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The Implications of Hyponitroxia in Cancer

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Abstract

Tumors are spatially heterogeneous, with regions of relative hypoxia and normoxia. The tumor microenvironment is an important determinant of both tumor growth and response to a variety of cytotoxic and targeted therapies. In the tumor microenvironment, reactive oxygen species and nitric oxide (NO) are important mediators of the level of expression of many transcription factors and signaling cascades that affect tumor growth and responses to therapy. The primary objective of this review is to explore and discuss the seemingly dichotomous actions of NO in cancer biology as both a tumor promoter and suppressor with an emphasis on understanding the role of persistently low NO concentrations or hyponitroxia as a key mediator in tumor progression. This review will also discuss the potential role of hyponitroxia as a novel therapeutic target to treat cancer and outline an approach that provides new opportunities for pharmacological intervention.

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Introduction

This review highlights the function of hyponitroxia as a proneoplastic effector, summarizes therapeutic strategies to increase intratumoral nitric oxide (NO) to mitigate, at least in part, the effect of hyponitroxia on angiogenesis and malignant progression, and makes the case for hyponitroxia a high-priority target in cancer therapy that may be as, if not more, important than hypoxia.

As in tumors, NO also plays an important role in normal tissues. Under physiological conditions, low levels of NO are produced from L-arginine by constitutively expressed NO synthase in neuronal cells (nNOS, also known as NOS1) and endothelial cells (eNOS or NOS3) [1], which contribute to the regulation of normal physiological processes through cell signaling (Figure 1). Higher levels of NO are produced by an inducible nitric oxide synthase (iNOS or NOS2) [1]. NO can stimulate pathways resulting in either cell growth or cell death, depending on the relative level of NO and a variety of associated factors [2].

The Hyponitroxia and Hypoxia Axis

In tumors, hyponitroxia is relative rather than absolute: low levels of NO (<100 nM) [3] are produced by three NOS enzymes described above [4]) and associated with the oxidative burst of macrophages. At the low concentrations of NO found in tumors, NO mediates redox signaling pathways linked to the proangiogenic activities of vascular endothelial growth factor and inhibition of thrombospondin 1 [5], promoting malignant conversion, tumor progression [6], and resistance to therapy in multiple cancers including prostate [7], colonic, lung [8], and mammary adenocarcinomas [8,9]. Other candidate oncogenic functions of NO include cell proliferation, invasion and metastasis, and stem cell renewal [3]. Hyponitroxia thus represents a modified form of hormesis [10], a dose-response model characterized by a beneficial effect at low doses and a detrimental effect at high doses. NO also exerts a direct effect on responses to hypoxia through changes in expression of hypoxia inducible factor, alpha subunit (HIF-1α). Mimicking and attenuating hypoxia [11], NO drives HIF-1α signaling, by inhibition of prolyl hydroxylase 2 [12], resulting in a more aggressive and resistant phenotype (Figure 2).

Hypoxia catalyzes the oncogenicity of NO: in addition to L-arginine, molecular oxygen is an essential substrate for the activity of NOSs, and exposure to low-oxygen tension limits endogenous NO production by these enzymes [13,14]. However, in the absence of complete anoxia, a rare state even in tumors, NO synthesis is only inhibited rather than abrogated [14], resulting in the constitutive induction of the enzyme guanyl cyclase (GC) [15] and the accumulation of its downstream mitogenic effector

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cyclic guanosine monophosphate. *S*-nitrosylation of caspases, leading to their inactivation, has also been proposed as a mechanism by which NO can block apoptosis and result in tumorigenesis [16]. In addition, hypoxia also redirects macrophage L-arginine metabolism from NOS to arginase [17], an enzyme that converts L-arginine to urea, leading to decreased arginine availability as a substrate for NO production.

Thus, as an inactivating mechanism for endogenous NO production, hypoxia acts as a protumorigenic stimulus, potentiating the destructive potential of NO [18], separate from its effects on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [19] and HIF-dependent transcriptional pathways.

However, the reverse is true as well: hyponitroxia exacerbates hypoxia through alterations in blood flow and oxygen consumption through NO mitochondria-mediated pathways [20,21]. Therefore, hypoxia and hyponitroxia are closely related and can affect a variety of downstream targets—either simultaneously or sequentially.

NO-Mediated Effects

In the past two decades or so, NO has been implicated in a wide array of potential effects on cancer suppression and progression. It is now generally agreed that NO has a highly context-dependent dose–response stimulation-inhibition relationship with cytotoxicity at high doses and mitogenicity at low doses [22]. Thus, NO has the ability to both promote and suppress cancer.

However, these binary either/or descriptions are an oversimplification. At low constitutive levels induced by hypoxia in tumors, NO levels are optimal for the mediation of aberrant, proliferative signaling. In contrast, levels either above or below this optimal range can have the opposite effect and activate signal transduction pathways that contribute to/result in growth inhibition or cell death.

The Threshold Dose and Cytotoxicity

NO is a radical with a free electron capable of interacting with reactive oxygen species (ROS) such as the superoxide anion to form a variety of highly reactive nitrogen oxides (NOx). The term *nitrosative stress* refers to the formation of NOx compounds such as peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2), and dinitrogen trioxide (N_2O_3) that are responsible for cytotoxic nitration and oxidation reactions [23] leading to apoptosis and cell death. In particular, the formation of peroxynitrite is a first-order reaction [23] dependent on the concentrations of NO and the superoxide anion and, therefore, on oxygen tension, because in the presence of hypoxia, both NO and ROS such as the superoxide anion will be less prevalent.

Xie et al [24] demonstrated that transfection of murine K-1735 melanoma cells with inducible NOS leading to the generation of high levels of NO resulted in suppression of tumorigenicity and metastasis. The cytotoxicity of higher concentrations of NO is consistent with the assumption that the toxic effect becomes apparent above a threshold dose of NOx. This balance between mitogenic and toxic effects of NO in tumor cells is potentially attributable to an increased susceptibility to free radical damage due to severe impairment of the antioxidant defense system [25] compared with healthy cells.

In cancer cells, reactive oxygen/nitrogen species "reprogram" the cellular metabolism toward a dependence on glucose use, termed the *Warburg effect*, a signature of virtually all tumors and the basis of fluorodeoxyglucose positron emission tomography imaging, to support anabolic proliferation. The fact that this core feature of tumors, metabolic reprogramming, is dependent on redox signaling implies that ROS/reactive nitrogen species (RNS) levels are higher in tumors than in healthy tissue, resulting in a differential sensitivity to oxidant stress [26]. Indeed, the presence of high levels of ROS in tumors has been linked with cell cycle arrest and apoptosis [27].

However, NOx cytotoxicity may not require superelevated doses but rather approximate "normalization" to physiological levels [27], because shifts in a particular direction can have important consequences. For example, Frederiksen et al. demonstrated that NO enrichment through low concentrations of the NO mimetics glyceryl trinitrate (GTN) and isosorbide dinitrate attenuated hypoxia-induced resistance to doxorubicin in prostate cancer mouse models.

At the other end of the spectrum, Kashiwagi and Jain [28] described radiosensitization in glioma xenografts through the normalizing effects of NOS inhibition on the tumor vasculature. The cytotoxicity of NO below a certain threshold is consistent with the assumption that lower concentrations of NO reduce signal transduction below a physiological baseline, leading to a loss of the aberrant induction of proangiogenic [5] signaling [29] networks that promote malignant progression (Figure 3).

This emerging background of conflicting preclinical evidence that both anti-NO–centered and pro-NO–centered therapeutic strategies are therapeutically effective has resulted in the initiation of human clinical trials with both NO donors and NO inhibitors such as

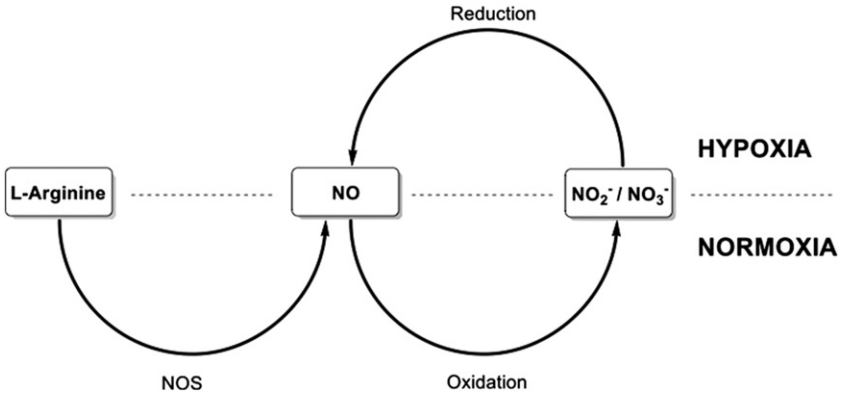

Figure 1. The nitrate-nitrite-NO pathway. NO is generated from the precursor L-arginine by the enzyme NOS under normoxic conditions. Under these conditions, NO is oxidized to nitrite and nitrate. Under hypoxia, nitrite is reduced by a variety of NOS-independent processes to form NO [2].