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Role of Nitric Oxide in Cancer Biology

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The role of nitric oxide (NO) in tumorigenesis is multifactorial. NO can participate in the complicated process of carcinogenesis by mediating DNA damage in early phases of tumorigenesis, as well as support tumor progression through the induction of angiogenesis and suppression of the immune response. This paper addresses the effects of NO on transcriptional regulation following DNA damage and cyclooxygenase expression in the multistep process of tumorigenesis.

Keywords: Nitric oxide, tumor, p53, cyclooxygenase

INTRODUCTION

A small, chemically simple and highly toxic gas, nitric oxide (NO) seems an unlikely biological jack-of-all-trades, as most of the body's functions are indeed regulated by extraordinarily large and complex proteins and compounds. NO exists in the gaseous state under normal atmospheric conditions. Over the past decade, it has been realized that this diatomic radical plays a variety of regulatory functions *in vivo*.[1] Interest in the importance of NO in medicine and biology escalated after the first paper on this topic, published in 1985 by Stuehr and Marletta, demonstrated NO synthesis by a mammalian cell.[2] Unsurprisingly, NO was named "Molecule of the Year" in 1992 in recognition of the tremendous significance of its role in biological systems.[3]

Nearly every cell type studied thus far has the capacity to synthesize NO by one of three distinct nitric oxide synthase (NOS) isoforms.[1] The effects brought forth by NO can vary greatly, depending on the site where NO is synthesized, the amount of NO formed, and the targets within the local environment. Beneficial responses, such as vasodilatation, inhibition of platelet aggregation, relaxation of smooth muscles and signal transmission in neuronal cells are induced by the production of small quantities of NO by constitutive NOS, namely endothelial and neuronal isoforms. These constitutive isoforms, which are expressed continuously, generate NO in conditions where intracellular Ca^{2+} level is increased and calmodulin is activated.[1] In contrast, the action of

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inducible NOS is independent of intracellular Ca^{2+} level and leads to the synthesis of high local concentrations of NO for prolonged periods of time. This isoform of NOS produces much larger quantities of NO in response to cytokines and endotoxins, which have been shown to be cytostatic or cytotoxic to tumor cells and a range of microorganisms.[4] The effects of NO are not all beneficial, as this free radical has also been identified as a deleterious agent in numerous pathophysiological conditions, including cancer. The anti-tumor properties of NO are highlighted by some authors, while others implicate NO in tumor promotion.

The objective of this review is to discuss these seemingly contradictory roles of NO in early and late stages of human carcinogenesis.

BIOCHEMICAL INTERACTIONS OF NO

The redox properties of NO influence its reactivity. The redox relationship of NO with other nitrogen oxides indicates that NO occupies a central and unique position in the redox scheme:[5]

$$N^{(+5)}O_3^{-} \overset{+2e^-}{\leftrightarrow} N^{(+3)}O_2^{-} \overset{+1e^-}{\leftrightarrow} N^{(+2)}O \overset{+1e^-}{\leftrightarrow} NH^{(+1)}O \overset{+2e^-}{\leftrightarrow} N^{(-1)}H_2OH \overset{+2e^-}{\leftrightarrow} N^{(-3)}H_3$$

Being a free radical, NO is capable of reacting with other free radicals. In basic conditions, NO is capable of oxidizing sulfhydryl groups, reacting with amines in organic solvents,[6] and with metal-containing proteins.[7,8] It is crucial to understand the NO chemistry, before one can fully grasp its physiological functions as well as its toxicity. The role of NO as a physiological messenger molecule and as a cytotoxic effector molecule under appropriate conditions is dependent on its chemical properties as well as concentration. Reactive nitrogen oxide species (RNOS) are produced by the reaction of NO with either molecular oxygen, one of the most common yet biologically reactive molecules, or with superoxide. Numerous biological molecules have been proven to be oxidized by RNOS,[9] therefore possibly accounting for certain types of NO-mediated toxicity.

The reactivity of NO is one of the primary determinants of its final effect on a given biological system. NO acts both directly and indirectly.[10] NO acts directly by reacting chemically with a specific biological target, mostly in conditions where constitutive NOS isoforms generate low levels of NO.[10] Thus, low levels of NO can react directly with certain heme-containing proteins such as guanylate cyclase, oxyhemoglobin, and cytochrome P450.[1] On the other hand, the reactive nitrogen oxide species mediate certain chemical reactions, where the local concentration of NO, produced by iNOS alone, is high. These constitute the indirect effects of NO. These chemical reactions bring about nitrosative and oxidative stresses in biological systems, both of which yield different types of DNA mutations.[11]

Nitrosamines generated in biological systems during nitrosative stress are potentially carcinogenic. It has been shown that these carcinogenic nitrosamines can be generated in the acidic conditions of the stomach.[12–17] Although NO itself does not directly interact with DNA or proteins, high concentrations of NO generated during inflammation as a response to any pathogen, can lead to the formation of RNOS, such as N_2O_3.[18] During inflammation, stimulated macrophages and neutrophils express iNOS and generate large amounts of NO. This promotes the formation of RNOS, which can then nitrosate amines.[19,20] Hence such nitrosation reactions appear to occur *in vivo*. Nitrosamines, formed under such conditions of chronic inflammation, can in turn lead to cancer.[21,22] Cells exposed to NO exhibit lesions consistent with the chemistry of deamination. This involves the conversion of cytosine to uracil, guanine to xanthine, methylcytosine to thymine, and adenine to hypoxanthine.[23,24] Single-stranded DNA appears to be far more susceptible to nitrosation than double-stranded DNA,[25]

suggesting that deamination occurs mainly during the replication and transcription of DNA. Such mechanisms involving the nitrosation of nucleic acids may contribute to *in vivo* spontaneous deamination.

Oxidative stress induced by RNOS is thought to be mediated primarily by the formation of peroxynitrite,[11,20,26] since this can induce DNA strand breaks *in vitro*.[27]

NO and RNOS may also act indirectly by affecting the enzymatic activity of several DNA repair proteins. RNOS have a high affinity for amino acids containing thiol residues in DNA repair proteins and they can induce nitrosation in their active sites.[28–20] Another important reaction is the inhibition of DNA-binding proteins containing zinc finger motifs[31] in the presence of NO under aerobic conditions. This occurs perhaps through the nitrosation of thiols by N_2O_3, subsequent ejection of zinc, and the consequent degradation of the structural integrity of the protein.[32]

Exposure of cells to NO results in an increased number of single-strand DNA breaks.[24] It has been shown that the effects of exposure to NO or RNOS include the inhibition of DNA ligase activity, leading to an accumulation of DNA breaks that occur either during transcription or repair. This increase in DNA breaks caused by the NO-mediated inhibition of ligase could in turn activate the tumor suppressor gene, p53.[33]

ROLE OF NO IN THE PROCESS OF CARCINOGENESIS

The etiology of cancer involves many factors, with the earliest oncogenic alterations occurring possibly decades before transformation. Tumors are formed in a process that consists of several stages: initiation, promotion, and progression. Via both its direct and indirect paths of action, NO is implicated in tumor promotion as well as tumor suppression. Evidently, NO plays a complex and sometimes contradictory role in carcinogenesis. The association between increased NOS expression and tumor progression as well as metastasis is proposed by some studies,[34–41] while others draw a link between tumor invasiveness and aggressiveness and decreased NOS activity.[42]

The involvement of NO in tumor biology has been a topic of much study in recent years. Interactions between the endothelial cells of tumor vasculature, tumor-infiltrating immune cells such as T lymphocytes and macrophages, and the tumor cells themselves play a regulatory role in tumor growth. The production of NO by most of these cellular components has been demonstrated.[43–46]

The role of NO in cancer is two-faceted: when produced at high concentrations, it has cytotoxic and cytostatic properties, resulting in DNA damage, the initial step in carcinogenesis. Tumor growth is enhanced via NO's anti-apoptotic effects and its role in the creation of neovasculature, under conditions of low to moderate NO concentration.[47]

INITIATION PHASE OF TUMORIGENESIS

The stage in tumor growth, the location of NO production and the concentration of NO determine the effects of NO in tumor biology. Tumor growth is a multistep process including the initiation phase and the enlargement of an established tumor. Accumulation of mutations occurs in tissues exposed to prolong high NO concentration, as a result of NO itself, or through the facilitation of other genotoxic agents, for instance during episodes of chronic inflammation or through exposure to mutagens in the environment. Inhibition of DNA synthesis via the inhibition of ribonucleotide reductase activity[48–50] and inhibition of mitochondrial respiration by reacting with iron–sulphur proteins such as aconitase[51–54] are a few examples of the numerous

mechanisms of NO cytostasis. By the formation of peroxynitrite, carcinogenic nitrosamines and the inhibition of systems required for the repairing of DNA damage, NO may aggravate DNA damage.[28,29,32,55–58] Exposure of cells to NO results in an increased number of single-strand DNA breaks.[24] Furthermore, NO or RNOS induced DNA damage, activates the tumor suppressor gene, p53.[33]

In the initiation phase of tumorigenesis, initial DNA damage causes the accumulation of the tumor suppressor gene, p53.[33] This acts as a checkpoint control in the cell cycle, permitting the repair of damaged DNA.[59] As p53 activation causes a block in G1/S transition in the cell cycle, apoptosis results in the case of severe DNA damage.[60,61] p53 thus plays an active role in the cellular response to endogenously produced DNA damage due to NO and RNOS.

It can be seen that NO-induced DNA damage results in wild-type p53 accumulation. Furthermore, it has been reported that increased expression of wild-type p53 downregulates iNOS expression via a negative feedback loop that results in the reduced production of NO.[59] In addition, prolonged transcription of wild-type p53 may cause methylation of p53 tumor suppressor gene, which favors G:C → A:T transitions in this gene due to NO.[21,23,24] It has been shown that G:C → A:T transition is one of the most common mutations in the p53 tumor suppressor gene in early phases of human carcinogenesis.[62] As a result of this process, the mutated p53 gene cannot protect cells and tissues from mutation in the event of severe DNA damage. Therefore, cells and tissues are allowed to multiply in the event of severe DNA damage since the mutated p53 gene loses its principal function in cell cycle control.

As the actions of NO and the p53 tumor suppressor gene in cell cycle control are inhibited, a functional derangement of the cell cycle and uncontrolled cellular proliferation ultimately result, thus contributing to the initial stage of tumorigenesis.

TUMOR-PROMOTING PROPERTIES OF NO IN ADVANCED TUMORS

When the tumor cells have reached a certain number, hypoxia occurs in the enlarging tumor and the creation of neovasculature becomes necessary for further tumor growth and survival. Wild-type p53 is a known inhibitor of tumor angiogenesis,[63] but the presence of hypoxia, arising from enlargement of the tumor, has been described as a selecting factor for mutant p53. Mutational inactivation of p53 generates a cell population that can tolerate the genotoxicity and cytostasis of sustained NO production.[64] Furthermore, low to moderate NO concentrations may even have anti-apoptotic properties in these cells. Therefore, the loss of p53 function in p53-mutated cells would permit both the growth of tumor cells as well as the release of angiogenic factors such as vascular endothelial growth factor in the presence of moderate NO concentrations.[65] At this stage, the production of iNOS, induced by hypoxia, stimulates the production of NO by tumor cells.[66] In this respect, NO production may be a part of the angiogenic switch in developing tumors, without which a lack of vascularization would result, hence limiting the size of the developing tumors (Figure 1). Thus, hypoxia and cytokines (e.g. tumor necrosis factor) produced by tumor cells induce iNOS expression in these cells. Subsequent production of NO then promotes tumor growth by stimulating angiogenesis,[47,67,68] increasing vascular permeability[69–72] and suppressing the immune response.[73,74] The suppression of leukocyte proliferation and infiltration is another systemic effect of NO of relevance in cancer biology. Several reports have indicated that NO produced by tumor cells may prevent the infiltration of leukocytes[73] and leukocyte adhesion.[68] Thus, NO, in addition to increasing the vascular permeability, downregulates the expression of some adhesion molecules, which are important for inflammatory and immune cell adhesion to vascular endothelium.[69]

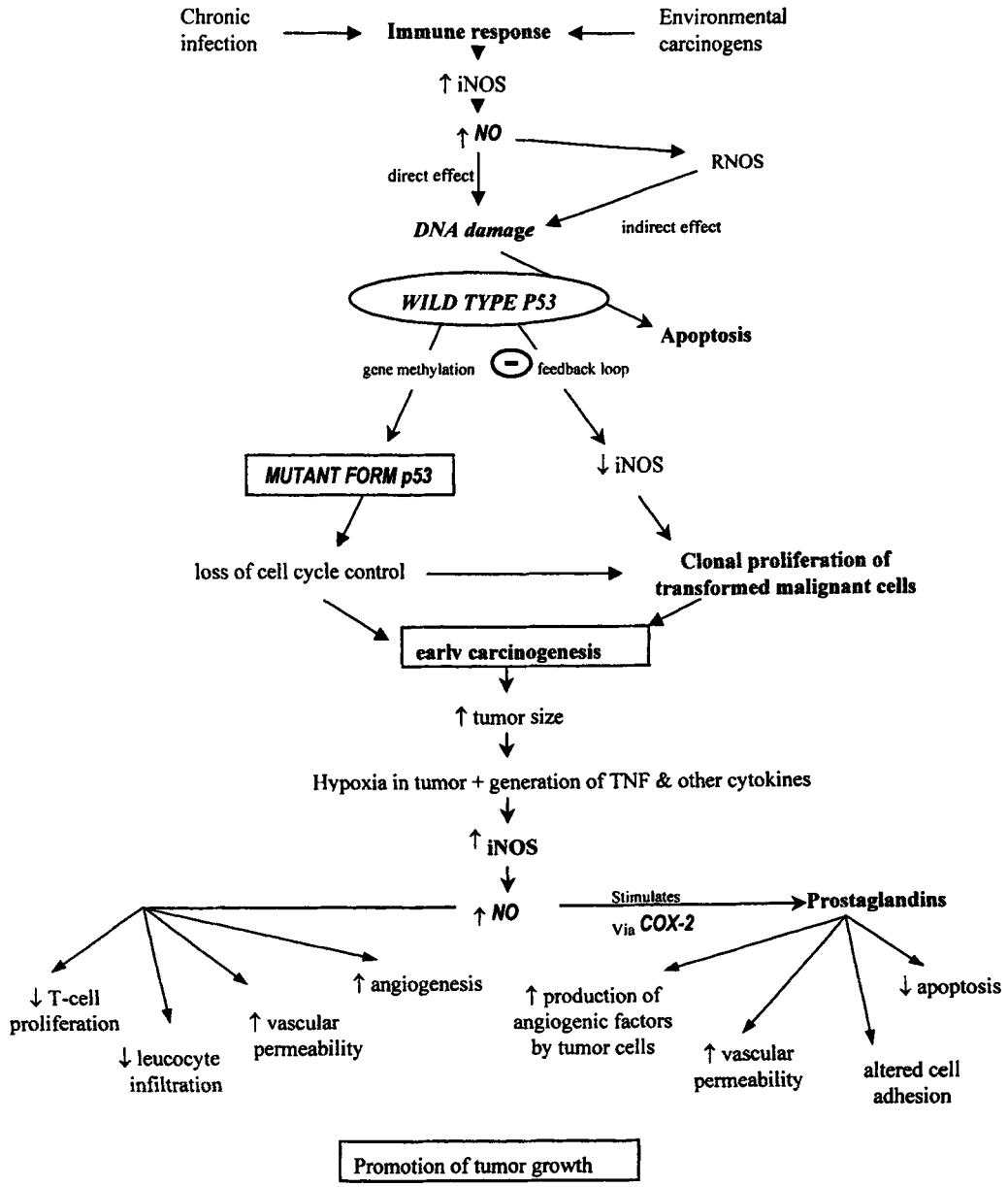

FIGURE 1 The involvement of molecular parameters in initial and late stages of carcinogenesis.

Another mechanism by which NO may promote tumor growth is by modulating the production of prostaglandins.[75] NO activates COX-2,[76] which in turn, stimulates the production of proangiogenic factors and prostaglandins. Prostaglandins increase vascular permeability supporting the development of neovasculature in tumors.[77,78] It has also been shown that the overexpression of COX-2 alters cell adhesion and protects cells against apoptosis by increasing the Bcl-2 protein production.[79,80] All these effects of NO and prostaglandins generated by COX-2 facilitate further tumor growth.

SUMMARY

Normal tissue homeostasis is maintained through the regulation of cell proliferation and apoptosis. It has been shown that in normal cells, wild-type p53 suppress both basal and cytokine-induced iNOS via a negative "feedback loop".[59] However, in tumor cells which express mutant p53, iNOS expression would be unchecked. These cells, lacking a functional p53 protein, are prevented from being eliminated in the event of severe DNA damage, and therefore proliferate more aggressively. Hypoxia, arising from enlargement in tumor size, has been described to be a selective factor for mutant p53 as hypoxia has both genotoxic as well as angiogenic properties.

The loss of wild-type p53 function or the expression of mutant p53 in the tumor cells would permit both the growth of tumor cells in the presence of moderate NO concentrations as well as the release of angiogenic factors such as the vascular endothelial growth factor. Besides supporting the development of neovasculature, increased iNOS levels in several solid tumors, such as human breast, brain, head and neck, and colon cancers,[41,65,81,82] may lead to a wild-type p53-mediated growth arrest in the epithelial cells close to the source of NO production. The resulting growth inhibition would subsequently exert a strong selection pressure, allowing those cells that contain mutant p53 to proliferate faster than those containing wild-type p53.[65] It has been reported that mutant p53-positive tumors are associated with poor prognosis and resistance to chemotherapy.[83–86] Furthermore, clonal selection and growth of these mutant p53-containing cells are further supported by the combination of prostaglandins and NO-induced angiogenesis, increased vascular permeability, immune suppression and reduced apoptosis (Figure 1).

It has been proposed that tumor-associated NO production, as well as cyclooxygenase-2 overexpression, promotes cancer progression by providing a selective growth advantage to tumor cells with mutant p53.[65] This is consistent with the hypothesis that NO and COX-2 are the cancer-promoting factors in human carcinogenesis. Therefore, according to this hypothesis, it may be possible in the future that the inhibitors of iNOS and COX-2 may have a therapeutic effect on these human tumors, which could be a direction of future research in the area of cancer treatment.

References

[1] S. Moncada, R.M.J. Palmer and E.A. Higgs, Nitric oxide: Physiology, pathophysiology and pharmacology. *Pharmacological Reviews* 1991; **43**: 109–141.

[2] D.J. Stuehr and M.A. Marletta, Mammalian nitrite biosynthesis: Mouse macrophages produce nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. *Proceedings of the National Academy of Science of the United States of America* 1985; **82**: 7738–7742.

[3] D.E. Koshland Jr., The molecule of the year. *Science* 1992; **258**: 1861.

[4] Y. Henry, M. Lepoivre, J.C. Drapier, C. Ducrocq, J.L. Boucher and A. Guissani, ERP characterization of molecular targets of NO in mammalian cells and organelles. *FASEB Journal* 1993; **7**: 1124–1134.

[5] J.S. Stamler, D.J. Singel and J. Loscalzo, Biochemistry of nitric oxide and its redox activated forms. *Science* 1992; **258**: 1898–1902.

[6] J.M. Fukuto, Chemistry of nitric oxide: Biologically relevant aspects. In: Ignarro, L. and Murad, F. (Eds.) *Nitric oxide – biochemistry, molecular biology and therapeutic implications. Advances in Pharmacology.* Academic Press, USA 1995; pp. 1–15.

[7] L.J. Ignarro, Heme-dependent activation of cytosolic guanylate cyclase by nitric oxide: A widespread signal transduction mechanism. *Biochemical Society Transactions* 1992; **20**: 465–469.

[8] L.J. Ignarro, Heme-dependent activation of soluble guanylate cyclase by nitric oxide: Regulation of enzyme activity by porphyrins and metallophorphyrins. *Seminars in Hematology* 1989; **26**: 63–76.

[9] R. Radi, J.S. Beckman, K.M. Bush and B.A. Freeman, Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics* 1991; **288**: 481–487.

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

[11] D.A. Wink, J.A. Cook, S. Kim, Y. Vodovotz, R. Pacelli, M.C. Krishna, A. Russo, J.B. Mitchell, D. Jourd'heuil, A.M. Miles and M.B. Grisham, Superoxide modulates the oxidation and nitrosation of thiols by nitric oxide derived reactive intermediates. *Journal of Biological Chemistry* 1997; **272**: 11 147–11 512.

[12] P. Correa, C. Cuello, L.F. Fajardo, W. Haenszel, O. Bolanos and B. de Ramirez, Diet and gastric cancer: Nutrition survey in a high-risk area. *Journal of the National Cancer Institute* 1983; **70**: 673–678.

[13] H. Marquardt, F. Rufino and J.H. Weisburger, Mutagenic activity of nitrite-treated foods: Human stomach cancer

may be related to dietary factors. *Science* 1977; **196**: 1000–1001.

[14] H.W. Taylor and W. Lijinski, Tumor induction in rats by feeding heptamethyleneimine and nitrite in water. *Cancer Research* 1975; **35**: 812–815.

[15] C.L. Lintas, A. Clark, J. Fox, S.R. Tannenbaum and P.M. Newberne, *In vivo* stability of nitrite and nitrosamine formation in the dog stomach: Effect of nitrite and amine concentration and of ascorbic acid. *Carcinogenesis* 1982; **3**: 161–165.

[16] J.H. Weisburger, H. Marquardt, N. Hirota, H. Mori and G.M. Williams, Induction of cancer of the glandular stomach in rats by an extract of nitrite-treated fish. *Journal of the National Cancer Institute* 1980; **64**: 163–167.

[17] S.S. Mirvish, S. Salmasi, S.M. Cohen, K. Patil and E. Mahboubi, Liver and forestomach tumors and other forestomach lesions in rats treated with morpholine and sodium nitrite, with and without sodium ascorbate. *Journal of the National Cancer Institute* 1983; **71**: 81–85.

[18] D.A. Wink, I. Hanbauer, M.B. Grisham, F. Laval, R.W. Nims, J. Laval, J.C. Cook, R. Pacelli, J. Liebmann, M.C. Krishna, M.C. Ford and J.B. Mitchell, The chemical biology of NO. Insights into regulation, protective and toxic mechanisms of nitric oxide. *Current Topics in Cellular Regulation* 1996; **34**: 159–187.

[19] M.A. Marletta, Mammalian synthesis of nitrite, nitrate, nitric oxide, and N-nitrosating agents. *Chemical Research in Toxicology* 1988; **1**: 249–257.

[20] A.M. Miles, M. Gibson, M. Krishna, J.C. Cook, R. Pacelli, D.A. Wink and M.B. Grisham, Effects of superoxide on nitric oxide-dependent N-nitrosation reactions. *Free Radical Research* 1995; **233**: 379–390.

[21] R.H. Liu and J.H. Hotchkiss, Potential genotoxicity of chronically elevated nitric oxide: A review. *Mutation Research* 1995; **339**: 73–89.

[22] H. Ohshima and H. Bartsch, Chronic infections and inflammatory processes as cancer risk factors: Possible role of nitric oxide in carcinogenesis. *Mutation Research* 1994; **305**: 253–264.

[23] D.A. Wink, K.S. Kasprzak, C.M. Maragos, R.K. Elespuru, M. Misra, T.M. Dunams, T.A. Cebula, W.H. Koch, A.W. Andrews, J.S. Allen and L.K. Keefer, DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 1991; **254**: 1001–1003.

[24] T. Nguyen, D. Brunson, C.L. Crespi, B.W. Penman, J.S. Wishnok and S.R. Tannenbaum, DNA damage and mutation in human cells exposed to nitric oxide *in vitro*. *Proceedings of the National Academy of Sciences of the United States of America* 1992; **89**: 3030–3034.

[25] K. Merchant, H. Chen, T.C. Gonzales, L.K. Keefer and B.R. Shaw, Deamination of single-stranded DNA cytosine residues in aerobic nitric oxide solution at micromolar total NO exposures. *Chemical Research in Toxicology* 1996; **9**: 891–896.

[26] T. DeRojas-Walker, S. Tamir, H. Ji, J.S. Wishnok and S.R. Tannenbaum, Nitric oxide induces oxidative damage in addition to deamination in macrophage DNA. *Chemical Research in Toxicology* 1995; **8**: 473–477.

[27] M.G. Salgo, K. Stone, G.L. Squadrito, J.R. Battista and W.A. Pryor, Peroxynitrite causes DNA nicks in plasmid pBR322. *Biochemical and Biophysical Research Communications* 1995; **210**: 1025–1030.

[28] C. Ling-Ling, T. Nakamura, Y. Nakatsu, K. Sakumi and H. Hayakawa, Specific amino acid sequences required for O^6-methylguanine-DNA-methyltransferase activity: Analysis of three residues at or near the methyl acceptor site. *Carcinogenesis* 1992; **13**: 837–843.

[29] P. Zak, K. Kliebl and F. Laval, Repair of O^6-methylguanine and O^4-methylthymine by human O^6-methylguanine-DNA-methyltransferase. *Journal of Biological Chemistry* 1994; **269**: 730–733.

[30] D.A. Wink, R.W. Nims, J.F. Darbyshire, D. Cristodoulou, I. Hanbauer, G.W. Cox, F. Laval, J. Laval, J.A. Cook, M.C. Krishna, W. DeGraff and J.B. Mitchell, 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. *Chemical Research in Toxicology* 1994; **7**: 519–525.

[31] D. Rhodes and A. Klug, Zinc fingers. *Scientific American* 1993; **268**: 56–65.

[32] D.A. Wink and J. Laval, The Fpg protein, a DNA repair enzyme, is inhibited by the biomediator nitric oxide *in vitro* and *in vivo*. *Carcinogenesis*, 1994; **15**: 2125–2129.

[33] U.K. Messmer, M. Ankarcrona, P. Nicotera and B. Brune, P53 expression in nitric oxide-induced apoptosis. *FEBS Letters* 1994; **355**: 23–26.

[34] L.L. Thomsen, D.W. Miles, L. Happerfield, L.G. Bobrow, R.G. Knowles and S. Moncada, Nitric oxide synthase activity in human breast cancer. *British Journal of Cancer* 1995; **72**: 41–44.

[35] L.L. Thomsen, F.G. Lawton, R.G. Knowles, J.E. Beesley, V. Riveros-Moreno and S. Moncada, Nitric oxide synthase activity in human gynecological cancer. *Cancer Research* 1994; **54**: 1352–1354.

[36] C.S. Cobbs, J.E. Brenman, K.D. Aldape, D.S. Bredt and M.A. Israel, Expression of nitric oxide synthase in human central nervous system tumors. *Cancer Research* 1995; **55**: 727–730.

[37] W. Radomski, D.C. Jenkins, L. Holmes and S. Moncada, Human colorectal adenocarcinoma cells: Differential nitric oxide synthesis determines their ability to aggregate platelets. *Cancer Research* 1991; **51**: 6073–6078.

[38] J. Prazma, P. Petrusz, W. Mims, S.S. Ball and M.C. Weissler, Immunohistochemical characterization of nitric oxide synthase activity in squamous cell carcinoma of the head and neck. *Otolaryngology and Head and Neck Surgery* 1995; **13**: 541–549.

[39] J. Folkman, Angiogenesis and angiogenesis inhibition: An overview. *EXS* 1997; **79**: 1–8.

[40] N. Weidner, J.P. Semple, W.R. Welch and J. Folkman, Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. *New England Journal of Medicine* 1991; **324**: 1–8.

[41] S. Ambs, W.P. Bennett, W.G. Merriam, M.O. Ogunfusika, S.M. Oser, A.M. Harrington, P.G. Shields, E. Felley-Bosco, S.P. Hussain and C.C. Harris, Relationship between p53 mutations and inducible nitric oxide synthase expression in human colorectal cancer. *Journal of the National Cancer Institute* 1999; **91**: 86–88.

[42] S.M. Moochhala, V.J.S. Chhatwall, S.T.F. Chan, S.S. Ngoi, Y.W. Chia and A. Rauff, Nitric oxide synthase activity and expression in human colorectal cancer. *Carcinogenesis* 1996; **17**: 1171–1174.

[43] R.M. Sutherland, J.S. Rasey and R.P. Hill, Tumor biology. *American Journal of Clinical Oncology* 1988; **11**: 253–274.

[44] E. Pipili-Synetos, A. Papageorgiou, E. Sakkoula, G. Sotiropoulou, G. Fotsis, G. Karakiulakis and M. Maragoudakis, Inhibition of angiogenesis, tumor growth and metastasis by the NO-releasing vasodilators,

isosorbide mononitrate and dinitrate. *British Journal of Pharmacology* 1995; **116**: 1829–1834.

[45] J.G. De Man, P.A. Pelckmans, G.E. Boeckxstaens, H. Bult, L. Oosterbosch, O. Hatjikondi, P. Ravazoula, D. Kardamakis, J. Dimopoulos and S. Papaioannou, *In vivo* experimental evidence that the nitric oxide pathway is involved in the X-ray-induced antiangiogenicity. *British Journal of Cancer* 1996; **74**: 1916–1923.

[46] M. Ziche, L. Morbidelli, E. Masini, S. Amerini, H.J. Granger, C.A. Maggi, P. Geppetti and F. Ledda, Nitric oxide mediates angiogenesis *in vivo* and endothelial cell growth and migration *in vitro* promoted by substance P. *Journal of Clinical Investigation* 1994; **94**: 2036–2044.

[47] D.C. Jenkins, I.G. Charles, L.L. Thomsen, D.W. Moss, L.S. Holmes, S.A. Baylis, P. Rhodes, K. Westmore, P.C. Emson and S. Moncada, Roles of nitric oxide in tumor growth. *Proceedings of the National Academy of Sciences of the United States of America* 1995; **92**: 4392–4396.

[48] M. Lepoivre, B. Chenais, A. Yapo, G. Lemaire, L. Thelander and J.P. Tenu, Alterations of ribonucleotide reductase activity following induction of the nitrite-generating pathway in adenocarcinoma cells. *Journal of Biological Chemistry* 1990; **265**: 14 143–14 149.

[49] M. Lepoivre, F. Fieschi, J. Coves, L. Thelander and M. Fontecave, Inactivation of ribonucleotide reductase by nitric oxide. *Biochemical and Biophysical Research Communications* 1991; **179**: 442–448.

[50] M. Lepoivre, J.M. Flaman and Y. Henry, Early loss of the tyrosyl radical in ribonucleotide reductase of adenocarcinoma cells producing nitric oxide. *Journal of Biological Chemistry* 1992; **267**: 22 994–23 000.

[51] J.R. Lancaster and J.B. Hibbs, EPR demonstration of iron-nitrosyl complex formation by cytotoxic activated macrophages. *Proceedings of the National Academy of Sciences of the United States of America* 1990; **87**: 1223–1227.

[52] J.C. Drapiers, H. Hirling, J. Wietzerbin, P. Kaildy and L.C. Kuhn, Biosynthesis of nitric oxide activates iron regulatory factor in macrophages. *EMBO Journal* 1993; **12**: 3643–3649.

[53] G. Weiss, B. Goossen, W. Doppler, K. Pantopoulos, G. Werner-Felmayer and M.W. Hentze, Translational regulation via iron-responsive elements by the nitric oxide/NO synthase pathway. *EMBO Journal* 1993; **12**: 3651–3657.

[54] C. Bouton, M. Raveau and J.C. Drapier, Modulation of iron regulatory protein function. Further roles of nitrogen and oxygen-derived reactive species. *Journal of Biological Chemistry* 1996; **271**: 2300–2306.

[55] M. Graziewicz, D.A. Wink and F. Laval, Nitric oxide inhibits DNA ligase activity. Potential mechanism for NO mediated DNA damage. *Carcinogenesis* 1996; **17**: 2501–2505.

[56] R. Radi, J.S. Beckman, K.M. Bush and B.A. Freeman, Peroxynitrate oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *Journal of Biological Chemistry* 1991; **266**: 4244–4250.

[57] J.P. Spencer, J. Wong, A. Jenner, O.I. Aruoma, C.E. Cross and B. Halliwell, Base modification and strand breakage in isolated calf thymus DNA and in DNA from human skin epidermal keratinocytes exposed to peroxynitrite or 3-morpholinosydnonimine. *Chemical Research in Toxicology* 1996; **9**: 1152–1158.

[58] H. Ohshima, Y. Yoshie, S. Auriol and I. Gilibert, Antioxidant and pro-oxidant actions of flavonoids: Effects on DNA damage induced by nitric oxide, peroxynitrite and nitroxyl anion. *Free radical Biology and Medicine* 1998; **25**: 1057–1065.

[59] K. Forrester, S. Ambs, S.E. Lupold, R.B. Kapust, E.A. Spillare, W.C. Weinberg, E. Felley-Bosco, X.W. Wang, D.A. Geller, E. Tzeng, T.R. Billiar and C.C. Harris, Nitric oxide-induced p53 accumulation and regulation of inducible nitric oxide synthase expression by wild-type p53. *Proceedings of the National Academy of Sciences of the United States of America* 1996; **93**: 2442–2447.

[60] S.J. Baker, S. Markowitz, E.R. Fearon, J.K.V. Willson and B. Vogelstein, Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science* 1990; **249**: 912–915.

[61] M.B. Kastan, O. Onykewere, D. Sidransky, B. Vogelstein and R.W. Craig, Participation of p53 protein in the cellular response to DNA damage. *Cancer Research* 1991; **51**: 6304–6311.

[62] S. Tsuji, M. Tsujii, W.H. Sun, E.S. Gunawan, H. Murata, S. Kawano and M. Hori, *Helicobacter pylori* and gastric carcinogenesis. *Journal of Clinical Gastroenterology* 1997; **25**: S186–S197.

[63] K.M. Dameron, O.V. Volpert, M.A. Tainsky and N. Bouck, Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* 1994; **265**: 1582–1584.

[64] T.G. Graeber, C. Osmanian, T. Jacks, D.E. Housman, C.J. Koch, S.W. Lowe and A.J. Giaccia, Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumors. *Nature* (*Lond.*) 1996; **379**: 88–91.

[65] S. Ambs, W.G. Merriam, M.O. Ogunfusika, W.P. Bennett, N. Ishibe, S.P. Hussain, E.E. Tzeng, D.A. Geller, T.R. Billiar and C.C. Harris, P53 and vascular endothelial growth factor regulate tumor growth of NOS2-expressing human carcinoma cells. *Nature Medicine* 1998; **4**: 1371–1376.

[66] G. Melillo *et al.*, A hypoxia-responsive element mediates a novel pathway of activation of the inducible nitric oxide synthase promoter. *Journal of Experimental Medicine* 1995; **182**: 1683–1693.

[67] G. Montrucchio, E. Lupia, A. de Martino, E. Battaglia, M. Arese, A.S. Tizzani, F. Bussolino and G. Camussi, Nitric oxide mediates angiogenesis induced *in vivo* by platelet-activating factor and tumor necrosis factor-α. *American Journal of Pathology* 1997; **151**: 557–563.

[68] N.Z. Wu, B. Klitzman, R. Dodge and M.W. Dewhirst, Diminished leukocyte-endothelium interaction in tumor microvessels. *Cancer Research* 1992; **52**: 4265–4268.

[69] B.V. Khan, D.G. Harrison, M.T. Olbrych, R.W. Alexander and R.M. Medford, Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proceedings of the National Academy of Sciences of the United States of America* 1996; **93**: 9114–9119.

[70] S. Nakano, K. Matsukado and K.L. Black, Increased brain tumor microvessel permeability after intracarotid bradykinin infusion is mediated by nitric oxide. *Cancer Research* 1996; **56**: 4027–4031.

[71] A. Orucevic and P.K. Lala, 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 Immunology, Immunotherapy* 1996; **42**: 38–46.

[72] C.S. Parkins, M.F. Dennis, M.R. Stratford, S.A. Hill and D.J. Chaplin, Ischemia reperfusion injury in tumors: The role of oxygen radicals and nitric oxide. *Cancer Research* 1995; **55**: 6026–6029.

[73] P. Lejeune, C.P. Lagade, N. Onier, D. Pinard, H. Ohshima and J.F. Jeannin, Nitric oxide involvement in tumor-induced immunosuppression. *Journal of Immunology* 1994; **152**: 5077–5083.
[74] C.D. Mills, Molecular basis of 'supressor' macrophages. Arginine metabolism via the nitric oxide synthetase pathway. *Journal of Immunology* 1991; **146**: 2719–2723.
[75] N.E. Hubbard and K.L. Erickson, Role of 5-lipoxygenase metabolites in the activation of peritoneal macrophages for tumoricidal function. *Cellular Immunology* 1995; **160**: 115–122.
[76] D. Salvemini, T.P. Misko, J.L. Masferrer, K. Seibert, M.G. Currie and P. Needleman, Nitric oxide activates cyclooxygenase enzymes. *Proceedings of the National Academy of Sciences of the United States of America* 1993; **90**: 7420–7444.
[77] T.E. Gardner, H. Naama and J.M. Daly, Peritoneal and splenic macrophage functions in the tumor-bearing host. *Journal of Surgical Research* 1995; **59**: 305–310.
[78] H. Maeda, Y. Noguchi, K. Sato and T. Akaike, Enhanced vascular permeability in solid tumor is mediated by nitric oxide and inhibited by both new nitric oxide scavenger and nitric oxide synthase inhibitor. *Japanese Journal of Cancer Research* 1994; **85**: 331–334.
[79] M. Tsujii and R.N. DuBois, Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 1995; **83**: 493–501.
[80] H. Sawaoka, S. Tsuji, M. Tsujii, E.S. Gunawan, A. Nakama, Y. Takei, K. Nagano, H. Matsui, S. Kawano and M. Hori, Expression of the cyclooxygenase-2 gene in gastric epithelium. *Clinical gastroenterology* 1997; **25**: S105–S110.
[81] L.L. Thomsen, D.W. Miles, L. Happerfield, L.G. Bobrow, R.G. Knowles and S. Moncada, Nitric oxide synthase activity in human breast cancer. *British Journal of Cancer* 1995; **72**: 41–44.
[82] E. Ellie, H. Loiseau, F. Lafond, J. Arsaut and J. Demotes-Mainard, Differential expression of inducible nitric oxide synthase mRNA in human brain tumors. *Neuroreport* 1995; **7**: 294–296.
[83] S. Cascinu, F. Graziano, E. Del Ferro, M.P. Staccioli, M. Ligi, A. Carnevali, P. Muretto and G. Catalano, Expression of p53 protein and resistance to preoperative chemotherapy in locally advanced gastric carcinoma. *Cancer* 1998; **83**: 1917–1922.
[84] B.V. Joypaul, D. Hopwood, E.L. Newman, S. Qureshi, A. Grant, S.A. Ogston *et al.*, The prognostic significance of the accumulation of p53 tumor suppressor gene protein in gastric adenocarcinoma. *British Journal of Cancer* 1994; **69**: 943–946.
[85] H.E. Gabbert, W. Muller, A. Schneiders, S. Meier and G. Hommel, The relationship of p53 expression to the prognosis of 418 patients with gastric carcinoma. *Cancer* 1995; **76**: 720–726.
[86] M. Victorzon, S. Nordling, C. Haglund, J. Lundin and P.J. Roberts, Expression of p53 protein as a prognostic factor in patients with gastric cancer. *European Journal of Cancer* 1996; **32A**: 215–220.