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S-Nitrosation Mediates Multiple Pathways That Lead to Tumor Progression in Estrogen Receptor–Negative Breast Cancer

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Abstract

Chronic inflammation within the tumor microenvironment is a major driver of tumor progression and poor prognosis. Inducible nitric oxide synthase (NOS2) is present in numerous solid tumors. Estrogen receptor–negative (ER−) patients with high expression of tumor NOS2 have a poorer outcome than patients with low expression of NOS2. Furthermore, expression of NOS2 is associated with the basal-like breast cancer phenotype. Using an *in vitro* model, we have found that nitrosation of critical thiols and nitration of tyrosines lead to the activation of membrane receptors such as epithelial growth factor receptor, Src, Ras, and CD63. These nitric oxide–mediated events in itiate oncogenic signaling pathways such as PI3K/Akt, Ras/ERK, β-catenin, nuclear factor-κB, and AP-1. These data suggest that NOS2 can serve as a major “nonmutatational driver” of ER− breast cancer.

Keywords

nitric oxide; cancer; nitrosative stress; S-nitrosylation

I. INTRODUCTION

Inducible nitric oxide (NO) synthase (NOS2) is associated with inflammation and plays a vital role in injury and the tissue restoration processes.[1] NOS2 is expressed in a variety of cells including macrophages and neutrophils and endothelial, glial, and epithelial cells.[2] NOS2 plays a major role in orchestrating an inflammatory response and participates in the eradication of parasites and tumors.[2] Unlike the constitutive NOS isoforms, NOS2 can generate high local levels of NO, ranging from 100 nM to 5 μM, for days.[1] This temporal and concentration range makes its biology unique compared with endothelial and neuronal NOS.

Expression of NOS2 is associated with both pro- and antitumor effects. For example, NOS2 plays a key role in promoting leukemic cell toxicity because of the prolonged high concentrations of NO produced.[3] Knockout models of NOS2, p53, and interleukin (IL)-10 show increased development of leukemia and thus are supportive of an antitumor role for

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NOS2. Furthermore, ectopic expression of NOS2 led to decreased tumorigenicity in a melanoma model.[4] Thus, for many years, it was thought that NOS2 only was involved in the eradication of tumors. However, tumor cells expressing elevated NOS2 have demonstrated increased aggressiveness and tumor metastasis.[5] More recently, NOS2 was reported to correlate with increased development of lung cancer.[6] A recent report suggests that the location of cells expressing NOS2 is an important determinant in cancer: the leukocytes contained NOS2 were associated with reduced tumor progression whereas NOS2 expressed in the tumor cell resulted in increased tumor progression.[7]

During the past several years, clinical investigations have shown that expression of NOS2 is elevated in patients with breast, non-small-cell lung, cervical, gastric, liver, esophageal, ovarian, glioma, melanoma, kidney, and colon tumors.[2] This indicates that an inflammatory environment may be involved in tumor progression. It is interesting that several studies have shown that NOS2 is an independent poor prognostic indic)ator, suggesting that NOS2 participates in tumor progression and metastasis.[8–11]

II. NOS2 IN BREAST TUMORS

Recent studies of breast cancer have shown that NOS2 is highly expressed in as many as 70% of all breast cancer patients.[8,12] NOS2 is an independent prognostic indicator of poor outcome in estrogen receptor-negative (ER−) but not estrogen receptor-positive (ER+) patients.[8] The hazard ratio (HR) for 10 years was 5.1, whereas at 5 years it was 6.1, indicating a rapid decline in outcome for patients with high expression of NOS2 in tumors. Despite the low presentation of lymph node metastasis, the patients who die seem to succumb to metastatic disease, indicating that expression of NOS2 may be an indicator of micrometastasis.

Within the ER− subtypes, expression of NOS2 corresponds to the expression of basal-like signature genes. Examination of gene expression profile from tumors with high NOS2 compared with low NOS2 shows that 44 genes are associated with high expression of NOS2.[8] Among these genes are *P-cadherin* and keratins associated with triple negative or basal-like breast cancer and the cancer stem cell marker *CD44.* There was also an increase in IL-8 and S100A8, indicating a proinflammatory microenvironment in tumors expressing high NOS2.

Is high expression of NOS2 a dosimeter or a causative agent? To answer this, NO donors able to deliver different fluxes of NO to a variety of different breast cancer cell types in an *in vitro* setting were used to recreate the possible NO tumor microenvironment.[13,14] Using cell culture models exposed to the diazeninium diolate and NO donor DETANO, which produce a similar environment of NO as NOS2 expression, increased the protein expression of P-cadherin, CD44, and keratins as well as IL-8 and S100A8 at 500 μM.[8] This amount of NO donor correlated with a flux of 300–500 nM NO at its peak. It is important that examination of these NO donor–induced protein changes showed a concomitant increase in protein expression, indicating that this level of NO recapitulates the protein expression profile in tumors expressing high NOS2.

III. *IN VITRO* NITROSATIVE SIGNALING IN BREAST CANCER CELLS

Comparing results from *in vitro* experiments with patient data can provide a powerful tool for investigating the chemical and molecular mechanisms of NO-mediated signaling. Genomic analyses of the promoter regions of the 44 genes associated with high expression of NOS2 revealed that expression of NOS2 correlates with an enrichment for genes regulated by β-catenin, nuclear factor (NF)-κB, AP-1, and Ets-1, suggesting that NOS2 is either upstream or downstream from these signaling pathways.[15–17]

Nuclear expression of β-catenin recently was found to be increased in basal-like patients and was related to poor outcomes.[18] Because β-catenin signaling also is related to NOS2 expression, we examined the effects of NO donors on β-catenin signaling. Similar to activation of the epithelial growth factor receptor (EGFR), approximately 200–300 nM NO resulted in increased nuclear translocation and transcriptional activity of β-catenin. Mechanistic studies revealed that NO activation of EGFR and Src initiate a molecular cascade via phosphatidylinositol 3-kinase (PI3K)/Akt to β-catenin.[15] This is consistent with a previous study linking expression of NOS2 with phosphorylation of breast cancer Akt. These observations indicate that NO-mediated activation of EGFR/Src and PI3K/Akt signaling may contribute to patient outcome.[12,19]

Using chemical inhibitors of reactive nitrogen species (RNS) revealed that a nitrosative species like N_2O_3 was responsible for NO-mediated activation of EGFR and Src.[15] Several reports have shown that critical thiols are targets of S-nitrosation.[20,21] S-nitrosylation (SNO) formation at Src cysteine 498 results in kinase activation.[22] S-nitrosation of EGFR has been reported to have inhibitory effects on EGFR kinase activity; however, the study used high concentrations of NO donors.[23] We observe a biphasic response to NO (EGFR activation peak ~ 300–500 nM NO), but activity decreases ≥1 μM, suggesting that at higher NO concentrations, nitrosative signaling levels become nitrosative stress levels.[15] These results point to a specific concentration of NO that is required to form RNS and for activation of these membrane proteins.

In addition to EGFR and Src, we observe S-nitrosation of the oncogenic signaling molecule Ras at similar NO concentrations. Lander et al[24] showed that Ras is a target of SNO and that Ras activity was increased upon modification of SNO. We show that 300- to 500-nM fluxes of NO also increase Ras activity and downstream activation of the mitogen-activated protein kinase kinase (MEK)/extracellular-signal-regulated kinase (ERK) pathway.[16] Ras activation initiates multiple signaling pathways involved in cancer progression. We recently showed that the oncogenic transcription factor Ets-1 is activated by the NO/Ras/MEK signaling axis. Furthermore, NO activation of Ets-1 corresponds to an enrichment of Ets-regulated genes in breast tumors expressing high NOS2 and that 100% of genes associated with NOS2 overexpression have Ets binding sites in their respective promoter regions. Therefore, NOS2 overexpression and NO signaling result in the nitrosative activation of 3 oncogenic signaling molecules, namely, EGFR, Src, and Ras; these can be referred to as “oncogenic nitrosyl receptors.”

Over the years, a map has begun to emerge showing that different NO concentrations activate specific and discrete pathways in cells.[13,14] The application of NO donors with cell culture models has revealed precise bands of NO concentrations whereby activation of specific molecular targets lead to signaling. This precise relationship to the concentration of NO indicates a new signaling paradigm in biological signaling. At the lowest levels, from <1 to 50 nM, is the level for the traditionally associated soluble guanylyl cyclasesignaling.[1] In MCF7, this can lead to activation of Raf-1 and ERK.[14] At higher levels (>100 nM), HIF-1 begins to be activated in a number of cells derived from tumors, macrophages, and endothelial cells.[25] Above 400 nM there is an increase in the phosphorylation of wild-type p53. Above 1 μM there is more ubiquitous nitrosative stress, which thereby increases decomposition of poly (ADP ribose) polymerase and other cysteines and zinc-finger proteins.[14,26,27] Although this level also is associated with cell death, nitrosation of Poly(ADP ribose) polymerase and caspases have been shown to be protective in some cases. This level of NO is associated with immune NO-mediated killing of pathogens and some types of cancer.

IV. NITRIC OXIDE AND MMP/TIMP-1

Matrix reorganization is a dynamic process that occurs during cancer progression and metastasis as the extracellular matrix is continually degraded and re-deposited to facilitate neo-vascularization, immune cell infiltration, and tumor growth and invasion. Matrix remodeling involves a variety of proteins including matrix metalloproteinases (MMPs) and their endogenous tissue inhibitor of matrix metalloproteinases (TIMP). In addition to matrix components, it is the process cytokines and growth factors of MMP that mediate cancer progression. Oxidation and nitrosation of the MMP cysteine switch results in the activation of MMP.[28,29] NO activates MMP-9 at concentrations ranging from 500 to 1500 nM NO.[30] CD44 is a marker of breast tumor metastasis and is upregulated by NO.[8,31] CD44 serves as a docking port for MMP-9 and MMP-9 colocalized with CD44 and has been identified at the leading edge of migrating cells.[31] TIMP-1 provides an additional biomarker in cancer. Macrophages treated with NO present reduced TIMP-1 protein and enhanced MMP-9 activity.[30,32] This is a critical component of a macrophage-mediated wound healing response.[30]

The tumor microenvironment encompasses an inflammatory environment, leading to the polarization of tumor-associated macrophages within the tumor stroma that favors increased angiogenesis and tumor growth. Moreover, the MMP/TIMP balance is important during tumor progression and metastasis, and we identified a positive correlation among NOS2, MMP-9, and TIMP-1 protein expression in patients with aggressive ER− breast tumors. Despite TIMP-1's highly efficient inhibition of MMP-9 activity, both MMP-9 and TIMP-1 predict poor disease-specific survival of cancer patients. Thus, we sought to elucidate a functional role for NO in this conundrum. Toward this end, we found that MMP-9 predicted poor survival in a breast cancer cohort. TIMP-1 also predicted poor survival among the same breast cancer cohort, which was restricted to tumors that expressed high NOS2 protein. In contrast, TIMP-1 did not predict poor breast cancer survival in patients whose tumors expressed low NOS2 protein.[33] In addition, an association between NOS2 expression and Akt phosphorylation status was reported in breast tumors, which was strongly enhanced by TIMP-1.[12,31] Further investigation of this pathway using NO donor-treated recombinant TIMP-1 protein and cell culture models led to the identification of the nitration of specific tyrosine residues within the TIMP-1 protein that correlated with its binding to the CD63 receptor and downstream activation of the PI3K/Akt/BAD pathway.[33] Using mass spectrometry, 2 nitrated tyrosine residues were identified near 2 key cysteine disulfide knots; these are critical for TIMP-1 binding and MMP-9 inactivation.[33] Moreover, a recent report associated TIMP-1 nitration with reduced binding and inhibition of MMP-9 in lipopolysaccharide-stimulated macrophages.[34] These observations offer a novel mechanism to decipher how TIMP-1 can predict poor cancer survival, which may involve, at least in part, NO-mediated posttranslational tyrosine nitration of TIMP-1 that redirects its function away from MMP-9 inhibition and toward TIMP-1/CD63 prosurvival signaling. In addition, the same NO flux directly activates MMP-9, which may facilitate tumor cell migration and invasion. Indeed, NOS2/MMP-9 colocalization has been identified at the leading edge of migrating cells.[35] Also, NOS2 deficient mice exhibit profoundly inhibited MMP-9 activity and leukocyte migration in an ischemia/reperfusion injury model.[36] Together, these results support a role for NOS2-derived NO in the regulation of the MMP/TIMP balance and function in inflammation and cancer.

The NO donor (DETA/NO) concentrations that mediated tyrosine nitration of recombinant TIMP-1 protein occurred in the same range of NO as that of other protumor growth pathways. TIMP-1 nitration was observed at fluxes as low as 200 nM of steady-state NO and increased at 500–1000 nM. Moreover, these conditions involved NO/O_2 intermediates. Nitrosation of tyrosine residues lead to nitrosotyrosine, which decomposes to 3-nitrotyrosine

with a half-life of 2–3 hours. This nitrosation mechanism as well as oxidative nitrosylation of tyrosine may be a major pathway to tyrosine nitration. Thus, levels of NO that activate MMP-9 via nitrosation of the thiol switch also blunt the inhibitory properties of TIMP-1. It seems that these conformational changes favor TIMP-1/CD63 interaction, downstream PI3K/Akt/BAD activation, and prosurvival signaling.[33] Because Src also is nitrosated under these conditions, these findings may represent a novel mechanism demonstrating an interplay of nitration and nitrosation mechanisms in the facilitation of prosurvival signaling pathways in cancer progression.

V. IMMUNOSUPPRESSION BY NITRIC OXIDE

There are several factors within the tumor microenvironment that lead to immunosuppression, including IL-10, transforming growth factor (TGF)-β, and vascular endothelial growth factor. These factors play a key role during the polarization of immune cells and their recruitment to the tumor environment. IL-10 from T_{reg} cells suppresses T-cell proliferation and TGF-β mediates increased CD25+ and FOX3P+, which scavenges IL-2. TGF-β also inhibits M1 polarized macrophages and promotes an immunosuppressive microenvironment.

Examination of the literature suggests that increased expression and activity of these factors occur again at NO concentrations in the range of 100–1000 nM. For example, HIF-1α is increased with >100 nM NO, which leads to increased production of vascular endothelial growth factor.[37] Activation of TGF-β in tumor cells occurs between 100 and 300 nM NO, whereas IL-10 has been shown to increase at 200–400 nM of steady-state NO.[38,39] Our preliminary data find that expression of IL-10 from Jurkat cells increases in response to 100–300 nM of steady-state NO. Thus, this level of NO, in addition to promoting tumor cell proliferation and migration, increases matrix remodeling proteins and protumorigenic cytokines and releases factors that polarize the immune system to favor immunosuppression.

VI. CHEMISTRY OF NITROSATION

The chemistry that occurs between 100 and 1000 nM NO is unique; the bulk of the nitrosative mechanisms stem from the autoxidation reaction in *in vitro* conditions. In activated murine macrophages, NO fluxes can reach the equivalent of ~1 mM of DETANO, indicating that a high flux of NO can be achieved from NOS2 within the cellular microenvironment. The autoxidation reaction of NO is relatively slow compared with the faster reaction of NO with superoxide O_2^-. However, prolonged fluxes of NO will produce RNS from the autoxidation reaction. The more facile chemistry of NO (e.g., with hemoproteins and reactive oxygen species) may be thought of as signaling sparks in specific rapid fashion compared with the NO/O_2 reaction, which is more like a slow burn. Lower NO flux over a prolonged period of time may provide spatial and molecular selectivity. The solubility of NO and RNS occur in the membrane or hydrophobic environments, respectively, in the cell. Because nitrosative species target thiols and, to a lesser extent, tyrosine, these molecules become ideal targets for electrophiles such as N_2O_3. Thus, receptors containing critical thiols that are susceptible to S-nitrosation by sustain fluxes of NOS2-derived NO can serve as triggers for nitrosative signaling mechanisms.

Acidic nitrite represents a potential source of nitrosation chemistry. Phagosomes, lysosomes, and autophagosomes are acidic intracellular compartments. Acidification of nitrite leads to the formation of NO_2/NO and N_2O_3, which may migrate to specific targets. Recent relationships between autophagy and the endoplasmic reticulum stress indicate that they can provide tumor suppression or promotion, depending on the circumstances. These organelles may provide an additional source of RNS capable of facilitating tumor progression.

VII. CONCLUSION

The genomic landscape recently has provided insight into several types of cancer. Specific mutations such as in *p53*, *Ras*, and *APC* have been identified in a number of epithelial related cancers, but these mutations seem not to be the whole story. The occurrence of other modifications activating specific signaling pathways may also be important drivers of cancer progression. In breast cancer, these pathways include PI3K/Akt and NF-κB. This genomic map may provide important insights into the protumorigenic properties of inflammatory molecules, including NO. Examination of the pathways associated with 100- to 1000-nM fluxes of NO and nitrosative signaling reveal the induction of PI3K as well NF-κB, as we have reported previously. Thus, NOS2-derived NO can function as a "nonmutational" driver for breast cancer progression through nitrosative chemistry.

Viewing the impact of NO on signaling pathways within the "nitrosative landscape," it becomes apparent that multiple targets and pathways are activated to promote cancer progression and metastasis. Thus, the presence of NOS2 in tumor cells can modify EGFR, Src, Ras, or TIMP-1 via a nitrosative mechanism to enhance the activation of many of critical pathways, including prosurvival signaling associated with mitogen-activated protein kinase, Akt, β-catenin, NF-κB, and TGF-β. These, in turn, increase chemoresistance, proliferation, and antiapoptosis as well as cancer stem cell factors and immunosuppression. By targeting specific cysteine and tyrosine residues, NOS2-derived NO activates specific signaling pathways via nitrosative chemistry to provide novel mechanisms associated with cancer progression.

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ABBREVIATIONS

ER−	estrogen receptor negative
ER+	estrogen receptor positive
IL	interleukin
MMP	matrix metalloprotein
NO	nitric oxide
NOS2	inducible nitric oxide synthase
PI3K	phosphatidylinositol 3-kinase
RNS	reactive nitrogen species
SNO	S-nitrosylation
TIMP	tissue inhibitor of metalloproteinase

References

1. Cheng, R.; Ridnour, LA.; Glynn, SA.; Switzer, CH.; Flores-Santana, W.; Hussain, P.; Thomas, DD.; Ambs, S.; Harris, CC.; Wink, DA.; Bonavida, B. Nitric oxide and cancer: an overview. In: Bonavida, B., editor. Nitric Oxide (NO) and Cancer (Cancer Drug Discovery and Development). New York: Springer; 2010. p. 3-20.

2. Wink DA, Hines HB, Cheng RYS, Switzer CH, Flores-Santana W, Vitek MP, Ridnour LA, Colton CA. Nitric oxide and redox mechanisms in the immune response. J Leukoc Biol. 2011; 89:873–891. [PubMed: 21233414]
3. Hussain SP, He P, Subleski J, Hofseth LJ, Trivers GE, Mechanic L, Hofseth AB, Bernard M, Schwank J, Nguyen G, Mathe E, Djurickovic D, Haines D, Weiss J, Back T, Gruys E, Laubach VE, Wiltrout RH, Harris CC. Nitric Oxide is a key component in inflammation-accelerated tumorigenesis. Cancer Res. 2008; 68:7130–7136. [PubMed: 18757428]
4. Shi Q, Xiong Q, Wang B, Le X, Khan NA, Xie K. Influence of Nitric oxide synthase ii gene disruption on tumor growth and metastasis. Cancer Res. 2000; 60:2579–2583. [PubMed: 10825124]
5. Thomsen LL, Miles DW. Role of nitric oxide in tumour progression: lessons from human tumours. Cancer Metastasis Rev. 1998; 17:107–118. [PubMed: 9544426]
6. Okayama H, Saito M, Oue N, Weiss JM, Stauffer J, Takenoshita S, Wiltrout RH, Hussain SP, Harris CC. NOS2 enhances KRAS-induced lung carcinogenesis, inflammation and microRNA-21 expression. Int J Cancer. 2012 May 23. [Epub ahead of print].
7. Lejeune P, Lagadec P, Onier N, Pinard D, Ohshima H, Jeannin JF. Nitric oxide involvement in tumor-induced immunosuppression. Increased NOS2 predicts poor survival in estrogen receptor-negative breast cancer patients. J Immunol. 1994; 152:5077–5083. [PubMed: 7513729]
8. Glynn SA, Boersma BJ, Dorsey TH, Yi M, Yfantis HG, Ridnour LA, Martin DN, Switzer CH, Hudson RS, Wink DA, Lee DH, Stephens RM, Ambs S. Increased NOS2 predicts poor survival in estrogen receptor-negative breast cancer patients. J Clin Invest. 2010; 120:3843–3854. [PubMed: 20978357]
9. Ekmekcioglu S, Ellerhorst J, Smid CM, Prieto VG, Munsell M, Buzaid AC, Grimm EA. Inducible nitric oxide synthase and nitrotyrosine in human metastatic melanoma tumors correlate with poor survival. Clin Cancer Res. 2000; 6:4768–4775. [PubMed: 11156233]
10. Eyler CE, Wu Q, Yan K, MacSwords JM, Chandler-Militello D, Misuraca KL, Lathia JD, Forrester MT, Lee J, Stamler JS, Goldman SA, Bredel M, McLendon RE, Sloan AE, Hjelmeland AB, Rich JN. Glioma stem cell proliferation and tumor growth are promoted by nitric oxide synthase-2. Cell. 2011; 146:53–66. [PubMed: 21729780]
11. Ambs S, Merriam WG, Bennett WP, Felley-Bosco E, Ogunfusika MO, Oser SM, Klein S, Shields PG, Billiar TR, Harris CC. Frequent nitric oxide synthase-2 expression in human colon adenomas: implication for tumor angiogenesis and colon cancer progression. Cancer Res. 1998; 58:334–341. [PubMed: 9443414]
12. Prueitt RL, Boersma BJ, Howe TM, Goodman JE, Thomas DD, Ying L, Pfiester CM, Yfantis HG, Cottrell JR, Lee DH, Remaley AT, Hofseth LJ, Wink DA, Ambs S. Inflammation and IGF-I activate the Akt pathway in breast cancer. Int J Cancer. 2007; 120:796–805. [PubMed: 17096325]
13. Ridnour LA, Thomas DD, Switzer C, Flores-Santana W, Isenberg JS, Ambs S, Roberts DD, Wink DA. Molecular mechanisms for discrete nitric oxide levels in cancer. Nitric Oxide. 2008; 19:73–76. [PubMed: 18472020]
14. Thomas DD, Ridnour LA, Isenberg JS, Flores-Santana W, Switzer CH, Donzelli S, Hussain P, Vecoli C, Paolocci N, Ambs S, Colton CA, Harris CC, Roberts DD, Wink DA. The chemical biology of nitric oxide: implications in cellular signaling. Free Radic Biol Med. 2008; 45:18–31. [PubMed: 18439435]
15. Switzer CH, Glynn SA, Cheng RYS, Ridnour LA, Green JE, Ambs S, Wink DA. S-Nitrosylation of EGFR and Src activates an oncogenic signaling network in human basal-like breast cancer. Molecular Cancer Research. 2012; 10:1203–1215. [PubMed: 22878588]
16. Switzer CH, Cheng RYS, Ridnour LA, Glynn SA, Ambs S, Wink DA. Ets-1 is a transcriptional mediator of oncogenic nitric oxide signaling in estrogen receptor-negative breast cancer. Breast Cancer Res. 2012; 14:R125. [PubMed: 22971289]
17. Switzer CH, Cheng RY, Ridnour LA, Murray MC, Tazzari V, Sparatore A, Del Soldato P, Hines HB, Glynn SA, Ambs S, Wink DA. Dithiolethiones inhibit NF-kappaB activity via covalent modification in human estrogen receptor-negative breast cancer. Cancer Res. 2012; 72:2394–2404. [PubMed: 22436383]

18. Khramtsov AI, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH. Wnt/{beta}-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. Am J Pathol. 2010; 176:2911–2920. [PubMed: 20395444]
19. Ambs S, Glynn SA. Candidate pathways linking inducible nitric oxide synthase to a basal-like transcription pattern and tumor progression in human breast cancer. Cell Cycle. 2011; 10:619–624. [PubMed: 21293193]
20. Sha Y, Marshall HE. S-nitrosylation in the regulation of gene transcription. Biochim Biophys Acta. 2012; 1820:701–711. [PubMed: 21640163]
21. Foster MW, Hess DT, Stamler JS. Protein S-nitrosylation in health and disease: a current perspective. Trends Mol Med. 2009; 15:391–404. [PubMed: 19726230]
22. Rahman MA, Senga T, Ito S, Hyodo T, Hasegawa H, Hamaguchi M. S-nitrosylation at cysteine 498 of c-Src tyrosine kinase regulates nitric oxide-mediated cell invasion. J Biol Chem. 2010; 285:3806–3814. [PubMed: 19948721]
23. Estrada C, Gomez C, Martin-Nieto J, De Frutos T, Jimenez A, Villalobo A. Nitric oxide reversibly inhibits the epidermal growth factor receptor tyrosine kinase. Biochem J. 1997; 326:369–376. [PubMed: 9291107]
24. Lander HM, Hajjar DP, Hempstead BL, Mirza UA, Chait BT, Campbell S, Quilliam LA. Structural basis for the nitric oxide-p21(ras) interaction. J Biol Chem. 1997; 272:4323–4326. [PubMed: 9020151]
25. Thomas DD, Espey MG, Ridnour LA, Hofseth LJ, Mancardi D, Harris CC, Wink DA. Hypoxic inducible factor 1α, extracellular signal-regulated kinase, and p53 are regulated by distinct threshold concentrations of nitric oxide. Proc Natl Acad Sci U S A. 2004; 101:8894–8899. [PubMed: 15178764]
26. Sidorkina O, Espey MG, Miranda KM, Wink DA, Laval J. Inhibition of poly(ADP-ribose) polymerase (PARP) by nitric oxide and reactive nitrogen oxide species. Free Radic Biol Med. 2003; 35:1431–1438. [PubMed: 14642390]
27. Kroncke KD, Carlberg C. Inactivation of zinc finger transcription factors provides a mechanism for a gene regulatory role of nitric oxide. FASEB J. 2000; 14:166–173. [PubMed: 10627291]
28. Fu X, Kassim SY, Parks WC, Heinecke JW. Hypochlorous acid oxygenates the cysteine switch domain of pro-matrilysin (MMP-7). A mechanism for matrix metalloproteinase activation and atherosclerotic plaque rupture by myeloperoxidase. J Biol Chem. 2001; 267:41279–41287. [PubMed: 11533038]
29. Krishnatry AS, Kamei T, Wang H, Qu J, Fung HL. Identification of nitroglycerin-induced cysteine modifications of pro-matrix metallo-proteinase-9. Rapid Commun Mass Spectrom. 2011; 25:2291–2298. [PubMed: 21766372]
30. Ridnour LA, Windhausen AN, Isenberg JS, Yeung N, Thomas DD, Vitek MP, Roberts DD, Wink DA. Nitric oxide regulates matrix metal-loproteinase-9 activity by guanylyl-cyclase-dependent and -independent pathways. Proc Natl Acad Sci U S A. 2007; 104:16898–16903. [PubMed: 17942699]
31. Yu Q, Toole BP, Stamenkovic I. Induction of apoptosis of metastatic mammary carcinoma cells *in vivo* by disruption of tumor cell surface CD44 function. J Exp Med. 1997; 186:1985–1996. [PubMed: 9396767]
32. Death AK, Nakhla S, McGrath KC, Martell S, Yue DK, Jessup W, Celermajer DS. Nitroglycerin upregulates matrix metalloproteinase expression by human macrophages. J Am Coll Cardiol. 2002; 39:1943–1950. [PubMed: 12084592]
33. Ridnour LA, Barasch K, Windhausen AN, Dorsey TH, Yfantis HG, Lee DH, Switzer CH, Glynn SA, Cheng RY, Heinecke J, Brueggemann E, Hines HB, Khanna C, Ambs S, Wink DA. Nitric Oxide synthase and breast cancer: role of TIMP-1 in NO-mediated Akt activation. PLoS One. 2012; 7:e44081. [PubMed: 22957045]
34. Patruno A, Pesce M, Marrone A, Speranza L, Grilli A, De Lutiis MA, Felaco M, Reale M. Activity of matrix metallo proteinases (MMPs) and the tissue inhibitor of MMP (TIMP)-1 in electromagnetic field-exposed THP-1 cells. J Cell Physiol. 2012; 227:2767–2774. [PubMed: 21928345]

35. Harris LK, McCormick J, Cartwright JE, Whitley GS, Dash PR. S-nitrosylation of proteins at the leading edge of migrating trophoblasts by inducible nitric oxide synthase promotes trophoblast invasion. Exp Cell Res. 2008; 314:1765–1776. [PubMed: 18394602]

36. Hamada T, Duarte S, Tsuchihashi S, Busuttil RW, Coito AJ. Inducible nitric oxide synthase deficiency impairs matrix metalloproteinase-9 activity and disrupts leukocyte migration in hepatic ischemia/reperfusion injury. Am J Pathol. 2009; 174:2265–2277. [PubMed: 19443702]

37. Brune B, Zhou J. The role of nitric oxide (NO) in stability regulation of hypoxia inducible factor-1alpha (HIF-1alpha). Curr Med Chem. 2003; 10:845–855. [PubMed: 12678687]

38. Niedbala W, Cai B, Liu H, Pitman N, Chang L, Liew FY. Nitric oxide induces CD4+CD25+ Foxp3 regulatory T cells from CD4+CD25 T cells via p53, IL-2, and OX40. Proc Natl Acad Sci U S A. 2007; 104:15478–15483. [PubMed: 17875988]

39. Vodovotz Y, Chesler L, Chong H, Kim SJ, Simpson JT, DeGraff W, Cox GW, Roberts AB, Wink DA, Barcellos-Hoff MH. Regulation of transforming growth factor beta1 by nitric oxide. Cancer Res. 1999; 59:2142–2149. [PubMed: 10232601]