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NO News is not Necessarily Good News in Cancer

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Abstract: The diatomic radical nitric oxide has been the focus of numerous studies involved with every facet of cancer. It has been implicated in carcinogenesis, progression, invasion, metastasis, angiogenesis, escape from immune surveillance, and modulation of therapeutic response. In recent years, an increasing number of studies have suggested the possible involvement of nitric oxide in multiple cancer types, including melanoma. It is perhaps not surprising that conflicting viewpoints have arisen as to whether nitric oxide is beneficial or deleterious in cancer. However, it has become clear that nitric oxide possesses modulatory properties in a number of signal transduction pathways that depend on concentration and context. Our laboratory has shown that tumor expression of inducible nitric oxide synthase in melanoma patients results in poor survival. Furthermore, we demonstrated that the removal of endogenous nitric oxide in melanoma cell lines led to increased sensitivity to cisplatin-induced apoptosis in a p53-dependent manner. Others have shown antiapoptotic properties of NO in melanoma cells. However, several studies also suggest that NO can inhibit metastasis and diminish resistance. Despite the apparently conflicting observations, it is evident that NO is involved in melanoma pathology. The purpose of this review is to summarize the current literature relating to the role of NO in cancer with particular emphasis on its relevance to therapeutic resistance in melanoma.

Recent evidence suggests the involvement of an intricate and complex interplay between reactive nitrogen species and reactive oxygen species. The importance of nitric oxide and its balance with other oxidative agents in the regulation of cancer cell response to therapies will be discussed. This balance may serve as an important focal point in determining patient response to therapy. The ability to control this balance could significantly influence outcome.

Keywords: Nitric oxide, NOS, cancer, drug resistance, prognostic markers, chemotherapy.

INTRODUCTION

Nitric oxide (NO) is an important pleiotropic mediator in many physiological and pathological processes, including vasodilatation, neurotransmission, host defense, platelet aggregation, iron metabolism, and peripheral mononuclear (PMN) and macrophage-mediated immunity. This tiny molecule is the product of the conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS) which exists as three isoforms; inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS) [1-3]. All three isoforms have been identified and named according to the cell type or conditions in which they were first described. These enzymes share about 50 % sequence homology and catalyze the NADPH- and O_2-dependent oxidation of L-arginine to NO and citrulline (see details in Table 1) [4-8]. NOSs are enzymes that are active only as homodimers. Dimerization is thought to activate the enzyme by sequestering iron, generating high-affinity binding sites for arginine and the essential cofactor tetrahydrobiopterin (BH_4), and allowing electron transfer from the reductase-domain flavins to the oxygenase-domain haem. Recent studies of NOS expression in tumor tissue indicate that NO can be involved both as a promoter and inhibitor in the etiology of cancer. Various NOS expression levels and activities have been detected in many different types of tumor cells, and have been associated with increased tumor grade, higher proliferation rate, altered expression of critical growth controlling signaling cascade components, and resistance to DNA-damaging therapeutics. This review will summarize the current literature relating to the role of NO in cancer with

Table 1. Human NOS Isoforms – General Characteristics

Human NOS Isoform	Gene Structure and Size	Chromosomal Location	Protein Size	Reference(s)
nNOS - NOS1	29 exons, 28 introns >200kbp	chromosome 12	161 kDa	4, 5
iNOS – NOS2	26 exons, 25 introns 37 kbp	chromosome 17	131 kDa	5,6
eNOS – NOS3	26 exons, 25 introns 21-22kbp	chromosome 7	133 kDa	7,8

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particular emphasis on endogenous iNOS regulated function and its relevance to resistance to therapeutic approaches in melanoma.

EXPRESSION OF NOS IN HUMAN TUMORS

Studies on the generation of NO by mammalian tissues and the expanded research on the role of NO in cancer have brought new efforts to bear on many areas of tumor biology research. Despite the initial findings that high levels of immune-cell generated NO can be cytotoxic for tumor cells, recent findings have shown that lower constitutive generation of NO may play a leading role in increased tumor growth. The study of NO and its role in tumor cell biology, and particularly the resistance to therapy, requires an understanding of the enzyme's activity and a clear identification of NOS localization in tumor tissues.

Solid tumors have a very complex biology and often exhibit unique mechanisms in different tumors. However, there is now a growing body of research in cellular expression of NOS isoforms in human cancers, including cancers of the breast, stomach, ovary, cervix, central nervous system, and melanoma [9-17]. Most of these studies have clearly documented with immunohistochemical studies, cellular localization either in tumor cells, stromal cells, or both depending on tumor type. The first report of NOS expression in human gynecological tumors showed predominantly localized expression in tumor cells [9]. Moreover, the same study showed that the lack of detectable activity in normal tissue suggested a relationship between NOS activity and malignancy. Subsequent studies focused on breast cancers and suggested that higher levels of NOS activity were localized in malignant tissue areas, rather than in benign tissue [10]. They have also observed a correlation between tumor grade and iNOS tissue expression; however unlike the gynecological tumors, iNOS was predominantly localized in the stromal components of breast tumor tissue. Analysis of the prognostic importance of NOS isoforms in breast cancer has been carried out in further investigations, which suggest that endogenous tumor cell mediated iNOS expression might have an inhibitory effect on the metastatic process in breast cancer, since the presence of tumor cell iNOS protein is inversely related to the tumor's metastatic potential [17]. Another early study compared the expression of all three NOS isoforms in brain tumors [18]. An increased expression of the nNOS and eNOS in astrocytic tumor cells was reported with the highest levels of expression being found in higher-grade tumors; iNOS, however, was less frequently detected and expressed at a lower level, predominantly in tumor endothelial cells. The data further indicates that central nervous system neoplasms express high levels of NOS, suggesting that NO production may be associated with pathophysiological processes important to these tumors. Studies on the level of expression and cellular localization of isoforms of NOS have also been performed in human stomach tumor tissues [13]. One study showed that the tumor tissues have 70% higher activity of NOS than that of normal tissues. Moreover, poorly differentiated adenocarcinoma tends to have higher activity than well differentiated and moderately differentiated tumor tissues. Overall the results of this study revealed that isoforms of NOS might contribute differentially to growth and progression of human stomach tumors.

The first study from our group focused on human melanoma and showed that iNOS and its product, NO, mediated post-translational protein modification end-product, nitrotyrosine (NT) expression by the melanoma cells. This strongly correlated with poor survival in patients with stage III disease, suggesting a pathway whereby iNOS might contribute to enhanced tumor progression [16]. Our initial findings suggested that iNOS expression might have potential as a prognostic marker, and that NO is a critical mediator of an aggressive tumor phenotype in human metastatic melanomas. Subsequently, we have also shown that tumor iNOS expression inversely correlates with tumor cells MDA7 expression [19], which is one of the novel markers that have been shown to be tumor suppressors in melanoma by various groups, as well as our group [19-21]. We have recently completed a larger series of Stage III melanoma patients' tumor iNOS expression analysis, and its significance on prognosis (our unpublished data). Our results strongly suggest that iNOS is an independent prognostic marker for Stage III melanomas. Besides our study, there are three relatively large-scale studies in breast cancer supporting iNOS expression in tumor cells and its correlation with disease progression [15, 22, 23]. In one of these large studies, Loibl *et al.* demonstrated that while none of the benign lesions were positive for iNOS (0/41), 67% *in situ* carcinomas (21/27) and 61% invasive lesions (33/54) showed iNOS tumor cell staining [23]. Therefore, based on various studies in different histological types of cancer, NOS expression is likely to play an active role in tumor biology. However, the precise function(s) of NO in cancer progression remain unclear; its presence in either tumor or stromal cells at the tumor site is likely to contribute to the altered biology of cancers.

ROLE OF NOS EXPRESSION AND NO PRODUCTION IN HUMAN TUMORS

Despite the apparent roles of eNOS and nNOS in important cell signaling mechanisms in the cardiovascular and nervous systems, the role of iNOS in cancer is more complicated, possibly due to the various concentrations and half-life of NO produced. NO production by eNOS and nNOS is dependant on transient increases in local calcium concentrations, and their activation results in a relatively short-term action. Conversely, NO is generated for longer periods and at higher levels as a result of iNOS expression, which is constitutively active at basal calcium levels due to its unusually high binding affinity for calmodulin (CaM). iNOS is therefore regulated at the level of transcription and translation and can be induced by inflammatory stimuli or appropriate cytokine stimulation. The induction of iNOS and the subsequent biological actions of NO are complex mechanisms, which may partly explain the contradicting results in NO biology obtained by different groups. As a major question, it is still not definite whether NO is protective or destructive to a cancer cell, which we propose may be either, based on the concentration and exposure of the cell to NO.

Recent studies have described NO involvement in various biological mechanisms, including cancer progression, metastasis, and tumor microcirculation and angiogenesis. Although the iNOS and NO data may appear

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confusing and conflicting, we propose a unifying view in which a dual role for the iNOS product NO exists [24]; lower levels (pM-nM) of NOS in tumors promote tumor growth while higher levels (μM) induce apoptosis or cell death. Anti-apoptotic activities of NO are observed at concentrations that are only 2-10% of those needed for the induction of apoptosis [25]. In an early study, human breast tissue was examined for iNOS protein, and a moderately low level, 20 pmoles/min/mg, was reported [10]. Our measurements of iNOS activity in human melanoma have confirmed the low levels as was predicted by the presence of the enzyme: up to 30 pmoles/min/mg was measured in the highest producers (Grimm, unpublished data). To date, only one report of human melanoma expressing iNOS has been published that addresses iNOS in primary cutaneous lesions and lymph nodes [12]. While over 80% of the tumors from these varied metastatic sites were positive, the conclusion offered was that iNOS presence is heterogeneous and may predict distant metastasis [12]. Our data extend this first report by Tschugguel [12]; we did not examine primary or cutaneous tissues, as all the patients for the preliminary data are from the metastasis of Stage III patients and the iNOS presence strongly correlated with poor survival [16]. Since this first report, an analysis of iNOS in benign and malignant cutaneous lesions was published, demonstrating that benign nevi do express iNOS but not NT, and NT was found to be readily increased in the more malignant and metastatic specimens, suggesting that iNOS is functionally inactive in benign lesions [26]. Salvucci *et al.* [27] confirmed that iNOS is also expressed in metastatic melanoma, but they did find iNOS in nevi; however blocking the iNOS protein with a specific inhibitor resulted in apoptosis in malignant cells but not melanocytes. Oshima [28] reviewed the data and hypothesized that chronic inflammatory processes and the associated NO provide a high risk for carcinogenesis. For example, the formation of major NO products peroxynitrite ($ONOO^-$) and NT are considered causal in the pathogenesis of ulcerative colitis and in gastritis due to *Helicobacter pylori*; these inflammatory clinical conditions are well-known as high risk factors for colon and stomach cancer [29, 30]. More recently, the existence of low levels of iNOS and the resultant NO has been related to tumor invasion [24], growth and vascularization through VEGF up-regulation [31] and resistance to apoptosis [32].

Role of NO in Cell Signaling

After discovering various biological effects of iNOS and NO, a number of investigators have begun to explore how NO-mediated posttranslational modifications of proteins may represent mechanisms of cellular signaling. These modifications include; nitrosylation of thiol and amine groups; nitration of tyrosine, tryptophan, amine, carboxylic acid, and phenylalanine groups; binding to metal centers; and oxidation of thiols and tyrosine [33]. Nitrosylation of thiols and nitration of tyrosine residues to produce nitrotyrosine are two well-studied mechanisms, and constitute current active areas of research in cancer biology. NO reacts with superoxide at near-diffusion kinetics to form $ONOO^-$, a potent nitrating agent [34]. Even though NO can affect cellular functions through posttranslational modifications of proteins directly and indirectly, the main physiological signaling pathway of NO is considered to be the activation of guanylate cyclase, formation of cGMP, and concomitant protein phosphorylation [35]. We will further discuss biological outcomes of these posttranslational protein modifications by NO, later in this review.

Many of the biological and pathological effects of NO are mediated through cell signaling pathways that are initiated by NO. Direct addition of NO or $ONOO^-$ to cells can result in activation of MAP kinases. Go *et al.* [36] have initially shown that shear stress induces the formation of both NO and O_2 within the cell and activates the MAP kinases extracellular signal regulated protein kinase (ERK) and N-terminal c-Jun kinase (JNK). In fact, Lander *et al.* have [37] reported the very first study on the effect of NO on protein kinase cascades showing that NO generating compounds stimulated a membrane-associated protein tyrosine phosphatase activity, which led to a dephosphorylation and activation of the src family protein tyrosine kinase $p56^{lck}$. The same group subsequently showed that NO activates all three MAPK cascades; the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) cascade, p38 MAPK cascade and the classical ERK/MAPK cascade [38]. Activation of the different MAPK cascades by NO may occur either by direct alterations to the kinases themselves or by modulation of an upstream factor such as a GTP-binding protein like p21ras [39]. NO has been shown to nitrate c-src, a part of a mitogenic signaling pathway, causing the dissociation of its autoregulatory loop and subsequently resulting in facilitated phosphorylation and activation of c-src. Tyrosine nitration of c-src in human pancreatic ductal adenocarcinoma was shown to increase tyrosine phosphorylation, c-src kinase activity, and to be associated with its downstream substrate cortactin [40]. S-nitrosylation of c-src has also been suggested [41]. In addition, Fos and Jun components of the AP-1 transcription factor were also shown to be susceptible to S-nitrosylation, which resulted in a decrease in DNA binding capacity [42].

Another class of protein kinases was shown to be directly modulated by NO, the Janus kinases (JAKs) [43]. Both JAK2 and JAK3 autokinase activity were found to be inhibited by NO, presumably by oxidation of crucial thiols to disulphides [44]. Very recent studies have also been focused on the PI3K and Akt pathway. Korkmaz *et al.* [45] have reported their data based on the background information that NO mediates cellular functions by partly activating soluble guanylate cyclase (sGC) that converts guanosine-5'-triphosphate (GTP) to cyclic guanosine-3',5'-monophosphate (cGMP). However, since membrane-bound GCs produce cGMP in response to a cellular signal, but not the NO-target enzyme sGC, the phosphorylation sites of NOS III, or their regulation by ERK1/2 and Akt/protein kinase B (Akt/PKB) in osteoclasts. Their results provide data showing that the phosphorylation of NOS III at Ser1177, and phosphorylated Akt/PKB are involved in regulation of NO production by NOS II in osteoclasts under basal conditions [45]. This pathway has also been explored in cardiovascular disease and recently, it has been suggested that the insulin-PI3K-Akt-NOS signaling pathway may play a significant role in central cardiovascular regulation *via* the insulin signal transduction pathway [46] or mitochondrial ATP-sensitive ($mitoK_{ATP}$) K^+ channels regulated pathway [47]. This study has shown that the $mitoK_{ATP}$ K^+ channel opener diazoxide

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activates Akt through the PI3 kinase signaling pathway, and iNOS and eNOS are downstream of Akt. Thus, NO may have widespread effects on phosphorylation-dependent and -independent intracellular signaling pathways. Whether tyrosine nitration and tyrosine phosphorylation are antagonistic or cooperative may depend on context [48]; and as with S-nitrosylation of cysteines, the factors regulating the specificity of tyrosine nitration by NO remains to be clarified [49].

In conclusion, these studies suggest that NO can deliver signals into all the major MAPK cascades including ERK, SAPK/JNK and p38 kinase cascades as well as the JAK/signal transducer and activator of transcription (STAT), and PI3K-Akt signaling pathways. These cascade events then trigger the phosphorylation of key nuclear proteins, including transcription factors such as c-Jun, and, finally, lead to alterations in gene expression. Fig. (1) summarizes the iNOS regulatory pathway in melanoma, potentially as a representative candidate for all NOS subtypes. Our laboratory has ongoing research on b-Raf mediated activation of these downstream molecules, which may activate the iNOS promoter in order to initiate NO production in melanoma cells (unpublished results). Initially, Tsao *et al.* have described activating mutations *NRAS* in 21% of 53 melanoma cell lines. Mutations of *NRAS* stabilize the protein when GTP rather than GDP binds it. This keeps NRAS in the activated state and NRAS-GTP phosphorylates BRAF, C-RAF, and PI3K. Potential downstream pathways to the malignant phenotype include the role of AKT, mTOR, MEK, and ERK tyrosine kinases in mediating growth and mitogenesis. More recently, Davies *et al.* [50] published that activating mutations in *BRAF* can be found in two thirds of melanoma tumors in primary culture and 70% of melanoma cell lines. Consequently Brose *et al.* [51] extended this finding in melanoma tumor samples and confirmed the high frequency of mutations in this enzyme. One point mutation, the substitution of adenosine for thymidine at position 1796 in exon 11, accounts for 80% of the *BRAF* mutations. Several of the less frequently described mutations also present this type of activation to a lesser degree. Changes at genomic, transcriptional and post-translational levels of G-proteins and protein kinases, such as Ras, B-Raf and their transcription factor effectors such as c-Jun, Stat3 and NF-κB affects TNF, Fas and TRAIL receptors, which play important roles in acquiring melanoma resistance to apoptosis.

Role in Apoptosis

Resistance of cancer to therapy is attributable in part to mechanisms such as oncogenic activation [52], altered drug uptake or transport, intracellular detoxification, altered DNA repair, and the dysregulation of the apoptotic pathway [53]. A number of common factors in chemoresistance in melanoma, including the expression of multiple drug resistance genes, alterations in glutathione detoxification systems, mutation in topoisomerase II, and changes in the Ras, Bcl-2 and p53 pathways, have been reviewed by Serrone and Hersey [54]. Dysregulation of the apoptotic-signaling pathway is a common feature of cancer [55]. For instance, melanoma cells were recently shown to commonly contain mutations in Apaf-1, a component of the apoptosome that is formed during mitochondria-mediated apoptosis. Additionally, active suppression of Apaf-1 expression was also seen [56].

An increasing amount of evidence suggests that iNOS has the potential to produce NO at toxic levels that may be exploited in achieving direct apoptosis. In general, NO

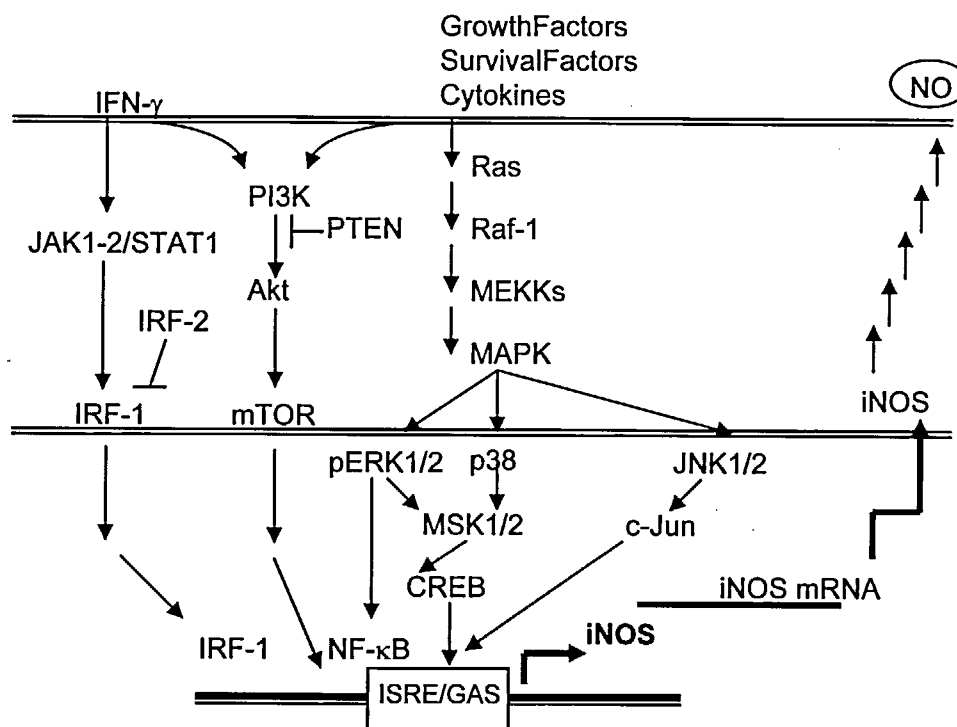

Fig. (1). Schematic Proposal of iNOS Regulatory Pathway in Melanoma.

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modulates the expression of Bcl-2 family members, p53, interleukin-1β-converting enzyme family proteases and the death receptor Fas. NO generated from NO donors or synthesized by NOS induces cell death via apoptosis in a variety of different cell types. There have been exciting advances in the understanding of the molecular relationship between apoptosis and NO. Overall, NO could be an important mediator to consider in the context of future therapeutic applications particularly considering apoptosis as a mechanism to regulate tumor progression.

The role of constitutive NO at lower levels is the main concern today of our research. In this role, NO leads to a resistance to apoptosis. One target is the cysteine protease family of caspases, which plays an important role in apoptotic signal transduction particularly in tumor necrosis factor-α receptor stimulation-mediated DNA fragmentation. The anti-apoptotic role of NO in melanoma can be mediated by the S-nitrosylation of these caspases at their active site cysteine residues [57]. This mechanism can inhibit initiator caspases, such as caspase-9 [58] and effector caspases, such as caspase 1 and 3 [59] as well as several additional caspase family members [60]. Another study has also shown that the activation of effector caspases, after triggering of apoptosis by CD95, can be inhibited by endogenous NO [61] as apoptosis triggered through CD95 requires caspase de-nitrosylation [62]. Information published more recently further supports the notion that NO production in human gastrointestinal carcinogenesis is involved in resistance to apoptosis, from evidence indicating blocking DNA repair enzymes by direct nitration, as well as reversibly inhibiting caspases by nitrosylation [63]. In an early study Haendeler *et al.* [25] demonstrated that the cellular regulatory processes of NO to protect cells from apoptosis may be independent of the oxidation-reduction (redox) state and that low concentrations of NO and ONOOq inhibit the cellular suicide program in some cells via S-nitrosylation of members of the caspase family. In addition, TNF-α -induced apoptosis has been shown to be inhibited by NO through induction of heat shock protein 70 expression [64]. It has further been reported that iNOS expression and NO production in UV-irradiated human skin led to the up-regulation of Bcl-2 and protection from apoptosis [65].

Another important facet of the potential anti-apoptotic mechanism of NO is the regulation of p53. Increased NO-generation in a cell may select mutant p53 cells and contribute to tumor angiogenesis by upregulating VEGF. Moreover, NO may modulate tumor DNA repair mechanisms by upregulating p53, poly(ADP-ribose) polymerase (PARP) and the DNA-dependent protein kinase (DNA-PK). Whether p53 plays a pivotal role in the melanoma resistance has been debated. Some data suggest that loss of p53 function may have some clinical correlation with resistance to therapy [66. The idea that p53 may confer resistance to DNA damage in tumor cells is supported by the finding that reduction of overexpressed p53 by human recombinant interferon-α2a in a cisplatin-resistant melanoma cell line released cells from G_1 arrest and abrogated cisplatin resistance [67]. Consistent with these observations, human colon cancer cell lines in which p53 signaling had been specifically disrupted by homologous recombination became more sensitive to DNA damaging chemotherapeutics while the same disruption rendered these cells resistant to antimetabolite 5-fluorouracil (5-FU) [68]. Part of the enhanced sensitivity to DNA damaging agents was explained by the failure of p53 to activate p21. An anti-apoptotic activity for p53 has also been suggested [69]. In accord with this idea, the work of Tang *et al.* [70] from our laboratory has demonstrated that the depletion of endogenously generated NO in melanoma cell lines containing wild-type p53 decreases cell growth, enhances cisplatin-induced apoptosis, and inhibits p53 accumulation and the subsequent induction of p21 by cisplatin. Moreover, this study showed that the increase in cisplatin-induced apoptosis in melanoma cells is caused by NO regulation of p53 expression, which exhibits a protective role against cisplatin treatment. These results support a similar model in which the retention of wild-type p53 confers resistance to DNA damaging agents in part by the induction of p21 as well as DNA repair mechanisms. Although the precise mechanism through which NO modulates the p53 signal transduction pathway remains to be clarified, data from this study suggest that NO is required for proper stabilization and activation of the p53 protein, which may exert a protective role against therapy.

Recently, NO was shown to inhibit Hdm2-p53 binding [71]. Hdm2 (Mdm2 in mouse) binds p53 to inhibit its transcriptional activity, ubiquitinates p53 to regulate its stability, and plays a pivotal role in determining p53 localization [72, 73]. Nitrosylation of cysteine 77 in the hydrophobic p53-binding pocket of Hdm2 is thought to interfere with p53 binding. Hdm2 has also been found to be upregulated in some melanoma cells containing wild-type p53 [74]. These findings suggest that NO is involved in the regulation of p53 stability, which may help to explain how the depletion of endogenous NO inhibits cisplatin-induced p53 accumulation in melanoma cells. Whether endogenous NO inhibits Hdm2-p53 interaction in melanoma cells remains to be determined.

The analysis of the cross-talk between the extrinsic and the intrinsic pathways of apoptosis has recently revealed new antiapoptotic roles in human melanoma cells for NO. From an immunologic stand point of NO-regulated apoptosis in melanoma, Salvucci *et al.* have already published their study showing that inhibition of iNOS activity by aminoguanidine leads to triggering of apoptosis in human melanoma cells but not in normal melanocytes, as documented by binding of annexin-V and by DNA fragmentation through TUNEL assays [27]. This study elegantly showed that the inhibition of NO synthesis in human melanoma activates the intrinsic pathway of apoptosis, as shown in the study by alteration of mitochondrial membrane potential and down-regulation of Bcl-2 protein level, eventually leading to enzymatic activation of effector caspases 1 and 3.

Role of NO in the Regulation of Oxidative Stress

The balance between reactive nitrogen species and reactive oxygen species has emerged as an increasingly important paradigm in cell physiology in the last several years. For example, NO appears to be largely antiapoptotic in hepatocytes, which are rather resistant to NO-associated toxicity, although NO-mediated protection of toxicity in the liver appears to depend on the redox state [75]. A subset of researchers in the field maintained that tyrosine nitration by peroxynitrite, a reaction product of NO and superoxide, was

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likely to be cytotoxic [76]. Yet, an increasing number of manuscripts have described the protective effects of NO in the context of oxidative stress. For example, NO depletion in pulmonary cells caused cell cycle alterations, apoptosis and oxidative stress [77]. Moreover, scavenging of mitochondrial superoxide by NO suppressed apoptosis in human colon cancer cells [78]. These results suggest that the formation of peroxynitrite from NO and superoxide is not necessarily toxic. More recently, careful kinetics and titration studies of NO and superoxide have offered some possible insights into how these factors influence NO chemistry within the cellular milieu [79].

Melanoma cells have been found to be under oxidative stress and contain relatively high levels of superoxide [80, 81]. In addition, melanoma cells appear to be able to buffer against the addition of superoxide, but are less tolerant of hydrogen peroxide treatment [82]. Our laboratory has observed that melanoma cells appear to be more resistant to exogenous sources of peroxynitrite than to exogenous NO alone, which supports the notion that NO generation by melanoma cells help to balance the oxidative stress and redirect the response from an apoptotic to an anti-apoptotic outcome. S-nitrosylation is also believed to be intimately involved in the regulation of several redox-sensitive transcription factors [83]. However, how specificity is built into S-nitrosylation by NO is still unclear [84].

It should be noted that under certain conditions, the oxygenase domain of NOS can generate superoxide without the generation of nitric oxide (NO) or L-citrulline. The level of BH_4 is inversely proportional to the rate of this uncoupled reaction. The level of L-arginine also appears to influence this reaction (reviewed in [85]).

NO in Resistance to DNA Damage-Inducing Therapeutics

NO may regulate tumor response to DNA damage by multiple mechanisms. NO may regulate the activity of p53 upstream effectors. In response to DNA damage, a number of kinases such as ataxia telangectasia mutant kinase (ATM), AT-mutated and Rad3-related kinase (ATR), and DNA-PK can phosphorylate p53 at multiple sites, which are important in p53 activity. In some cases, NO itself was shown to induce DNA damage [86] and lipid peroxidation, which could lead to apoptosis [87]. However, NO has also been implicated in protecting cells against DNA damage and lipid peroxidation in some cases [88]. For instance, NO was shown to activate DNA-PKs and protect cells from DNA damage inducing chemotherapeutics [89]. There is also evidence that NO inhibits the activity of DNA damage activated kinases [90]. Thus, whether and how NO regulates DNA damage activated kinases in melanoma cells remain to be clarified.

Role in Angiogenesis and Tumor Microcirculation

Angiogenesis is a complex process of new capillary formation on the basis of already existing blood vessels. The formation of a functioning vasculature requires the coordinated interaction between endothelial cells, extracellular matrix, and surrounding cells. Physiologically, it is a very strictly regulated process, which results in a balance between stimulatory (angiogenic) and inhibitory (angiostatic) factors to control the correct development of blood vessels [91]. The major physiological stimuli for angiogenesis include tissue ischemia and hypoxia, inflammation, and shear stress [92]. A number of specific factors are known to stimulate or inhibit angiogenesis, including vascular growth factors, inflammatory cytokines, adhesion molecules, and NO. In human tumors NOS expression and activity correlate with tumor growth and aggressiveness, through angiogenesis stimulation and regulation of angiogenic factor expression. The prototypical angiogenic factor, vascular endothelial growth factor (VEGF), is a circulating glycoprotein that promotes blood vessel growth in response to ischemia and other stimuli [93]. Relative to most other solid tumors, human melanomas maintain high microvessel density and a large fraction of immature vascular buds [94]. The overexpression of VEGF is associated with increased metastatic potential and poor prognosis [95]. This presents a unique therapeutic opportunity, which can be manageable by the regulation of NO.

Angiogenic factors induce the release of NO from endothelial cells, which mediates a multiplicity of processes involved in angiogenesis. These NO-modulated processes include endothelial cell survival, proliferation, migration, and interaction with the extracellular matrix. Hood *et al.* [96] demonstrated the significance of NO by showing that capillary formation is prevented by inhibitors of NO synthase, and that VEGF upregulates endothelial NO synthase expression and NO production. Similarly, NO has been shown to be an important downstream mediator of other angiogenic factors, including basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), and angiopoietin-1 [92]. NO stimulates endothelial proliferation and migration, functions as an endothelial survival factor, and may augment angiogenesis through vasodilation-induced increases in local blood flow. Moreover, the competitive inhibitor of the NOS pathway asymmetric dimethylarginine (ADMA) acts as an endogenous inhibitor of angiogenesis [97]. Conversely, agents that increase NO synthesis, such as low dose statins, enhance angiogenesis. Statins have been shown to promote angiogenesis by decreasing caveolin abundance and *via* activation of the endothelial protein kinase Akt/PKB, effects that might also increase NOS activity [98-100]. In addition to increasing NOS activity, statins are known to increase the endothelial expression of NO synthase [101]. Ambs *et al.* used recombinant iNOS expressing human carcinoma cell lines containing mutant p53 [102] to look at tumor growth. Their study demonstrated that an NO-mediated up-regulation of VEGF corresponded with increased vascularization in xenograft tumours. Therefore, it is possible that NO generated by NOS may promote new blood vessel formation by up-regulating VEGF. This neovasculaturization not only enhances the ability of the tumor to grow, but also increases its invasiveness and metastatic ability. Tumor cell invasiveness is promoted by endogenous low levels of NO [103], possibly in part due to increased angiogenesis based on observations in which mice genetically incapable of producing iNOS had decreased VEGF and tumor-associated vascularity [104]. Therefore, modulation of the NO synthase pathway could become a new approach for anti-angiogenic mechanisms.

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REGULATION OF NOS ACTIVITY

Many researchers have studied induction of iNOS activity in different systems. Cytokine treatments using IL-2 and IL-1β have been shown to induce NOS in humans [105-107]. As a result, increased NO production has been found to be associated with toxic effects of cytokine treatment, which were mainly circulatory changes and hypotension as detailed in these studies. Such *in vivo* studies measure plasma nitrite and nitrate levels (NOx) as a systemic indication of increased expression of iNOS since the unstable NO rapidly oxidizes to nitrite and predominantly nitrate. The major problem on using plasma NOx levels as an indicator of enzyme activity is the specificity of the cellular origin, which may not reflect tissue site but only systemic increase as a result of indirect stimulation. However, Moilanen *et al.* showed that 5,6-dimethyl-xanthenone-4-acetic acid (5,6-MeXAA) induced iNOS expression correlated with substantial increases in plasma NOx concentrations that peaked at 8-12 h after 5,6-MeXAA. Moreover, this increased plasma NO levels inhibited by a NOS inhibitor N-iminoethyl-L-ornithine (L-NIO), indicating that it was due to enhanced production of NO [108]. Baylis and Vallance [109] reviewed this issue in great detail and suggested that NOx presents a useful and straightforward index of NO generation, but interpretation of results should be made with caution. They recommended that plasma samples and 24-h urine samples should be collected only after a suitable period of fasting or with individuals/animals on a controlled diet. Moreover, plasma and urine levels together should allow some estimation of approximate total body NO generation and allow calculation of NOx clearance that may vary between individuals and between diseases. Even with accurate measurement of NOx generation, NOx may not reflect biologically active NO, and it is quite possible to conceive of situations in which NOx levels increase while biologic activity of NO decreases. They carefully suggest that measures of NOx should only be used as part of a panel of functional and biochemical measures.

More extensive research on the regulation of iNOS has been performed in cultured cell lines. These *in vitro* studies have shown that three major pro-inflammatory cytokines are known to induce iNOS in human macrophages and non-melanoma tumors; these are the IL-1s, TNFα and IFNγ. These cytokines regulate many NF-κB inducible genes that control expression of other cytokines, cell adhesion molecules, immunoregulatory molecules, and proinflammatory mediators. The promoter of the *iNOS* gene is approximately 16 kB in length and remains only partially characterized. It contains at least four cis-acting NF-κB elements responsible for iNOS induction in lung and liver cell lines [110]. In addition, interferon-γ-responsive elements (IRF-1, ISRE, GAS), and TNF-responsive elements are also present, which suggest a complex transcriptional regulation of iNOS by various signaling pathways (reviewed in [111]). More recently, chromatin *in situ* studies have identified constitutive and inducible sites that function in a tissue-specific manner, suggesting that published studies may not reflect the situation in melanoma [112]. Recently, Richmond *et al.* reported that NF-κB is constitutively active in approximately 60% of human melanoma cell lines, which has been confirmed by our preliminary data and leads to test whether the constitutive iNOS is a result of the constitutive NF-κB [113-114]. In our recently published study, we demonstrate that the treatment of human melanoma cells with curcumin induces G2/M-phase cell cycle arrest and apoptosis and that these mechanisms are related to reduction of NF-κB activity and alteration in the levels of iNOS, p53, p21Cip1, p27Kip1, and CHK2 [115]. In this study, we have demonstrated that human melanoma cells (A375 and MeWo) constitutively expressed the iNOS protein and mRNA. Moreover, our data clearly verified that NOS activity was present in both A375 and MeWo cells. We further demonstrated that curcumin inhibits iNOS activity and leads to the NO depletion, which in turn suppressed melanoma proliferation. However, the link between constitutive NF-κB and constitutive iNOS in melanoma is currently unknown and requires further exploration.

The expression of Cox-2 and iNOS and thereby production of prostaglandins and NO are regulated by the same set of cytokines. The most notable ones are IFNγ and IL-1β, both *in vitro* [116] and *in vivo* [117] . It is also known that the CXCL1 (GROα) and CXCL8 (IL-8) chemokines are highly expressed in many melanomas and form autocrine loops that drive NF-κB in a constitutively active mode [118]; however, their relationship to iNOS expression is not known at this time. More is known about the relation between NF-κB activation mediated NO inducers, which clearly includes heat shock responses. Several inducers of the heat shock response, such as hyperthermia, inhibit NF-κB activation and NO formation. An early study by Feinstein *et al.* [119] shown that heat shock protein 70 suppresses astroglial-iNOS expression by decreasing NF-κB activation. However, it has been recently shown that the antioxidant lipoic acid inhibits NF-κB activation and nitric oxide formation completely independent of the heat shock proteins [120].

The inhibition of iNOS expression can alter the biology of cancers, as others and we have shown. A growing list of NOS inhibitors are currently available with an increasing specificity for individual type of NOSs (see details in Table 2) [5, 121-122]. Original *in vivo* tumor studies widely used N-monomethyl-L-arginine (L-NMMA) and N-nitro-L-arginine methyl ester (L-NAME). However, many studies have used inhibitors that lack any selectivity and they have concluded the actions of iNOS using such inhibitors as selective. As an example, AG is about 10-fold selective for iNOS over eNOS but it is minimally selective over nNOS. Moreover, it has numerous other biological activities, including inhibition of polyamine metabolism and catalase, as well as causing the production of advanced glycosylation products [123].

The first described NOS inhibitors were L-arginine analogs. The most widely studied compounds are guanidine mono- or di-substituted analogues of L-arginine, most notably L-NMMA [124] and L-NAME [125]. They bind at the arginine-binding site and are competitive with arginine. Although, arginine-binding sites of NOS isoforms show high degree of similarity, highly selective iNOS inhibitors have recently been developed that compete with arginine and binds to this site of the molecule [121, 126]. The acetamidine derivatives of L-ornithine (L-*N*-iminoethylornithine, L-NIO) [127] and L-lysine (L-NIL),

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Table 2. Common NOS Inhibitors

nNOS Inhibitors	Molecular Weight
3-Bromo-7-nitroindazole	242.03
6-Nitroindazole	163.14
N^{ω}-Propyl-L-arginine	216.28
eNOS Inhibitors	**Molecular Weight**
L-NIO	246.14
iNOS Inhibitors	**Molecular Weight**
S-(2-Aminoethyl)isothiourea dihydrobromide	281.02
Aminoguanidine hydrochloride	110.55
AMT hydrocholoride	166.67
L-Canavanine sulphate	274.25
EIT hydrobromide	185.09
2-Iminopiperidine hydrochloride	134.61
Isopropylisothiourea hydrobromide	199.11
S-Methyl isothiourea sulphate	278.38
L-NIL hydrochloride	223.70
1400W dihydrochloride	250.17
AR-C102222	
Non-selective NOS Inhibitors	**Molecular Weight**
S-Methyl-L-thiocitrulline dihydrochloride	278.26
L-NAME hydrochloride	269.69
7-NINA	185.12
7-Nitroindazole	163.14
L-NMMA	248.28
L-NNA	219.20
TRIM	212.17
Aminopteridine	440.47
GW273629	
GW274150	

Data adapted from Refs 5, 122, and 123

[128] analogues of the NOS substrate L-arginine, are potent inhibitors of iNOS, with both compounds shows more selectivity over the constitutive isoforms. Substrate based inhibitor 1400W is about 10,000-fold selective for iNOS over eNOS and 30-fold selective for over nNOS [129]. GW 273629 and GW274150 are other compounds that belong to the same group, as substrate-based inhibitors [121]. Both are hetero-substituted analogues and homologues of L-NIL, and have similar potency to L-NIL with significantly increased isoform selectivity [130]. AR-C102222 is an alternative chemical compound that has been developed recently as a highly selective inhibitor for iNOS [121].

Blocking of enzyme dimerization lead to development of another group of NOS inhibitors. A series of potent and selective iNOS inhibitors has been shown to prevent iNOS dimerization in cells and inhibit iNOS *in vivo*. These inhibitors are now shown to block dimerization of purified human iNOS monomers. Novel pyrimidineimidazole-based iNOS inhibitors are shown to inhibit dimerization of purified iNOS monomers [131]. This particular study has revealed the mechanism whereby inhibitors bind to a heme-containing iNOS monomer species to form an inactive iNOS monomer-heme-inhibitor complex in a pterin- and L-arginine-independent manner.

Cofactor blockers such as aminopteridine, which affects calmodulin binding, have been engineered as another group of NOS inhibitors. Matter *et al.* have published a series of inhibitors, which targets the BH_4-binding site [132]. Their results enhance our understanding of electrostatic, hydrophobic, and steric requirements for ligand binding and provide a guide for the design of novel pteridine-based NOS inhibitors to those regions that show tenfold selectivity for nNOS over the other two isoforms. NOS inhibitors as therapeutic targets for cancer with particular emphasis on iNOS, will be discussed in the next section.

INOS AS A THERAPEUTIC TARGET FOR CANCER

Although NO literature shows quite contradictory studies showing beneficial or harmful effects of endogenous NO, it seems clear that blocking NO has the potential to produce therapeutic benefit for cancer. The difficulties, we face today, are isoforms specificity for different species, targeting the specific tissue or even specific cells, and degree and the duration of the inhibition. In the case of cancer, most of the interest has been focused on iNOS inhibitors, since a growing number of studies show that iNOS expression and NO production are beneficial for cancer cell survival. New targets in drug design in cancer include cytostatic agents and angiogenesis inhibitors. Down-regulation of inflammatory prostaglandin synthesis by inhibition of COX-2 and inhibition of the iNOS, are currently examined in experimental models and clinical trials. COX-2 and iNOS are important enzymes that mediate inflammatory processes. Mounting evidence in recent years has shown that up-regulation of COX-2 and/or iNOS is associated with the pathophysiology of certain types of human cancers. Since inflammation is closely linked to tumor promotion, substances with potent anti-inflammatory activities are anticipated to exert chemopreventive effects on carcinogenesis, particularly in the promotion stage [133]. Recent studies have demonstrated that NF-κB is involved in regulation of COX-2 and iNOS expression. Several chemopreventive chemicals have been shown to inhibit COX-2 and iNOS expression by blocking improper NF-κB activation. Multiple lines of compelling evidence indicate that ERK1/2 and p38 MAP kinase are key elements of the intracellular signaling cascades responsible for NF-κB activation in response to the external stimuli. It has been also shown that endogenous prostaglandin E2 (PGE2) resulting from COX-2 expression in a highly metastatic murine breast cancer cell line upregulates IFNγ + LPS-induced iNOS expression and NO production [134]. This action of PGE2 is mediated through the EP4 receptor in a

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cAMP-dependent manner, and both nonselective and selective COX-2 inhibitors suppressed IFNγ + LPS-induced-induced NO production.

Another potential therapeutic approach on overcoming of iNOS regulated anti-apoptotic characteristics of cancer cells might be the inhibition of cell cycle regulators. Flavopiridol, an inhibitor of cyclin-dependent kinases, induces *in vitro* apoptosis of malignant cells from B-cell chronic lymphocytic leukemia (B-CLL). It has been reported that NO, produced by iNOS, spontaneously expressed by the B-CLL cells, contributed to their deficiency in apoptosis [33]. In the following work by the same group, Billard *et al.* [135], show that *ex vivo* treatment of leukemic cells from B-CLL patients with flavopiridol results in the inhibition of iNOS expression, and NO production. They have shown that these effects were accompanied by membrane, mitochondrial and nuclear events of apoptosis. Flavopiridol exposure also results in the stimulation of caspase 3 activity and in caspase-dependent cleavage of $p27^{kip1}$, a negative regulator of the cell cycle, which is overexpressed in B-CLL. Thus, flavopiridol is capable of downregulating both iNOS and $p27^{kip1}$ expression in B-CLL cells. Furthermore, flavopiridol-promoted apoptosis could partly be reverted by an NO donor, suggesting that inhibition of the NO pathway could participate in the apoptotic effects of flavopiridol on the leukemic cells. A recent study by the same group has shown that downregulation of iNOS and NO is a pathway common for flavones and polyphenols, two distinct families of phytoalexins [136]. Their data suggest that inhibition of the NO pathway could be one of the mechanisms involved in the proapoptotic properties of these phytoalexins in leukemia B-cells.

CONCLUSION

The discovery of the generation of NO by mammalian cells and the revelation of some of its biological roles in cancer has brought a new era to cancer biology research. The leading studies have allowed us to understand potential effects and responses of tissue and tumors to NO-targeted treatments. Now, the field of NO therapeutics is starting a critical and exciting period in cancer research. Selective inhibitors of iNOS have been identified and developed for use in clinical trials. Both universities and companies have devoted -and are devoting- exclusive time for intense research and financial support to treat a diverse range of diseases, including asthma, arthritis, inflammatory diseases, and potentially cancer. From initial clinical applications, we will first learn how much inhibition we should target and how much specificity towards an isoform is achievable. Consequently, we need to learn about the nature and duration of the effects. Eventually, in targeted diseases for which iNOS inhibition might be beneficial, there will be the need to measure clinical outcomes and responses that are specific to this inhibition. We will be able to explore more specific inhibitors for diseases and combination treatment approaches in our future therapeutic modalities.

ABBREVIATIONS

5,6-MeXAA = 5,6-Dimethylxanthenone-4-acetic acid
ADMA = Asymmetric dimethylarginine
ATM = Ataxia telangectasia mutant kinase
ATR = AT-mutated and Rad3-related kinase
B-CLL = B-cell chronic lymphocytic leukemia
bFGF = Basic fibroblast growth factor
cGMP = Cyclic guanosine-3',5'-monophosphate
eNOS = Endothelial NOS
GTP = Guanosine-5'-triphosphate
iNOS = Inducible NOS
JAKs = Janus kinases
L-NAME = N-nitro-L-arginine methyl ester
L-NMMA = N-monomethyl-L-arginine
L-NIO = L-*N*-iminoethylornithine
L-NIL = L-lysine
L-NIO = N-iminoethyl-L-ornithine
nNOS = Neuronal NOS
NO = Nitric Oxide
NOS = Nitric oxide synthase
NT = Nitrotyrosine
PGE2 = Prostaglandin E2
PMN = Peripheral mononuclea
sGC = Soluble guanylate cyclase
TGF-β = Transforming growth factor–β
VEGF = Vascular endothelial growth factor

REFERENCES

[1] Knowles, R. G.; Moncada, S. Nitric oxide synthases in mammals. *Biochem. J.* 1994, *298*(Pt 2), 249-258.
[2] Marletta, M. A. Nitric oxide synthase: aspects concerning structure and catalysis. *Cell* 1994, *78*, 927-930.
[3] Nathan, C.; Xie, Q. W. Nitric oxide synthases: roles, tolls, and controls. *Cell* 1994, *78*, 915-918.
[4] Hall, A. V.; Antoniou, H.; Wang, Y.; Cheung, A. H.; Arbus, A. M.; Olson, S. L.; Lu, W. C.; Kau, C. L.; Marsden, P. A. Structural organization of the human neuronal nitric oxide synthase gene (NOS1). *J. Biol. Chem.* 1994, *269*, 33082-33090.
[5] Alderton, W. K.; Cooper, C. E.; Knowles, R. G. Nitric oxide synthases: structure, function and inhibition. *Biochem. J.* 2001, *357*, 593-615.
[6] Charles, I. G.; Palmer, R. M. J.; Hickery, M. S.; Bayliss, M. T.; Chubb, A. P.; Hall, V. S.; Moss, D. W.; Moncada, S. Cloning, Characterization,and and expressions of a cDNA endocing an inducible nitric oxide synthase from the human chondrocyte. *Proc. Natl. Acad. Sci. USA* 1993, *90*, 11419-11423.
[7] Janssens, S. P.; Shimouchi, A.; Quertermous, T.; Bloch, D. B.; Bloch, K. D. Cloning and expression of a cDNA encoding human endothelium-derived relaxing factor/nitric oxide synthase. *J. Biol. Chem.* 1992, *267*, 14519-14522. [Published Erratum Appears in *J. Biol. Chem.* 1992, *267*, 22694].
[8] Marsden, P. A.; Schappert, K. T.; Chen, H. S.; Flowers, M.; Sundell, C. L.; Wilcox, J. N.; Lamas, S.; Michel, T. Molecular cloning and characterization of human endothelial nitric oxide synthase. *FEBS Lett.* 1992, *307*, 287-293.
[9] Thomsen, L. L.; Lawton, F. G.; Knowles, R. G.; Beesley, J. E.; Riveros-Moreno, V.; Moncada, S. Nitric oxide synthase activity in human gynecological cancer. *Cancer Res.* 1994, *54*, 1352-1354.

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[10] Thomsen, L. L.; Miles, D. W.; Happerfield, L.; Bobrow, L. G.; Knowles, R. G.; Moncada, S. Nitric oxide synthase activity in human breast cancer. *Br. J. Cancer* 1995, *72*, 41-44.

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

[12] Tschugguel, W.; Pustelnik, T.; Lass, H.; Mildner, M.; Weninger, W.; Schneeberger, C.; Jansen, B.; Tschachler, E.; Waldhor, T.; Huber, J. C.; Pehamberger, H. Inducible nitric oxide synthase (inos) expression may predict distant metastasis in human melanoma [Published Erratum Appears in *Br. J. Cancer* 1999, *80*(8), 1302]. *Br. J. Cancer* 1999, *79*, 1609-1612.

[13] Koh, E.; Noh, S. H.; Lee, Y. D.; Lee, H. Y.; Han, J. W.; Lee, H. W.; Hong, S. Differential expression of nitric oxide synthase in human stomach cancer. *Cancer Lett.* 1999, *146*, 173-180.

[14] Baltaci, S.; Orhan, D.; Gogus, C.; Turkolmez, K.; Tulunay, O.; Gogus, O. Inducible nitric oxide synthase expression in benign prostatic hyperplasia, low- and high-grade prostatic intraepithelial neoplasia and prostatic carcinoma. *BJU International* 2001, *88*, 100-103.

[15] Vakkala, M.; Kahlos, K.; Lakari, E.; Paakko, P.; Kinnula, V.; Soini, Y. Inducible nitric oxide synthase expression, apoptosis, and angiogenesis in *in situ* and invasive breast carcinomas. *Clin. Cancer Res.* 2000, *6*, 2408-2416.

[16] Ekmekcioglu, S.; Ellerhorst, J.; Smid, C. M.; Prieto, V. G.; Munsell, M.; Buzaid, A. C.; Grimm, E. A. Inducible nitric oxide synthase and nitrotyrosine in human metastatic melanoma tumors correlate with poor survival. *Clin. Cancer Res.* 2000, *6*, 4768-4775.

[17] Tschugguel, W.; Schneeberger, C.; Unfried, G.; Czerwenka, K.; Weninger, W.; Mildner, M.; Gruber, D. M.; Sator, M. O.; Waldhor, T.; Huber, J. C. Expression of inducible nitric oxide synthase in human breast cancer depends on tumor grade. *Breast Cancer Res. Treat.* 1999, *56*, 143-149.

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

[19] Ekmekcioglu, S.; Ellerhorst, J. A.; Mumm, J. B.; Zheng, M.; Broemeling, L.; Prieto, V. G.; Stewart, A. L.; Mhashilkar, A. M.; Chada, S.; Grimm, E. A. Negative association of melanoma differentiation-associated gene (Mda-7) and inducible nitric oxide synthase (INOS) in human melanoma: MDA-7 regulates INOS expression in melanoma cells. *Mol. Cancer Ther.* 2003, *2*, 9-17.

[20] Ekmekcioglu, S.; Ellerhorst, J.; Mhashilkar, A. M.; Sahin, A. A.; Read, C. M.; Prieto, V. G.; Chada, S.; Grimm, E. A. Down-regulated melanoma differentiation associated gene (Mda-7) expression in human melanomas. *Intl. J. Cancer* 2001, *94*, 54-59.

[21] Ellerhorst, J. A.; Prieto, V. G.; Ekmekcioglu, S.; Broemeling, L.; Yekell, S.; Chada, S.; Grimm, E. A. Loss of MDA-7 expression with progression of melanoma. *J. Clin. Oncol.* 2002, *20*, 1069-1074.

[22] Reveneau, S.; Arnould, L.; Jolimoy, G.; Hilpert, S.; Lejeune, P.; Saint-Giorgio, V.; Belichard, C.; Jeannin, J. F. Nitric oxide synthase in human breast cancer is associated with tumor grade, proliferation rate, and expression of progesterone receptors. *Lab. Invest.* 1999, *79*, 1215-1225.

[23] Loibl, S.; von Minckwitz, G.; Weber, S.; Sinn, H. P.; Schini-Kerth, V. B.; Lobysheva, I.; Nepveu, F.; Wolf, G.; Strebhardt, K.; Kaufmann, M. Expression of endothelial and inducible nitric oxide synthase in benign and malignant lesions of the breast and measurement of nitric oxide using electron paramagnetic resonance spectroscopy. *Cancer* 2002, *95*, 1191-1198.

[24] Jenkins, D. C.; Charles, I. G.; Thomsen, L. L.; Moss, D. W.; Holmes, L. S.; Baylis, S. A.; Rhodes, P.; Westmore, K.; Emson, P. C.; Moncada, S. Roles of Nitric oxide in tumor growth. *Proc. Natl. Acad. Sci. USA* 1995, *92*, 4392-4396.

[25] Haendeler, J.; Weiland, U.; Zeiher, A. M.; Dimmeler, S. effects of redox-related congeners of NO on apoptosis and caspase-3 activity. *Nitric Oxide* 1997, *1*, 282-293.

[26] Ahmed, B.; Van Den Oord, J. J. Expression of the inducible isoform of nitric oxide synthase in pigment cell lesions of the skin. *Br. J. Dermatol.* 2000, *142*, 432-440.

[27] Salvucci, O.; Carsana, M.; Bersani, I.; Tragni, G.; Anichini, A. Antiapoptotic role of endogenous nitric oxide in human melanoma cells. *Cancer Res.* 2001, *61*, 318-326.

[28] Oshima, H.; Bartsch, H. chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat. Res.* 1994, *305*, 253-264.

[29] Singer, I. I.; Kawka, D. W.; Scott, S.; Weidner, J. R.; Mumford, R. A.; Riehl, T. E.; Stenson, W. F. Expression of inducible nitric oxide synthase and nitrotyrosine in colonic epithelium in inflammatory bowel disease. *Gastroenterology* 1996, *111*, 871-885.

[30] Mannick, E. E.; Bravo, L. E.; Zarama, G.; Realpe, J. L.; Zhang, X. J.; Ruiz, B.; Fontham, E. T.; Mera, R.; Miller, M. J.; Correa, P. Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in *Helicobacter pylori* gastritis: effect of antibiotics and antioxidants. *Cancer Res.* 1996, *56*, 3238-3243.

[31] Ziche, M.; Morbidelli, L.; Choudhuri, R.; Zhang, H. T.; Donnini, S.; Granger, H. J.; Bicknell, R. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. *J. Clin. Invest.* 1997, *99*, 2625-2634.

[32] Zhao, H.; Dugas, N.; Mathiot, C.; Delmer, A.; Dugas, B.; Sigaux, F.; Kolb, J. P. B-Cell chronic lymphocytic leukemia cells express a functional inducible nitric oxide synthase displaying anti-apoptotic activity. *Blood* 1998, *92*, 1031-1043.

[33] Gow, A. J.; Farkouh, C. R.; Munson, D. A.; Posencheg, M. A.; Ischiropoulos, H. Biological significance of nitric oxide-mediated protein modifications. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2004, *287*, L262-L268.

[34] Pryor, W. A.; Squadrito, G. L. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am. J. Physiol.* 1995, *268*, L699-L722.

[35] Schmidt, H. H. H. W. NO-, CO and -OH endogenous soluble guanylyl cyclase-activating factors. *FEBS Lett.* 1992, *307*, 102-107.

[36] Go, Y. M.; Patel, R. P.; Maland, M. C.; Park, H.; Beckman, J. S.; Darley-Usmar, V. M.; Jo, H. Evidence for peroxynitrite as a signaling molecule in flow-dependent activation of C-Jun NH(2)-terminal kinase. *Am. J. Physiol.* 1999, *277*, H1647-H1653.

[37] Lander, H. M.; Sehajpal, P.; Levine, D. M.; Novogrodsky, A. Activation of human peripheral blood mononuclear cells by nitric oxide-generating compounds. *J. Immunol.* 1993, *150*, 1509-1516.

[38] Lander, H. M.; Jacovina, A. T.; Davis, R. J.; Tauras, J. M. Differential activation of mitogen-activated protein kinases by nitric oxide-related species. *J. Biol. Chem.* 1996, *271*, 19705-19709.

[39] Lander, H. M.; Ogiste, J. S.; Pearce, S. F.; Levi, R.; Novogrodsky, A. Nitric oxide-stimulated guanine nucleotide exchange on p21ras. *J. Biol. Chem.* 1995, *270*, 7017-7020.

[40] MacMillan-Crow, L. A.; Greendorfer, J. S.; Vickers, S. M.; Thompson, J. A. Tyrosine nitration of c-src tyrosine kinase in human pancreatic ductal adenocarcinoma. *Arch. Biochem. Biophys.* 2000, *377*, 350-356.

[41] Akhand, A. A.; Pu, M.; Senga, T.; Kato, M.; Suzuki, H.; Miyata, T.; Hamaguchi, M.; Nakashima, I. Nitric oxide controls src kinase activity through a sulfhydryl group modification-mediated Tyr-527-independent and Tyr-416-linked mechanism. *J. Biol. Chem.* 1999, *274*, 25821-25826.

[42] Nikitovic, D.; Holmgren, A.; Spyrou, G. Inhibition of AP-1 DNA binding by nitric oxide involving conserved cysteine residues in Jun and Fos. *Biochem. Biophys. Res. Commun.* 1998, *242*, 109-112.

[43] Beck, K. F.; Eberhardt, W.; Frank, S.; Huwiler, A.; Messmer, U. K.; Muhl, H.; Pfeilschifter, J. Inducible NO synthase: role in cellular signalling. *J. Exp. Biol.* 1999, *202*, 645-653.

[44] Duhe, R. J.; Evans, G. A.; Erwin, R. A.; Kirken, R. A.; Cox, G. W.; Farrar, W. L. Nitric oxide and thiol redox regulation of Janus kinase activity. *Proc. Natl. Acad. Sci. USA* 1998, *95*, 126-131.

[45] Korkmaz, Y.; Baumann, M. A.; Schroder, H.; Behrends, S.; Addicks, K.; Raab, W. H.; Bloch, W. Localization of the NO-cGMP signaling pathway molecules, NOS III-phosphorylation sites, ERK1/2, and Akt/PKB in osteoclasts. *J. Periodontol.* 2004, *75*, 1119-1125.

[46] Huang, H. N.; Lu, P. J.; Lo, W. C.; Lin, C. H.; Hsiao, M.; Tseng, C. J. *In situ* Akt phosphorylation in the nucleus tractus solitarii is involved in central control of blood pressure and heart rate. *Circulation* 2004, *110*, 2476-2483.

[47] Wang, Y.; Ahmad, N.; Kudo, M.; Ashraf, M. Contribution of Akt and endothelial nitric oxide synthase to diazoxide-induced late

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preconditioning. *Am. J. Physiol. Heart Circ. Physiol.* 2004, *287*, H1125-H1131.

[48] Mondoro, T. H.; Shafer, B. C.; Vostal, J. G. Peroxynitrite-induced tyrosine nitration and phosphorylation in human platelets. *Free Radic. Biol. Med.* 1997, *22*, 1055-1063.

[49] Souza, J. M.; Daikhin, E.; Yudkoff, M.; Raman, C. S.; Ischiropoulos, H. Factors determining the selectivity of protein tyrosine nitration. *Arch. Biochem. Biophys.* 1999, *371*, 169-178.

[50] Davies, H.; Bignell, G. R.; Cox, C.; Stephens, P.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M. J.; Bottomley, W.; Davis, N.; Dicks, E.; Ewing, R.; Floyd, Y.; Gray, K.; Hall, S.; Hawes, R.; Hughes, J.; Kosmidou, V.; Menzies, A.; Mould, C.; Parker, A.; Stevens, C.; Watt, S.; Hooper, S.; Wilson, R.; Jayatilake, H.; Gusterson, B. A.; Cooper, C.; Shipley, J.; Hargrave, D.; Pritchard-Jones, K.; Maitland, N.; Chenevix-Trench, G.; Riggins, G. J.; Bigner, D. D.; Palmieri, G.; Cossu, A.; Flanagan, A.; Nicholson, A.; Ho, J. W.; Leung, S. Y.; Yuen, S. T.; Weber, B. L.; Seigler, H. F.; Darrow, T. L.; Paterson, H.; Marais, R.; Marshall, C. J.; Wooster, R.; Stratton, M. R.; Futreal, P. A. Mutations of the BRAF gene in human cancer. *Nature* 2002, *417*, 949-954.

[51] Brose, M. S.; Volpe, P.; Feldman, M.; Kumar, M.; Rishi, I.; Gerrero, R.; Einhorn, E.; Herlyn, M.; Minna, J.; Nicholson, A.; Roth, J. A.; Albelda, S. M.; Davies, H.; Cox, C.; Brignell, G.; Stephens, P.; Futreal, P. A.; Wooster, R.; Stratton, M. R.; Weber, B. L. BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res.* 2002, *62*, 6997-7000.

[52] Yu, D. The role of oncogenes in drug resistance. *Cytotechnology* 1998, *27*, 283-292.

[53] Grossman, D.; Altieri, D. C. Drug resistance in melanoma: mechanisms, apoptosis, and new potential therapeutic targets. *Cancer Metast. Rev.* 2001, *20*, 3-11.

[54] Serrone, L.; Hersey, P. The Chemoresistance of Human Malignant Melanoma: an Update. *Melanoma Res.* 1999, *9*, 51-58.

[55] Soengas, M. S.; Lowe, S. W. Apoptosis and melanoma chemoresistance. *Oncogene* 2003, *22*, 3138-3151.

[56] Soengas, M. S.; Capodieci, P.; Polsky, D.; Mora, J.; Esteller, M.; Opitz-Araya, X.; McCombie, R.; Herman, J. G.; Gerald, W. L.; Lazebnik, Y. A.; Cordon-Cardo, C.; Lowe, S. W. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. *Nature* 2001, *409*, 207-211.

[57] Melino, G.; Bernassola, F.; Knight, R. A.; Corasaniti, M. T.; Nistico, G.; Finazzi-Agro, A. S-Nitrosylation regulates apoptosis. *Nature* 1997, *388*, 432-433.

[58] Torok, N. J.; Higuchi, H.; Bronk, S.; Gores, G. J. Nitric oxide inhibits apoptosis downstream of cytochrome c release by nitrosylating caspase 9. *Cancer Res.* 2002, *62*, 1648-1653.

[59] Rossig, L.; Fichtlscherer, B.; Breitschopf, K.; Haendeler, J.; Zeiher, A. M.; Mulsch, A.; Dimmeler, S. Nitric oxide inhibits caspase-3 by s-nitrosation *in vivo*. *J. Biol. Chem.* 1999, *274*, 6823-6826.

[60] Li, J.; Billiar, T. R.; Talanian, R. V.; Kim, Y. M. Nitric oxide reversibly inhibits seven members of the caspase family via s-nitrosylation. *Biochem. Biophys. Res. Commun.* 1997, *240*, 419-424.

[61] Mannick, J. B.; Miao, X. Q.; Stamler, J. S. Nitric oxide inhibits Fas-induced apoptosis. *J. Biol. Chem.* 1997, *272*, 24125-24128.

[62] Mannick, J. B.; Hausladen, A.; Liu, L.; Hess, D. T.; Zeng, M.; Miao, Q. X.; Kane, L. S.; Gow, A. J.; Stamler, J. S. Fas-induced caspase denitrosylation. *Science* 1999, *284*, 651-654.

[63] Jaiswal, M.; LaRusso, N. F.; Gores, G. J. Nitric oxide in gastrointestinal epithelial cell carcinogenesis: linking inflammation to oncogenesis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2001, *281*, G626-G634.

[64] Kim, Y. M.; de Vera, M. E.; Watkins, S. C.; Billiar, T. R. Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor-alpha-induced apoptosis by inducing heat shock protein 70 expression. *J. Biol. Chem.* 1997, *272*, 1402-1411.

[65] Suschek, C. V.; Krischel, V.; Bruch-Gerharz, D.; Berendji, D.; Krutmann, J.; Kroncke, K. D.; Kolb-Bachofen, V. Nitric oxide fully protects against uva-induced apoptosis in tight correlation with Bcl-2 up-regulation. *J. Biol. Chem.* 1999, *274*, 6130-6137.

[66] Li, G.; Tang, L.; Zhou, X.; Tron, V.; Ho, V. Chemotherapy-induced apoptosis in melanoma cells is p53 dependent. *Melanoma Res.* 1998, *8*, 17-23.

[67] Davol, P. A.; Goulette, F. A.; Frackelton, A. R. Jr.; Darnowski, J. W. Modulation of p53 expression by human recombinant interferon-alpha2a correlates with abrogation of cisplatin resistance in a human melanoma cell line. *Cancer Res.* 1996, *56*, 2522-2526.

[68] Bunz, F.; Hwang, P. M.; Torrance, C.; Waldman, T.; Zhang, Y.; Dillehay, L.; Williams, J.; Lengauer, C.; Kinzler, K. W.; Vogelstein, B. Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *J. Clin. Invest.* 1999, *104*, 263-269.

[69] Davis, D. W.; Weidner, D. A.; Holian, A.; McConkey, D. J. Nitric oxide-dependent activation of p53 suppresses bleomycin-induced apoptosis in the lung. *J. Exp. Med.* 2000, *192*, 857-869.

[70] Tang, C. H.; Grimm, E. A. Depletion of endogenous nitric oxide enhances cisplatin-induced apoptosis in a p53-dependent manner in melanoma cell lines. *J. Biol. Chem.* 2004, *279*, 288-298.

[71] Schonhoff, C. M.; Daou, M. C.; Jones, S. N.; Schiffer, C. A.; Ross, A. H. Nitric oxide-mediated inhibition of hdm2-p53 binding. *Biochemistry* 2002, *41*, 13570-13574.

[72] Ashcroft, M.; Vousden, K. H. Regulation of p53 stability. *Oncogene* 1999, *18*, 7637-7643.

[73] Zhang, Y.; Xiong, Y. Control of p53 ubiquitination and nuclear export by MDM2 and ARF. *Cell Growth Differ.* 2001, *12*, 175-186.

[74] Poremba, C.; Yandell, D. W.; Metze, D.; Kamanabrou, D.; Bocker, W.; Dockhorn-Dworniczak, B. Immunohistochemical detection of p53 in melanomas with rare p53 gene mutations is associated with Mdm-2 overexpression. *Oncol. Res.* 1995, *7*, 331-339.

[75] Li, J.; Billiar, T. R. The anti-apoptotic actions of nitric oxide in hepatocytes. *Cell Death Differ.* 1999, *6*, 952-955.

[76] Szabo, C. Multiple pathways of peroxynitrite cytotoxicity. *Toxicol. Lett.* 2003, *140-141*, 105-112.

[77] Janssen, Y. M.; Soultanakis, R.; Steece, K.; Heerdt, E.; Singh, R. J.; Joseph, J.; Kalyanaraman, B. Depletion of nitric oxide causes cell cycle alterations, apoptosis, and oxidative stress in pulmonary cells. *Am. J. Physiol.* 1998, *275*, L1100-L1109.

[78] Wenzel, U.; Kuntz, S.; De Sousa, U. J.; Daniel, H. Nitric oxide suppresses apoptosis in human colon cancer cells by scavenging mitochondrial superoxide anions. *Int. J. Cancer* 2003, *106*, 666-675.

[79] Espey, M. G.; Thomas, D. D.; Miranda, K. M.; Wink, D. A. Focusing of nitric oxide mediated nitrosation and oxidative nitrosylation as a consequence of reaction with superoxide. *Proc. Natl. Acad. Sci. USA* 2002, *99*, 11127-11132.

[80] Sander, C. S.; Hamm, F.; Elsner, P.; Thiele, J. J. Oxidative stress in malignant melanoma and non-melanoma skin cancer. *Br. J. Dermatol.* 2003, *148*, 913-922.

[81] Bittinger, F.; Gonzalez-Garcia, J. L.; Klein, C. L.; Brochhausen, C.; Offner, F.; Kirkpatrick, C. J. Production of superoxide by human malignant melanoma cells. *Melanoma Res.* 1998, *8*, 381-387.

[82] Meyskens, F. L. Jr.; Chau, H. V.; Tohidian, N.; Buckmeier, J. Luminol-enhanced chemiluminescent response of human melanocytes and melanoma cells to hydrogen peroxide stress. *Pigment Cell Res.* 1997, *10*, 184-189.

[83] Marshall, H. E.; Merchant, K.; Stamler, J. S. Nitrosation and oxidation in the regulation of gene expression. *FASEB J.* 2000, *14*, 1889-1900.

[84] Stamler, J. S.; Toone, E. J.; Lipton, S. A.; Sucher, N. J. (S)NO Signals: translocation, regulation, and a consensus motif. *Neuron* 1997, *18*, 691-696.

[85] Alderton, W. K.; Cooper, C. E.; Knowles, R. G. Nitric oxide synthases: structure, function and inhibition. *Biochem. J.* 2001, *357*, 593-615.

[86] Lala, P. K.; Chakraborty, C. Role of nitric oxide in carcinogenesis and tumour progression. *Lancet Oncol.* 2001, *2*, 149-156.

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

[88] Hogg, N.; Kalyanaraman, B. Nitric oxide and lipid peroxidation. *Biochim. Biophys. Acta* 1999, *1411*, 378-384.

[89] Xu, W.; Liu, L.; Smith, G. C.; Charles, G. Nitric oxide upregulates expression of DNA-PKcs to protect cells from DNA-damaging anti-tumour agents. *Nat. Cell Biol.* 2000, *2*, 339-345.

[90] Jaiswal, M.; LaRusso, N. F.; Burgart, L. J.; Gores, G. J. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res.* 2000, *60*, 184-190.

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[91] Josko, J.; Mazurek, M. Transcription factors having impact on vascular endothelial growth factor (VEGF) gene expression in angiogenesis. *Med. Sci. Monit.* 2004, *10*, RA89-RA98.

[92] Fam, N. P.; Verma, S.; Kutryk, M.; Stewart, D. J. Clinician guide to angiogenesis. *Circulation* 2003, *108*, 2613-2618.

[93] Yancopoulos, G. D.; Davis, S.; Gale, N. W.; Rudge, J. S.; Wiegand, S. J.; Holash, J. Vascular-specific growth factors and blood vessel formation. *Nature* 2000, *407*, 242-248.

[94] Kashani-Sabet, M.; Sagebiel, R. W.; Ferreira, C. M.; Nosrati, M.; Miller, J. R. III. Tumor vascularity in the prognostic assessment of primary cutaneous melanoma. *J. Clin. Oncol.* 2002, *20*, 1826-1831.

[95] Ugurel, S.; Rappl, G.; Tilgen, W.; Reinhold, U. Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. *J. Clin. Oncol.* 2001, *19*, 577-583.

[96] Hood, J. D.; Meininger, C. J.; Ziche, M.; Granger, H. J. VEGF upregulates ecNOS message, protein, and NO production in human endothelial cells. *Am. J. Physiol.* 1998, *274*, H1054-H1058.

[97] Cooke, J. P. NO and angiogenesis. *Atheroscler. Suppl.* 2003, *4*, 53-60.

[98] Urbich, C.; Dernbach, E.; Zeiher, A. M.; Dimmeler, S. Double-edged role of statins in angiogenesis signaling. *Circulation Res.* 2002, *90*, 737-744.

[99] Brouet, A.; Sonveaux, P.; Dessy, C.; Moniotte, S.; Balligand, J. L.; Feron, O. Hsp90 and caveolin are key targets for the proangiogenic nitric oxide-mediated effects of statins. *Circulation Res.* 2001, *89*, 866-873.

[100] Kureishi, Y.; Luo, Z.; Shiojima, I.; Bialik, A.; Fulton, D.; Lefer, D. J.; Sessa, W. C.; Walsh, K. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nat. Med.* 2000, *6*, 1004-1010.

[101] Wolfrum, S.; Jensen, K. S.; Liao, J. K. Endothelium-dependent effects of statins. *Arterioscler. Thromb. Vasc. Biol.* 2003, *23*, 729-736.

[102] Ambs, S.; Merriam, W. G.; Ogunfusika, M. O.; Bennett, W. P.; Ishibe, N.; Hussain, S. P.; Tzeng, E. E.; Geller, D. A.; Billiar, T. R.; Harris, C. C. p53 and vascular endothelial growth factor regulate tumor growth of NOS2-expressing human carcinoma cells. *Nat. Med.* 1998, *4*, 1371-1376.

[103] Orucevic, A.; Bechberger, J.; Green, A. M.; Shapiro, R. A.; Billiar, T. R.; Lala, P. K. Nitric-oxide production by murine mammary adenocarcinoma cells promotes tumor-cell invasiveness. *Int. J. Cancer* 1999, *81*, 889-896.

[104] Wang, B.; Shi, Q.; Abbruzzese, J. L.; Xiong, Q.; Le, X.; Xie, K. A novel, clinically relevant animal model of metastatic pancreatic adenocarcinoma biology and therapy. *Int. J. Pancreatol.* 2001, *29*, 37-46.

[105] Hibbs, J. B. J.; Westenfelder, C.; Taintor, R.; Vavrin, Z.; Kablitz, C.; Baranowski, R. L.; Ward, J. H.; Menlove, R. L.; McMurry, M. P.; Kushner, J. P. Evidence for cytokine-inducible nitric oxide synthesis from l-arginine in patients receiving interleukin-2 therapy. *J. Clin. Invest.* 1992, *89*, 867-877. [Published Erratum Appears in *J. Clin. Invest.* 1992, 90, 295].

[106] Citterio, G.; Pellegatta, F.; Lucca, G. D.; Fragasso, G.; Scaglietti, U.; Pini, D.; Fortis, C.; Tresoldi, M.; Rugarli, C. Plasma nitrate plus nitrite changes during continuous intravenous infusion interleukin 2. *Br. J. Cancer* 1996, *74*, 1297-1301.

[107] Ogilvie, A. C.; Hack, C. E.; Wagstaff, J.; van Mierlo, G. J.; Erenberg, A. J.; Thomsen, L. L.; Hoekman, K.; Rankin, E. M. IL-1 beta does not cause neutrophil degranulation but does lead to IL-6, IL-8, and nitrite/nitrate release when used in patients with cancer. *J. Immunol.* 1996, *156*, 389-394.

[108] Moilanen, E.; Thomsen, L. L.; Miles, D. W.; Happerfield, D. W.; Knowles, R. G.; Moncada, S. Persistent induction of nitric oxide synthase in tumours from mice treated with the anti-tumour agent 5,6-dimethylxanthenone-4-acetic acid. *Br. J. Cancer* 1998, *77*, 426-433.

[109] Baylis, C.; Vallance, P. Measurement of nitrite and nitrate levels in plasma and urine--what does this measure tell us about the activity of the endogenous nitric oxide system? *Curr. Opin. Nephrol. Hypertension* 1998, *7*, 59-62.

[110] Taylor, B. S.; de Vera, M. E.; Ganster, R. W.; Wang, Q.; Shapiro, R. A.; Morris, S. M. J.; Billiar, T. R.; Geller, D. A. Multiple NF-KappaB enhancer elements regulate cytokine induction of the human inducible nitric oxide synthase gene. *J. Biol. Chem.* 1998, *273*, 15148-15156.

[111] Geller, D. A.; Billiar, T. R. Molecular biology of nitric oxide synthases. *Cancer Metast. Rev.* 1998, *17*, 7-23.

[112] Mellott, J. K.; Nick, H. S.; Waters, M. F.; Billiar, T. R.; Geller, D. A.; Chesrown, S. E. Cytokine-induced changes in chromatin structure and *in vivo* footprints in the inducible NOS promoter. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2001, *280*, L390-L399.

[113] Dhawan, P.; Richmond, A. A novel NF-Kappa B-inducing kinase-MAPK signaling pathway up-regulates NF-Kappa B activity in melanoma cells. *J. Biol. Chem.* 2002, *277*, 7920-7928.

[114] Richmond, A. NF-Kappa B, Chemokine Gene Transcription and Tumour Growth. *Nat. Rev. Immunol.* 2002, *2*, 664-674.

[115] Zheng, M.; Ekmekcioglu, S.; Walch, E. T.; Tang, C. H.; Grimm, E. A. Inhibition of nuclear factor-kappa b and nitric oxide by curcumin induces G2/M cell cycle arrest and apoptosis in human melanoma cells. *Melanoma Res.* 2004, *14*, 165-171.

[116] Misko, T. P.; Trotter, J. L.; Cross, A. H. Mediation of inflammation by encephalitogenic cells: interferon [gamma] induction of nitric oxide synthase and cyclooxygenase 2. *J. Neuroimmunol.* 1995, *61*, 195-204.

[117] Tabatabaie, T.; Vasquez, A. M.; Moore, D. R.; Floyd, R. A.; Kotake, Y. Direct administration of interleukin-1 and interferon-gamma to rat pancreas leads to the *in vivo* production of nitric oxide and expression of inducible nitric oxide synthase and inducible cyclooxygenase. *Pancreas* 2001, *23*, 316-322.

[118] Yang, J.; Richmond, A. Constitutive IkappaB kinase activity correlates with nuclear factor-kappab activation in human melanoma cells. *Cancer Res.* 2001, *61*, 4901-4909.

[119] Feinstein, D. L.; Galea, E.; Aquino, D. A.; Li, G. C.; Xu, H.; Reis, D. J. Heat shock protein 70 suppresses astroglial-inducible nitric-oxide synthase expression by decreasing NFkappaB activation. *J. Biol. Chem.* 1996, *271*, 17724-17732.

[120] Demarco, V. G.; Scumpia, P. O.; Bosanquet, J. P.; Skimming, J. W. Alpha-lipoic acid inhibits endotoxin-stimulated expression of INOS and nitric oxide independent of the heat shock response in RAW 264.7 cells. *Free Radic. Res.* 2004, *38*, 675-682.

[121] Vallance, P.; Leiper, J. Blocking NO synthesis: how, where and why? *Nat. Rev. Drug Discov.* 2002, *1*, 939-950.

[122] Matter, H.; Kotsonis, P. Biology and chemistry of the inhibition of nitric oxide synthases by pteridine-derivatives as therapeutic agents. *Med. Res. Rev.* 2004, *24*, 662-684.

[123] Crowell, J. A.; Steele, V. E.; Sigman, C. C.; Fay, J. R. Is inducible nitric oxide synthase a target for chemoprevention? *Mol. Cancer Ther.* 2003, *2*, 815-823.

[124] Rees, D. D.; Palmer, R. M.; Hodson, H. F.; Moncada, S. A specific inhibitor of nitric oxide formation from l-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.* 1989, *96*, 418-424.

[125] Moore, P. K.; al Swayeh, O. A.; Chong, N. W.; Evans, R. A.; Gibson, A. L-NG-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation *in vitro*. *Br. J. Pharmacol.* 1990, *99*, 408-412.

[126] Li, H.; Raman, C. S.; Glaser, C. B.; Blasko, E.; Young, T. A.; Parkinson, J. F.; Whitlow, M.; Poulos, T. L. Crystal structures of zinc-free and -bound heme domain of human inducible nitric-oxide synthase. implications for dimer stability and comparison with endothelial nitric-oxide synthase. *J. Biol. Chem.* 1999, *274*, 21276-21284.

[127] Rees, D. D.; Palmer, R. M.; Schulz, R.; Hodson, H. F.; Moncada, S. Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.* 1990, *101*, 746-752.

[128] Moore, W. M.; Webber, R. K.; Jerome, G. M.; Tjoeng, F. S.; Misko, T. P.; Currie, M. G. L-N6-(1-iminoethyl)lysine: a selective inhibitor of inducible nitric oxide synthase. *J. Med. Chem.* 1994, *37*, 3886-3888.

[129] Garvey, E. P.; Oplinger, J. A.; Furfine, E. S.; Kiff, R. J.; Laszlo, F.; Whittle, B. J.; Knowles, R. G. 1400W is a slow, tight binding, and highly selective inhibitor of inducible nitric-oxide synthase *in vitro* and *in vivo*. *J. Biol. Chem.* 1997, *272*, 4959-4963.

[130] Young, R. J.; Beams, R. M.; Carter, K.; Clark, H. A. R.; Coe, D. M.; Chambers, C. L.; Davies, P. I.; Dawson, J.; Drysdale, M. J.; Franzman, K. W. Inhibition of inducible nitric oxide synthase by acetamidine derivatives of hetero-substituted lysine and homolysine. *Bioorg. Med. Chem. Lett.* 2000, *10*, 597-600.

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[131] Blasko, E.; Glaser, C. B.; Devlin, J. J.; Xia, W.; Feldman, R. I.; Polokoff, M. A.; Phillips, G. B.; Whitlow, M.; Auld, D. S.; McMillan, K.; Ghosh, S.; Stuehr, D. J.; Parkinson, J. F. Mechanistic studies with potent and selective inducible nitric-oxide synthase dimerization inhibitors. *J. Biol. Chem.* 2002, *277,* 295-302.

[132] Matter, H.; Kotsonis, P.; Klingler, O.; Strobel, H.; Frohlich, L. G.; Frey, A.; Pfleiderer, W.; Schmidt, H. H. Structural requirements for inhibition of the neuronal nitric oxide synthase (NOS-I): 3D-QSAR analysis of 4-oxo- and 4-amino-pteridine-based inhibitors. *J. Med. Chem.* 2002, *45,* 2923-2941.

[133] Surh, Y. J.; Chun, K. S.; Cha, H. H.; Han, S. S.; Keum, Y. S.; Park, K. K.; Lee, S. S. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. *Mutat. Res.* 2001, *480-481,* 243-268.

[134] Timoshenko, A. V.; Lala, P. K.; Chakraborty, C. PGE2-mediated upregulation of INOS in murine breast cancer cells through the activation of EP4 receptors. *Int. J. Cancer* 2004, *108,* 384-389.

[135] Billard, C.; Kern, C.; Tang, R.; Ajchenbaum-Cymbalista, F.; Kolb, J. P. Flavopiridol downregulates the expression of both the inducible NO synthase and p27(Kip1) in malignant cells from B-cell chronic lymphocytic leukemia. *Leukemia* 2003, *17,* 2435-2443.

[136] Billard, C. H. R. I.; Quiney, C. L. A. I.; Tang, R. U. O. P.; Kern, C. A. T. H.; Ajchenbaum-Cymbalista, F. L. O. R.; Dauzonne, D. A. N. I.; Kolb, J. P. The inducible NO iynthase is downregulated during apoptosis of malignant cells from B-cell chronic lymphocytic leukemia induced by flavopiridol and polyphenols. *Ann. NY Acad. Sci.* 2003, *1010,* 381-383.

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