $NO^{\bullet}$ delivery using an $NO^{\bullet}$ donor (nitrosocaptopril) enhanced the transmembrane uptake of Taxol and increased its cytotoxic effect in two prostate cancer cell lines in-vitro, but had neither of these effects in two neuroblastoma cell lines (Jia et al 2003). The mechanisms responsible for these differential effects remain obscure, but a role for P-glycoprotein mediated drug transport has been suggested (Jia et al 2003).

Can in-vitro chemosensitization by $NO^{\bullet}$ be translated into in-vivo models of cancer? Few studies exist, but one group has studied the combination of a $NO^{\bullet}$ donor 3,3-Bis(nitroxymethyl)oxetane with the conventional cytotoxics cyclophosphamide and doxorubicin in mouse models of lung cancer, melanoma and leukaemia (Konovalova et al 2003). The results were impressive: combining daily (for the first nine days) intraperitoneal injection of the $NO^{\bullet}$ donor with cisplatin, cyclophosphamide or cyclophosphamide plus doxorubicin markedly prolonged survival of leukaemia-bearing animals compared with the cyototoxic drugs alone. In combination with cyclophosphamide the $NO^{\bullet}$ donor also enhanced the inhibition of metastasis from subcutaneously implanted melanomas compared with cyclophosphamide alone; $NO^{\bullet}$ therapy inhibited the development of resistance to cyclophosphamide in leukaemic cells. The work of this group illustrates the potential of $NO^{\bullet}$ therapy and there is a pressing need for further studies in this area to develop this concept for clinical application.

While there is overwhelming evidence that $NO^{\bullet}$ can act as a chemosensitizer in combination with many cytotoxic agents currently in clinical use, there may be exceptions. iNOS derived $NO^{\bullet}$ was shown to confer resistance in a rat glioma cell line against the carbamoylating action of chloroethylynitrosourea (Yin et al 2001) via a mechanism thought to involve at least in part *S*-nitrosoglutathione (Yang et al 2004), a potent antioxidant derived from interaction of $NO^{\bullet}$ and glutathione. It will be important, therefore, to gain a fuller understanding of the mechanistic basis of $NO^{\bullet}$ chemosensitization before extensive clinical testing of the concept is introduced as there may be the possibility of adverse interactions with some drugs in some tumours.

## Conclusions

The evidence presented clearly demonstrates the enormous potential of $NO^{\bullet}$ as an anticancer agent if it can be reliably targeted to tumours at high concentrations. Spontaneous and bioreductive $NO^{\bullet}$ donors have been shown to elicit in-vitro

many of the anticancer effects already described, but their use in-vivo at the doses required would result in unacceptable systemic effects, predominantly hypotension, and so existing compounds in this class must be considered unsuitable for clinical use. Indirect activation of NOS by IFN gamma has also been used in-vitro, but the systemic toxicity of IFN is already well documented. NOS activation must therefore be confined to the tumour volume, and gene therapy offers the potential for this targeting specificity combined with high $NO^{\bullet}$-generating capability. Several authors have demonstrated the effectiveness of iNOS gene transfer in-vitro to tumour cells as a method of suppressing tumorigenicity or metastatic potential in-vivo. This leads us to believe that more sophisticated targeting and gene activation could result in highly effective tumour control, particularly when combined with radiotherapy or chemotherapy to exploit all of the therapeutic potential of $NO^{\bullet}$. Overcoming the problems associated with gene therapy, such as delivery, tumour targeting and toxicity of viral vectors, should allow exploitation of $NO^{\bullet}$. To date, systemic delivery of targeted iNOS gene therapy has yet to be reported.

## References


Albina, J. E., Reichner, J. S. (1998) Role of nitric oxide in mediation of macrophage cytotoxicity and apoptosis. *Cancer Metastasis Rev*. **17**: 39–53

Andrew, P. J., Harant, H., Lindley, I. J. (1995) Nitric oxide regulates IL-8 expression in melanoma cells at the transcriptional level. *Biochem. Biophys. Res. Commun*. **214**: 949–956

Azizzadeh, B., Yip, H. T., Blackwell, K. E., Horvath, S., Calcaterra, T. C., Buga, G. M., Ignarro, L. J., Wang, M. B. (2001) Nitric oxide improves cisplatin cytotoxicity in head and neck squamous cell carcinoma. *Laryngoscope* **111:** 1896–1900

Babal, P., Pechanova, O., Bernatova, I., Stvrtina, S. (1997) Chronic inhibition of NO synthesis produces myocardial fibrosis and arterialmedia hyperplasia. *Histol. Histopathol*. **12**: 623–629

Bal-Price, A., Brown, G. C. (2000) Nitric-oxide-induced necrosis and apoptosis in PC12 cells mediated by mitochondria. *J. Neurochem*. **75**: 1455–1464

Brennan, P. A. (2000) The actions and interactions of nitric oxide in solid tumours. *Eur. J. Surg. Oncol*. **26**: 434–437

Bulut, A. S., Erden, E., Sak, S. D., Doruk, H., Kursun, N., Dincol, D. (2005) Significance of inducible nitric oxide synthase expression in benign and malignant breast epithelium: an immunohistochemical study of 151 cases. *Virchows Arch*. **447**: 24–30

Chao, J. I., Kuo, P. C., Hsu, T. S. (2004) Down-regulation of survivin in nitric oxide-induced cell growth inhibition and apoptosis of the human lung carcinoma cells. *J. Biol. Chem*. **279**: 20267–20276

Charalambous, C., Pen, L. B., Su, Y. S., Milan, J., Chen, T. C., Hofman, F. M. (2005) Interleukin-8 differentially regulates migration of tumor-associated and normal human brain endothelial cells. *Cancer Res*. **65**: 10347–10354

Chen, H. H., Su, W. C., Chou, C. Y., Guo, H. R., Ho, S. Y., Que, J., Lee, W. Y. (2005) Increased expression of nitric oxide synthase and cyclooxygenase-2 is associated with poor survival in cervical cancer treated with radiotherapy. *Int. J. Radiat. Oncol. Biol. Phys*. **63**: 1093–1100

Chin, K., Kurashima, Y., Ogura, T., Tajiri, H., Yoshida, S., Esumi, H. (1997) Induction of vascular endothelial growth factor by nitric oxide in human glioblastoma and hepatocellular carcinoma cells. *Oncogene* **15**: 437–442

Chinje, E. C., Stratford, I. J. (1997) Role of nitric oxide in growth of solid tumours: a balancing act. *Essays Biochem*. **32**: 61–72

Chung, P., Cook, T., Liu, K., Vodovotz, Y., Zamora, R., Finkelstein, S., Billiar, T., Blumberg, D. (2003) Overexpression of the human inducible nitric oxide synthase gene enhances radiation-induced apoptosis in colorectal cancer cells via a caspase-dependent mechanism. *Nitric Oxide* **8**: 119–126

Cook, T., Wang, Z., Alber, S., Liu, K., Watkins, S. C., Vodovotz, Y., Billiar, T. R., Blumberg, D. (2004) Nitric oxide and ionizing radiation synergistically promote apoptosis and growth inhibition of cancer by activating p53. *Cancer Res*. **64**: 8015–8021

Dedon, P. C., Tannenbaum, S. R. (2004) Reactive nitrogen species in the chemical biology of inflammation. *Arc h. Biochem. Biophys*. **423**: 12–22

De Santo, C., Serafini, P., Marigo, I., Dolcetti, L., Bolla, M., Del Soldato, P., Melani, C., Guiducci, C., Colombo, M. P., Iezzi, M., Musiani. P., Zanovello, P., Bronte, V. (2005) Nitroaspirin corrects immune dysfunction in tumor-bearing hosts and promotes tumor eradication by cancer vaccination. *Proc. Natl. Acad. Sci. USA* **102**: 4185–4190

de Wilt, J. H., Manusama, E. R., van Etten, B., van Tiel, S. T., Jorna, A. S., Seynhaeve, A. L., ten Hagen, T. L., Eggermont, A. M. (2000) Nitric oxide synthase inhibition results in synergistic antitumour activity with melphalan and tumour necrosis factor alpha-based isolated limb perfusions. *Br. J. Cancer* **83**: 1176–1182

Dewey, D. L. (1960) Effect of oxygen and nitric oxide on the radiosensitivity of human cells in tissue culture. *Nature* **186**: 780–782

Duenas-Gonzalez, A., Isales, C. M., del Mar Abad-Hernandez, M., Gonzalez-Sarmiento, R., Sangueza, O., Rodriguez-Commes, J. (1997) Expression of inducible nitric oxide synthase in breast cancer correlates with metastatic disease. *Mod. Pathol*. **10**: 645–649

Ekmekcioglu, S., Tang, C. H., Grimm, E. A. (2005) NO news is not necessarily good news in cancer. *Curr. Cancer Drug Targets* **5**: 103–115

Evig, C. B., Kelley, E. E., Weydert, C. J., Chu, Y., Buettner, G. R., Burns, C. P. (2004) Endogenous production and exogenous exposure to nitric oxide augment doxorubicin cytotoxicity for breast cancer cells but not cardiac myoblasts. *Nitric Oxide* **10**: 119–129

Fabbri, F., Brigliadori, G., Ulivi, P., Tesei, A., Vannini, I., Rosetti, M., Bravaccini, S., Amadori, D., Bolla, M., Zoli, W. (2005) Pro-apoptotic effect of a nitric oxide-donating NSAID, NCX 4040, on bladder carcinoma cells. *Apoptosis* **10**: 1095–1103

Folkes, L. K., Wardman, P. (2004) Kinetics of the reaction between nitric oxide and glutathione: implications for thiol depletion in cells. *Free Radic. Biol. Med*. **37**: 549–556

Folkman, J. (1990) What is the evidence that tumors are angiogenesis dependent? *J. Natl. Cancer Inst*. **82**: 4–6

Ford, E., Hughes, M. N., Wardman, P. (2002) Kinetics of the reactions of nitrogen dioxide with glutathione, cysteine, and uric acid at physiological pH. *Free Radic. Biol. Med*. **32**: 1314–1323

Franchi, A., Santucci, M., Masini, E., Sardi, I., Paglierani, M., Gallo, O. (2002) Expression of matrix metalloproteinase 1, matrix metalloproteinase 2, and matrix metalloproteinase 9 in carcinoma of the head and neck. *Cancer* **95**: 1902–1910

Franchi, A., Massi, D., Santucci, M., Masini, E., Degl'innocenti, D. R., Magnelli, L., Fanti, E., Naldini, A., Ardinghi, C., Carraro, F., Gallo, O. (2005) Inducible nitric oxide synthase activity correlates with lymphangiogenesis and vascular endothelial growth factor-C expression in head and neck squamous cell carcinoma. *J. Pathol*. **208**: 439–445

Frank, S., Stallmeyer, B., Kampfer, H., Kolb, N., Pfeilschifter, J. (1999a) Nitric oxide triggers enhanced induction of vascular endothelial growth factor expression in cultured keratinocytes

(HaCaT) and during cutaneous wound repair. *FASEB J*. **113**: 2002–2014

Frank, S., Stallmeyer, B., Kampfer, H., Schaffner, C., Pfeilschifter, J. (1999b) Differential regulation of vascular endothelial growth factor and its receptor fms-like-tyrosine kinase is mediated by nitric oxide in rat renal mesangial cells. *Biochem. J*. **338**: 367–374

Fukumura, D., Yuan, F., Endo, M., Jain, R. K. (1997) Role of nitric oxide in tumor microcirculation. Blood flow, vascular permeability, and leukocyte-endothelial interactions. *Am. J. Pathol*. **150**: 713–725

Gansauge, S., Nussler, A. K., Beger, H. G., Gansauge, F. (1998) Nitric oxide-induced apoptosis in human pancreatic carcinoma cell lines is associated with a G1-arrest and an increase of the cyclin-dependent kinase inhibitor p21WAF1/CIP1. *Cell Growth Differ*. **9**: 611–617

Gao, J., Liu, X., Rigas, B. (2005) Nitric oxide-donating aspirin induces apoptosis in human colon cancer cells through induction of oxidative stress. *Proc. Natl. Acad. Sci. USA* **102**: 17207–17212

Gatti, G., Simsek, S., Zurrida, S., Kurne, A., Giannetti, I., Demirer, S., Smeets, A., Caldarella, P., Vento, A. R., Giraldo, A., Luini, A. (2004) Possible role of nitric oxide in the biology of breast carcinoma: review of the available literature. *Breast* **13**: 1–6

Gray, L. H., Conger, A. D., Ebert, M., Hornsey, S., Scott, O. C. (1953) The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br. J. Radiol*. **26**: 638–648

Gray, L. H., Green, F. O., Hawes, C. A. (1958) Effect of nitric oxide on the radiosensitivity of tumour cells. *Nature* **182**: 952–953

Graziewicz, M., Wink, D. A., Laval, F. (1996) Nitric oxide inhibits DNA ligase activity. Potential mechanisms for NO mediated DNA damage. *Carcinogenesis* **17**:2501–2505

Griffin, R. J., Makepeace, C. M., Hur, W. J., Song, C. W. (1996) Radiosensitization of hypoxic tumor cells in vitro by nitric oxide. *Int. J. Radiat. Oncol. Biol. Phys*. **36**: 377–383

Guo, J.-P., Panday, M. M., Consigny, P. M., Lefer, A. M. (1995) Mechanisms of vascular preservation by a novel NO donor following carotid artery intimal injury. *Am. J. Physiol*. **269**: H1122–H1131

Gursoy-Ozdemir, Y., Bolay, H., Saribas, O., Dalkara, T. (2000) Role of endothelial nitric oxide generation and peroxynitrite formation in reperfusion injury after focal cerebral ischemia. *Stroke* **31**: 1974–1980

Halliwell, B., Zhao, K., Whiteman, M. (1999) Nitric oxide and peroxynitrite. The ugly, the uglier and the not so good: a personal view of recent controversies. *Free Radic. Res*. **31**: 651–669

Hara, A., Okayasu, I. (2004) Cyclooxygenase-2 and inducible nitric oxide synthase expression in human astrocytic gliomas: correlation with angiogenesis and prognostic significance. *Acta Neuropathol (Berl)* **108**: 43–48

Harada, K., Supriatno, Kawaguchi, S., Tomitaro, O., Yoshida, H., Sato, M. (2004) Overexpression of iNOS gene suppresses the tumorigenicity and metastasis of oral cancer cells. *In Vivo* **18**: 449–455

Harmey, J. H., Bucana, C. D., Lu, W., Byrne, A. M., McDonnell, S., Lynch, C., Bouchier-Hayes, D., Dong, Z. (2002) Lipopolysaccharide-induced metastatic growth is associated with increased angiogenesis, vascular permeability and tumor cell invasion. *Int. J. Cancer* **101**: 415–422

Harris, A. L. (2002) Hypoxia – a key regulatory factor in tumour growth. *Nat. Rev. Cancer* **2**: 38–47

Hegardt, P., Widegren, B., Li, L., Sjogren, B., Kjellman, C., Sur, I., Sjogren, H. O. (2001) Nitric oxide synthase inhibitor and IL-18 enhance the anti-tumor immune response of rats carrying an intrahepatic colon carcinoma. *Cancer Immunol. Immunother*. **50**: 491–501

Hellmuth, M., Paulukat, J., Ninic, R., Pfeilschifter, J., Muhl, H. (2004) Nitric oxide differentially regulates pro- and anti-angiogenic markers in DLD-1 colon carcinoma cells. *FEBS Lett*. **563**: 98–102

Hino, M., Kohchi, C., Nishizawa, T., Yoshida, A., Nakata, K., Inagawa, H., Hori, H., Makino, K., Terada, H., Soma, G. (2005) Innate-immune therapy for lung carcinoma based on tissue-macrophage activation with lipopolysaccharide. *Anticancer Res*. **25**: 3747–3754

Howard-Flanders, P. (1957) Effect of nitric oxide on the radiosensitivity of bacteria. *Nature* **180**: 1191–1192

Hu, D. E., Dyke, S. O., Moore, A. M., Thomsen, L. L., Brindle, K. M. (2004) Tumor cell-derived nitric oxide is involved in the immune-rejection of an immunogenic murine lymphoma. *Cancer Res*. **64**: 152–161

Huguenin, S., Vacherot, F., Kheuang, L., Fleury-Feith, J., Jaurand, M. C., Bolla, M., Riffaud, J. P., Chopin, D. K. (2004) Antiproliferative effect of nitrosulindac (NCX 1102), a new nitric oxide-donating non-steroidal anti-inflammatory drug, on human bladder carcinoma cell lines. *Mol. Cancer Ther*. **3**: 291–298

Huguenin, S., Vacherot, F., Fleury-Feith, J., Riffaud, J. P., Chopin, D. K., Bolla, M., Jaurand, M. C. (2005) Evaluation of the antitumoral potential of different nitric oxide-donating non-steroidal anti-inflammatory drugs (NO-NSAIDs) on human urological tumor cell lines. *Cancer Lett*. **218**: 163–170

Hussain, S. P., Trivers, G. E., Hofseth, L. J., He, P., Shaikh, I., Mechanic, L. E., Doja, S., Jiang, W., Subleski, J., Shorts, L., Haines, D., Laubach, V. E., Wiltrout, R. H., Djurickovic, D., Harris, C. C. (2004) Nitric oxide, a mediator of inflammation, suppresses tumorigenesis. *Cancer Res*. **64**: 6849–6853

Isenberg, J. S, Ridnour, L. A., Perruccio, E. M., Espey, M. G., Wink, D. A., Roberts, D. D. (2005) Thrombospondin-1 inhibits endothelial cell responses to nitric oxide in a cGMP dependent manner. *Proc. Natl. Acad. Sci. USA* **102**: 13141–13146.

Ishii, Y., Ogura, T., Tatemichi, M., Fujisawa, H., Otsuka, F., Esumi, H. (2003) Induction of matrix metalloproteinase gene transcription by nitric oxide and mechanisms of MMP-1 gene induction in human melanoma cell lines. *Int. J. Cancer* **103**: 161–168

Jadeski, L. C., Lala, P. K. (1999) Nitric oxide synthase inhibition by N(G)-nitro-L-arginine methyl ester inhibits tumor-induced angiogenesis in mammary tumors. *Am. J. Pathol*. **155**: 1381–1390

Jadeski, L. C., Hum, K. O., Chakraborty, C., Lala, P K. (2000) Nitric oxide promotes murine mammary tumour growth and metastasis by stimulating tumour cell migration, invasiveness and angiogenesis. *Int. J. Cancer* **86**: 30–39

Jadeski, L. C., Chakraborty, C., Lala, P. K. (2003) Nitric oxide-mediated promotion of mammary tumour cell migration requires sequential activation of nitric oxide synthase, guanylate cyclase and mitogen-activated protein kinase. *Int. J. Cancer* **106**: 496–504

Janssens, M. Y., Van den Berge, D. L., Verovski, V. N., Monsaert, C., Storme, G. A. (1998) Activation of inducible nitric oxide synthase results in nitric oxide-mediated radiosensitization of hypoxic EMT-6 tumor cells. *Cancer Res*. **58**: 5646–5648

Janssens, M. Y., Verovski, V. N., Van den Berge, D. L., Monsaert, C., Storme, G. A. (1999) Radiosensitization of hypoxic tumour cells by S-nitroso-N-acetylpenicillamine implicates a bioreductive mechanism of nitric oxide generation. *Br. J. Cancer* **79**: 1085–1089

Jia, L., Schweizer, J., Wang, Y., Cerna, C., Wong, H., Revilla, M. (2003) Effect of nitric oxide on cytotoxicity of Taxol: enhanced Taxol transcellular permeability. *Biochem. Pharmacol*. **66**: 2193–2199

Jones, M. K, Tsugawa, K., Tarnawski, A. S., Baatar, D. (2004) Dual actions of nitric oxide on angiogenesis: possible roles of PKC, ERK, and AP-1. *Biochem. Biophys. Res. Commun*. **318**: 520–528

Jordan, B. F., Misson, P., Demeure, R., Baudelet, C., Beghein, N., Gallez, B. (2000) Changes in tumor oxygenation/perfusion induced by the NO donor, isosorbidedinitrate, in comparison with carbogen: monitoring by EPR and MRI. *Int. J. Radiat. Oncol. Biol. Phys*. **48**: 565–570

Jourd'heuil, D., Jourd'heuil, F. L., Kutchukian, P. S., Musah, R. A., Wink, D. A., Grisham, M. B. (2001) Reaction of superoxide and

nitric oxide with peroxynitrite. Implications for peroxynitrite-mediated oxidation reactions in vivo. *J. Biol. Chem.* **276**: 28799–28805

Juang, S. H., Xie, K., Xu, L., Wang, Y., Yoneda, J., Fidler, I. J. (1997) Use of retroviral vectors encoding murine inducible nitric oxide synthase gene to suppress tumorigenicity and cancer metastasis of murine melanoma. *Cancer Biother. Radiopharm.* **12**: 167–175

Juang, S. H., Xie, K., Xu, L., Shi, Q., Wang, Y., Yoneda, J., Fidler, I. J. (1998) Suppression of tumorigenicity and metastasis of human renal carcinoma cells by infection with retroviral vectors harboring the murine inducible nitric oxide synthase gene. *Hum. Gene Ther.* **9**: 845–854

Jurasz, P., Sawicki, G., Duszyk, M., Sawicka, J., Miranda, C., Mayers, I., Radomski, M. W. (2001) Matrix metalloproteinase 2 in tumor cell-induced platelet aggregation: regulation by nitric oxide. *Cancer Res.* **61**: 376–382

Kanagy, N. L. (1997) Increased vascular responsiveness to alpha 2-adrenergic stimulation during NOS inhibition-induced hypertension. *Am. J. Physiol.* **273**: H2756–H2764

Kashfi, K., Rigas, B. (2005) Molecular targets of nitric-oxide-donating aspirin in cancer. *Biochem. Soc. Trans.* **33**: 701–704

Kashiwagi, S., Izumi, Y., Gohongi, T., Demou, Z. N., Xu, L., Huang, P. L., Buerk, D. G., Munn, L. L., Jain, R. K., Fukumura, D. (2005) NO mediates mural cell recruitment and vessel morphogenesis in murine melanomas and tissue-engineered blood vessels. *J. Clin. Invest.* **115**: 1816–1827

Kasper, H. U., Wolf, H., Drebber, U., Wolf, H. K., Kern, M. A. (2004) Expression of inducible nitric oxide synthase and cyclooxygenase-2 in pancreatic adenocarcinoma: correlation with microvessel density. *World J. Gastroenterol.* **10**: 1918–1922

Kennovin, G. D., Hirst, D. G., Stratford, M. R. L., Flitney, F. W. (1994) Inducible nitric oxide synthase is expressed in tumour-associated vasculature: inhibition retards tumour growth. In: Moncada, S., Feelisch, M., Busse, R., Higgs, E. A. (eds) *The Biology of nitric oxide*. Portland Press, London, pp 259–263

Konovalova, N. P., Goncharova, S. A., Volkova, L. M., Rajewskaya, T. A., Eremenko, L. T., Korolev, A. M. (2003) Nitric oxide donor increases the efficiency of cytostatic therapy and retards the development of drug resistance. *Nitric Oxide* **8**: 59–64

Korbelik, M., Parkins, C. S., Shibuya, H., Cecic, I., Stratford, M. R., Chaplin, D. J. (2000) Nitric oxide production by tumour tissue: impact on the response to photodynamic therapy. *Br. J. Cancer* **82**: 1835–1843

Kroncke, K. D. (2001) Cysteine-$Zn^{2+}$ complexes: unique molecular switches for inducible nitric oxide synthase-derived NO. *FASEB J.* **15**: 2503–2507

Kroncke, K. D., Klotz, L. O., Suschek, C. V., Sies, H. (2002) Comparing nitrosative versus oxidative stress toward zinc finger-dependent transcription. Unique role for NO. *J. Biol. Chem.* **277**: 13294–13301

Kuroki, M. (2004) Gene therapy in cancer via use of a retrovector having a tumor specificity and expressing inducible nitric oxide synthase. *Methods Mol. Biol.* **279**: 201–211

Kuroki, M., Arakawa, F., Khare, P. D., Kuroki, M., Liao, S., Matsumoto, H., Abe, H., Imakiire, T. (2000) Specific targeting strategies of cancer gene therapy using a single-chain variable fragment (scFv) with a high affinity for CEA. *Anticancer Res.* **20**: 4067–4071

Kwak, J. Y., Han, M. K., Choi, K. S., Park, I. H., Park, S. Y., Sohn, M. H., Kim, U. H., McGregor, J. R., Samlowski, W. E., Yim, C. Y. (2000) Cytokines secreted by lymphokine-activated killer cells induce endogenous nitric oxide synthesis and apoptosis in DLD-1 colon cancer cells. *Cell Immunol.* **203**: 84–94

Lala, P. K., Chakraborty, C. (2001) Role of nitric oxide in carcinogenesis and tumour progression. *Lancet Oncol.* **2**: 149–156

Laskin, J. D., Heck, D. E., Gardner, C. R., Laskin, D. L. (2001) Prooxidant and antioxidant functions of nitric oxide in liver toxicity. *Antioxid. Redox Signal.* **3**: 261–271

Lau, Y.-T., Ma, W.-C. (1996) Nitric oxide inhibits migration of cultured endothelial cells. *Biochem. Biophys. Res. Commun.* **221**: 670–674

Laval, F., Wink D. A. (1997) Inhibition by nitric oxide of the repair protein O6–methylguanin-DNA-methyltransferase. *Carcinogenesis* **15**: 443–447

Le, X., Wei, D., Huang, S., Lancaster, J. R., Xie, K. (2005) Nitric oxide synthase II suppresses the growth and metastasis of human cancer regardless of its up-regulation of protumor factors. *Proc Natl Acad Sci USA* **102**: 8758–8763

Lechner, M., Lirk, P., Rieder, J. (2005) Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. *Semin. Cancer Biol.* **15**: 277–289

Leiro, J., Iglesias, R., Parama, A., Sanmartin, M. L., Ubeira, F. M. (2001) Respiratory burst responses of rat macrophages to microsporidian spores. *Exp. Parasitol.* **98**: 1–9

Li, H., Hu, J., Xin, W., Zhao, B. (2000) Production and interaction of oxygen and nitric oxide free radicals in PMA stimulated macrophages during the respiratory burst. *Redox Rep.* **5**: 353–358

Li, W., Xu, R. J., Jiang, L. H., Shi, J., Long, X., Fan, B. (2005) Expression of cyclooxygenase-2 and inducible nitric oxide synthase correlates with tumor angiogenesis in endometrial carcinoma. *Med. Oncol.* **22**: 63–70

Liu, J., Li, C., Qu, W., Leslie, E., Bonifant, C. L., Buzard, G. S., Saavedra, J. E., Keefer, L. K., Waalkes, M. P. (2004) Nitric oxide prodrugs and metallochemotherapeutics: JS-K and CB-3-100 enhance arsenic and cisplatin cytolethality by increasing cellular accumulation. *Mol. Cancer Ther.* **3**: 709–714

Loibl, S., Buck, A., Strank, C., von Minckwitz, G., Roller, M., Sinn, H. P., Schini-Kerth, V., Solbach, C., Strebhardt, K., Kaufmann, M. (2005) The role of early expression of inducible nitric oxide synthase in human breast cancer. *Eur. J. Cancer* **41**: 265–271

Marrogi, A. J., Travis, W. D., Welsh, J. A., Khan, M. A., Rahim, H., Tazelaar, H., Pairolero, P., Trastek, V., Jett, J., Caporaso, N. E., Liotta, L. A., Harris, C. C. (2000) Nitric oxide synthase, cyclooxygenase 2, and vascular endothelial growth factor in the angiogenesis of non-small cell lung carcinoma. *Clin. Cancer Res.* **6**: 4739–4744

Matsumoto, H., Hayashi, S., Hatashita, M., Ohnishi, K., Shioura, H., Ohtsubo, T., Kitai, R., Ohnishi, T., Kano, E. (2001) Induction of radioresistance by a nitric oxide-mediated bystander effect. *Radiat. Res.* **155**: 387–396

Matsunaga, T., Weihrauch, D. W., Moniz, M. C., Tessmer, J., Warltier, D. C., Chilian, W. M. (2002) Angiostatin inhibits coronary angiogenesis during impaired production of nitric oxide. *Circulation* **105**: 2185–2191

Meyer, R. E., Shan, S., DeAngelo, J., Dodge, R. K., Bonaventura, J., Ong, E. T., Dewhirst, M. W. (1995) Nitric oxide synthase inhibition irreversibly decreases perfusion in the R3230Ac rat mammary adenocarcinoma. *Br. J. Cancer* **71**: 1169–1174

Mitchell, J. B., Wink, D. A., DeGraff, W., Gamson, J., Keefer, L. K., Krishna, M. C. (1993) Hypoxic mammalian cell radiosensitization by nitric oxide. *Cancer Res.* **53**: 5845–5848

Mitchell, J. B., Cook, J. A., Krishna, M. C., DeGraff, W., Gamson, J., Fisher, J., Christodoulou, D., Wink, D. A. (1996) Radiation sensitisation by nitric oxide releasing agents. *Br. J. Cancer* **27**(Suppl.): S181–184

Mitchell, J. B., DeGraff, W., Kimi, S., Cook, J. A., Gamson, J., Christodoulou, D., Feelisch, M., Wink, D. A. (1998) Redox generation of nitric oxide to radiosensitize hypoxic cells. *Int. J. Radiat. Oncol. Biol. Phys.* **42**: 795–798

Moncada, S. (1999) Nitric oxide: discovery and impact on clinical medicine. *J. R. Soc. Med.* **92**: 164–169

Moncada, S., Higgs, E. A. (1991) Endogenous nitric oxide: physiology, pathology and clinical relevance. *Eur. J. Clin. Invest.* **21**: 361–374

Mortensen, K., Skouv, J., Hougaard, D. M., Larsson, L. I. (1999) Endogenous endothelial cell nitric-oxide synthase modulates

apoptosis in cultured breast cancer cells and is transcriptionally regulated by p53. *J. Biol. Chem*. **274**: 37679–37684

Murohara, T., Asahara, T., Silver, M., Bauters, C., Masuda, H., Kalka, C., Kearney, M., Chen, D., Symes, J. F., Fishman, M. C., Huang, P. L., Isner, J. M. (1998) Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. *J. Clin. Invest*. **101**: 2567–2578

Nabah, Y. N., Mateo, T., Cerda-Nicolas, M., Alvarez, A., Martinez, M., Issekutz, A. C., Sanz, M. J. (2005) L-NAME induces direct arteriolar leukocyte adhesion, which is mainly mediated by angiotensin-II. *Microcirculation* **12**: 443–453

Orucevic, A., Lala, P. K. (1996) Effects of *N*(G)-Nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthesis, on IL-2-induced LAK cell generation in vivo and in vitro in healthy and tumor-bearing mice. *Cell Immunol*. **169**: 125–132

Oshima, T., Imada, T., Nagashima, Y., Cho, H., Shiozawa, M., Rino, Y., Takanashi, Y. (2001) Role of nitric oxide in human gastric cancer cells treated with 5 fluorouracil. *Oncol. Rep*. **8**: 847–849

Osorio, J. C., Recchia, F. A. (2000) The role of nitric oxide in metabolism regulation: from basic sciences to the clinical setting. *Intensive Care Med*. **26**: 1395–1398

Palmer, R. M., Ferrige, A. G., Moncada, S. (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327**: 524–526

Pan, J. W., Zhan, R. Y., Tong, Y., Zhou, Y. Q., Zhang, M. (2005) Expression of endothelial nitric oxide synthase and vascular endothelial growth factor in association with neovascularization in human primary astrocytoma. *J. Zhejiang Univ. Sci. B*. **6**: 693–698

Pechanova, O., Bernatova, I., Babal, P., Martinez, M. C., Kysela, S., Stvrtina, S., Andriantsitohaina, R. (2004) Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension. *J. Hypertens*. **22**: 1551–1559

Pervin, S., Singh, R., Gau, C. L., Edamatsu, H., Tamanoi, F., Chaudhuri, G. (2001) Potentiation of nitric oxide-induced apoptosis of MDA-MB-468 cells by farnesyltransferase inhibitor: implications in breast cancer. *Cancer Res*. **61**: 4701–4706

Phillips, P. G., Birnby, L. M. (2004) Nitric oxide modulates caveolin-1 and matrix metalloproteinase-9 expression and distribution at the endothelial cell/tumor cell interface. *Am. J. Physiol. Lung Cell. Mol. Physiol*. **286**: L1055–L1065

Pipili-Synetos, E., Sakkoula, E., Haralabopoulos, G., Andriopoulou, P., Peristeris, P., Maragoudakis, M. E. (1994) Evidence that nitric oxide is an endogenous antiangiogenic mediator. *Br. J. Pharmacol*. **111**: 894–902

Powell, J. A., Mohamed, S. N., Kerr, J. S., Mousa, S. A. (2000) Antiangiogenesis efficacy of nitric oxide donors. *J. Cell Biochem*. **80**: 104–114

Puhakka, A., Kinnula, V., Napankangas, U., Saily, M., Koistinen, P., Paakko, P., Soini, Y. (2003) High expression of nitric oxide synthases is a favorable prognostic sign in non-small cell lung carcinoma. *APMIS* **111**: 1137–1146

Raspollini, M. R., Amunni, G., Villanucci, A., Boddi, V., Baroni, G., Taddei, A., Taddei, G. L. (2004) Expression of inducible nitric oxide synthase and cyclooxygenase-2 in ovarian cancer: correlation with clinical outcome. *Gynecol. Oncol*. **92**: 806–812

RayChaudhury, A., Frischer, H., Malik, A. B. (1996) Inhibition of endothelial cell proliferation and bFGF-induced phenotypic modulation by nitric oxide. *J. Cell Biochem*. **63**: 125–134

Ridnour, L. A., Isenberg, J. S., Espey, M. G., Thomas, D. D., Roberts, D. D., Wink, D. A. (2005) Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1. *Proc. Natl. Acad. Sci. USA* **102**: 13147–13152

Royle, J. S., Ross, J. A., Ansell, I., Bollina, P., Tulloch, D. N., Habib, F.K. (2004) Nitric oxide donating nonsteroidal anti-inflammatory drugs induce apoptosis in human prostate cancer cell systems and human prostatic stroma via caspase-3. *J. Urol*. **172**: 338–344

Rubio, L., Vera-Sempere, F. J., Lopez-Guerrero, J. A., Padilla, J., Moreno-Baylach, M. J. (2005) A risk model for non-small cell lung cancer using clinicopathological variables, angiogenesis and oncoprotein expression. *Anticancer Res*. **25**: 497–504

Schimmel, M., Bauer, G. (2001) Selective apoptosis induction in transformed fibroblasts by B cell lines: involvement of reactive oxygen and nitrogen species. *Int. J. Oncol*. **19**: 299–304

Shao, C., Stewart, V., Folkard, M., Michael, B. D., Prise, K. M. (2003) Nitric oxide-mediated signaling in the bystander response of individually targeted glioma cells. *Cancer Res* **63**: 8437–8442

Shizukuda, Y., Tang, S., Yokota, R., Ware, J. A. (1999) Vascular endothelial growth factor-induced endothelial cell migration and proliferation depend on a nitric oxide-mediated decrease in protein kinaseCdelta activity. *Circ. Res*. **85**: 247–256

Sidorkina, O., Espey, M. G., Miranda, K. M., Wink, D. A., Laval, J. (2003) Inhibition of poly(ADP-RIBOSE) polymerase (PARP) by nitric oxide and reactive nitrogen oxide species. *Free Radic. Biol. Med*. **35**: 1431–1438

Sokolov, M. V., Smilenov, L. B., Hall, E. J., Panyutin, I. G., Bonner, W. M., Sedelnikova, O. A. (2005) Ionizing radiation induces DNA double-strand breaks in bystander primary human fibroblasts. *Oncogene* **24**: 7257–7265

Soler, M. N., Bobe, P., Benihoud, K., Lemaire, G., Roos, B. A., Lausson, S. (2000) Gene therapy of rat medullary thyroid cancer by naked nitric oxide synthase II DNA injection. *J. Gene Med*. **2**: 344–352

Somers, M. J., Harrison, D. G. (1999) Reactive oxygen species and the control of vasomotor tone. *Curr. Hypertens. Rep*. **1**: 102–108

Son, K. K., Hall, K. J. (2000) Nitric oxide-mediated tumor cell killing of cisplatin-based interferon-gamma gene therapy in murine ovarian carcinoma. *Cancer Gene Ther*. **7**: 1324–1328

Sonveaux, P., Brouet, A., Havaux, X., Gregoire, V., Dessy, C., Balligand, J. L., Feron, O. (2003) Irradiation-induced angiogenesis through the up-regulation of the nitric oxide pathway: implications for tumor radiotherapy. *Cancer Res*. **63**: 1012–1019

Srivastava, R. C., Farookh, A., Ahmad, N., Misra, M., Hasan, S. K., Husain, M. M. (1996) Evidence for the involvement of nitric oxide in cisplatin-induced toxicity in rats. *Biometals* **9**: 139–142

Swaroop, G. R., Malcolm, G. P., Kelly, P. A., Ritchie, I., Whittle, I. R. (1998) Effects of nitric oxide modulation on tumour blood flow and microvascular permeability in C6 glioma. *Neuroreport* **9**: 2577–2581

Szabo, C., Ohshima, H. (1997) DNA damage induced by peroxynitrite: subsequent biological effects. *Nitric Oxide* **1**: 373–385

Thomsen, L. L., Scott, J. M., Topley, P., Knowles, R. G., Keerie, A. J., Frend, A. J. (1997) Selective inhibition of inducible nitric oxide synthase inhibits tumor growth in vivo: studies with 1400W, a novel inhibitor. *Cancer Res*. **57**: 3300–3304

Tozer, G. M., Everett, S. A. (1997) Nitric oxide in tumour biology and cancer therapy. Part 1: Physiological aspects. *Clin. Oncol. (R Coll Radiol)* **9**: 282–293

Tozer, G. M., Prise, V. E., Chaplin, D. J. (1997) Inhibition of nitric oxide synthase induces a selective reduction in tumor blood flow that is reversible with L-arginine. *Cancer Res*. **57**: 948–955

Tozer, G. M., Prise, V. E., Motterlini, R., Poole, B. A., Wilson, J., Chaplin, D. J. (1998) The comparative effects of the NOS inhibitor, Nomega-nitro-L-arginine, and the haemoxygenase inhibitor, zinc protoporphyrin IX, on tumour blood flow. *Int. J. Radiat. Oncol. Biol. Phys*. **42**: 849–853

Tschugguel, W., Pustelnik, T., Lass, H., Mildner, M., Weninger, W., Schneeberger, C., Jansen, B., Tschachler, E., Waldhor, T., Huber, J. C., Pehamberger, H. (1999) Inducible nitric oxide synthase (iNOS) expression may predict distant metastasis in human melanoma. *Br. J. Cancer* **79**: 1609–1612

Tse, G. M., Wong, F. C., Tsang, A. K., Lee, C. S., Lui, P. C., Lo, A. W., Law, B. K., Scolyer, R. A., Karim, R. Z., Putti, T. C. (2005)

Stromal nitric oxide synthase (NOS) expression correlates with the grade of mammary phyllodes tumour. *J. Clin. Pathol*. **58**: 600–604

Wakabayashi, Y., Shono, T., Hori, S., Matsushima, K., Ono, M., Kuwano, M. (1995) Dual pathways of tubular morphogenesis of vascular endothelial cells by human glioma cells: VEGF/bFGF and IL-8. *Jpn. J. Cancer Res*. **86**: 1189–1197

Wang, G. Y., Ji, B., Wang, X., Gu, J. H. (2005) Anti-cancer effect of iNOS inhibitor and its correlation with angiogenesis in gastric cancer. *World J. Gastroenterol*. **11**: 3830–3833

Wang, Z., Cook, T., Alber, S., Liu, K., Kovesdi, I., Watkins, S. K., Vodovotz, Y., Billiar, T. R., Blumberg, D. (2004) Adenoviral gene transfer of the human inducible nitric oxide synthase gene enhances the radiation response of human colorectal cancer associated with alterations in tumor vascularity. *Cancer Res*. **64**: 1386–1395

Watanabe, K., Hess, A., Michel, O., Yagi, T. (2000) Nitric oxide synthase inhibitor reduces the apoptotic change in the cisplatin-treated cochlea of guinea pigs. *Anticancer Drugs* **11**: 731–735

Wei, D., Richardson, E. L., Zhu, K., Wang, L., Le, X., He, Y., Huang, S., Xie, K. (2003) Direct demonstration of negative regulation of tumor growth and metastasis by host-inducible nitric oxide synthase. *Cancer Res*. **63**: 3855–3859

Williams, E. L., Djamgoz, M. B. (2005) Nitric oxide and metastatic cell behaviour. *Bioessays* **27**: 1228–1238

Wink, D. A., 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

Wink, D. A., Hanbauer, I., Krishna, M. C., DeGraff, W., Gamson, J., Mitchell, J. B. (1993) Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *Proc. Natl. Acad. Sci. USA*. **90**: 9813–9817

Wink, D. A., Cook, J. A., Christodoulou, D., Krishna, M. C., Pacelli, R., Kim, S., DeGraff, W., Gamson, J., Vodovotz, Y., Russo, A., Mitchell, J. B. (1997) Nitric oxide and some nitric oxide donor compounds enhance the cytotoxicity of cisplatin. *Nitric Oxide* **1**: 88–94

Wink, D. A., Vodovotz, Y., Laval, J., Laval, F., Dewhirst, M. W., Mitchell, J. B. (1998) The multifaceted roles of nitric oxide in cancer. *Carcinogenesis* **19**: 711–721

Worthington, J., Robson, T., O'Keeffe, M., Hirst, D. G. (2002) Tumour cell radiosensitization using constitutive (CMV) and radiation inducible (WAF1) promoters to drive the iNOS gene: a novel suicide gene therapy. *Gene Ther*. **9**: 263–269

Worthington, J., McCarthy, H. O., Barrett, E., Adams, C., Robson, T., Hirst, D. G. (2004) Use of the radiation-inducible WAF1 promoter to drive iNOS gene therapy as a novel anti-cancer treatment. *J. Gene Med*. **6**: 673–680

Worthington, J., Robson, T., Scott, S., Hirst, D. (2005) Evaluation of a synthetic CArG promoter for nitric oxide synthase gene therapy of cancer. *Gene Ther*. **12**: 1417–1423

Wu, J., Akaike, T., Hayashida, K., Okamoto, T., Okuyama, A., Maeda, H. (2001) Enhanced vascular permeability in solid tumor involving peroxynitrite and matrix metalloproteinases. *Jpn. J. Cancer Res*. **92**: 439–451

Xiong, Y., Yuan, L. W., Deng, H. W., Li, Y. J., Chen, B. M. (2001) Elevated serum endogenous inhibitor of nitric oxide synthase and endothelial dysfunction in aged rats. *Clin. Exp. Pharmacol. Physiol*. **28**: 842–847

Yamaguchi, K., Saito, H., Oro, S., Tatebe, S., Ikeguchi, M., Tsujitani, S. (2005) Expression of inducible nitric oxide synthase is significantly correlated with expression of vascular endothelial growth factor and dendritic cell infiltration in patients with advanced gastric carcinoma. *Oncology* **68**: 471–478

Yang, D. I., Yin, J. H., Ju, T. C., Chen, L. S., Hsu, C. Y. (2004) Nitric oxide and BCNU chemoresistance in C6 glioma cells: role of S-nitrosoglutathione. *Free Radic. Biol. Med*. **36**: 1317–1328

Yin, J. H., Yang, D. I., Chou, H., Thompson, E. M., Xu, J., Hsu, C. Y. (2001) Inducible nitric oxide synthase neutralizes carbamoylating potential of 1,3-bis(2-chloroethyl)-1-nitrosourea in C6 glioma cells. *J. Pharmacol. Exp. Ther*. **297**: 308–315

Zhang, X. M., Xu, Q. (2001) Metastatic melanoma cells escape from immunosurveillance through the novel mechanism of releasing nitric oxide to induce dysfunction of immunocytes. *Melanoma Res*. **11**: 559–567

Ziche, M., Morbidelli, L. (2000) Nitric oxide and angiogenesis. *J. Neurooncol*. **50**: 139–148