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STATE-OF-THE-ART PAPER

Effects of Nitric Oxide on Cell Proliferation

Novel Insights

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Nitric oxide (NO) has been suggested to be a pathophysiological modulator of cell proliferation, cell cycle arrest, and apoptosis. In this context, NO can exert opposite effects under diverse conditions. Indeed, several studies have indicated that low relative concentrations of NO seem to favor cell proliferation and antiapoptotic responses and higher levels of NO favor pathways inducing cell cycle arrest, mitochondria respiration, senescence, or apoptosis. Here we report the effects of NO on both promotion and inhibition of cell proliferation, in particular in regard to cardiovascular disease, diabetes, and stem cells. Moreover, we focus on molecular mechanisms of action involved in the control of cell cycle progression, which include both cyclic guanosine monophosphate–dependent and –independent pathways. This growing field may lead to broad and novel targeted therapies against cardiovascular diseases, especially concomitant type 2 diabetes, as well as novel bioimaging NO-based diagnostic tools. (J Am Coll Cardiol 2013;62:89–95) © 2013 by the American College of Cardiology Foundation

Nitric oxide (NO) is a signaling molecule that regulates many functions, such as vascular tone, blood pressure, neurotransmission, immune response, and oxidation-sensitive mechanisms (1–4). NO may act as an autocrine or paracrine messenger, and its production and degradation are cell type dependent. NO synthesis is catalyzed from L-arginine by a family of nitric oxide synthases (NOSs), varying from picomolar/nanomolar range for short periods to micromolar range for protracted periods (3). NO reacts with molecules such as oxygen, superoxide or metals, nucleic acids, and proteins. The major reactions involving NO support its rapid oxidation into nitrate and nitrite, which are now considered not inert end products but actors of a reverse pathway that represents an alternative source of NO when the endogenous L-arginine/NOS pathway is dysfunctional (5,6).

The effect of NO production on cellular processes is also dependent on its concentration and on the presence of other free radicals. Peroxynitrites, generated from reaction with a superoxide, can interact with several cellular components and are implicated in NO signaling mechanisms involving protein modifications (6). Lower concentrations of NO have been suggested to exert a direct effect on processes such as cell proliferation and survival, whereas higher concentrations have an indirect effect through both oxidative and nitrosative stresses (3,5,6). Free radical interactions also influence NO signaling. One of the consequences of reactive oxygen species (ROS) generation is reduced concentrations of NO (1). The resulting reactive nitrogen species can also have biological effects and increase oxidative and nitrosative stress responses. Overall, cellular responses are differentially regulated by specific NO concentrations, with lower NO concentrations (picomolar to nanomolar) generally promoting cell survival and proliferation and higher concentrations (micromolar) favoring cell cycle arrest, apoptosis, and senescence (6) (Fig. 1A). However, observed divergent effects can be explained by cellular context, cell cycle point, and oncogenic state (6). The molecular mechanisms underlying the proliferative action of NO at low concentrations are not yet clear, but key molecules and pathways involved in NO-mediated inhibition have been better studied. Some basic distinct concentrations of NO have also been proposed for activity, such as for cyclic guanosine monophosphate (cGMP)-mediated processes (30 nmol/l) and nitrosative stress (1 μmol/l) (6).

Molecular Mechanisms of NO Involved in Progression of the Cell Cycle

NO blocks the progression of the cell cycle primarily at the G_1/S transition. Hence, NO-induced G_1 arrest has been observed in vascular smooth muscle cells (VSMCs) and

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Abbreviations and Acronyms

BMC = bone marrow cell(s)
cGMP = cyclic guanosine monophosphate
EGFR = epidermal growth factor receptor
eNOS = endothelial nitric oxide synthase
EPC = endothelial progenitor cell(s)
GC = guanylate cyclase
NO = nitric oxide
NOS = nitric oxide synthase
ROS = reactive oxygen species
T2DM = type 2 diabetes mellitus
VSMC = vascular smooth muscle cell(s)

other cell lines (7–9). In other contexts and after treatment with NO donors of the nonsteroidal anti-inflammatory drug family, the entry into the G_2/M phase was NO sensitive (8,10,11).

As expected, many cell cycle proteins ($p21^{Cip1/Waf1}$, p27, cyclins, Cdk2, pRb, and the like) are good candidates for final molecular targets of NO, as revealed by exogenous NO, endothelial nitric oxide synthase (eNOS) transfection, and whole expression studies (7,9,12–18). NO is also able to regulate cell proliferation by targeting important mitogenic receptors and their downstream pathways, such as epidermal growth factor receptor (EGFR) signaling pathway (8,19). Moreover, 26S proteasome and apoptosis factors represent other important NO targets (20,21) (Fig. 1B).

cGMP-dependent and -independent pathways. A canonical NO pathway involves the selective activation of soluble guanylate cyclase (GC), the generation of cGMP, and the activation of specific cGMP-dependent protein kinases (1,22). However, further mechanisms have emerged that mainly include the formation of NO-induced post-translational modifications (8,22,23). Overall, these modifications activate pathways that are cGMP independent (Fig. 1B).

The involvement of cGMP in growth inhibition has been described in VSMCs, in which NO activates GC with a subsequent increase in cGMP leading to the phosphorylation of a vasodilator-stimulated phosphoprotein and subsequent inhibition of the epidermal growth factor signaling pathway (24,25). Recent findings indicate that other nuclear effects of NO altering the cell cycle can occur. A mechanism involved in the regulation of the cell cycle is the direct interaction of NO-sensitive GC with chromosomes during mitosis (26). More recently, NO has been shown to modulate chromatin folding in human endothelial cells through class II histone deacetylases (27).

The antiproliferative effect of NO does not necessarily require cGMP as an intermediate effector because it was also shown in a fibroblast cell line lacking soluble GC (19) or with GC inhibitors (16,28,29). Inhibition of the G_1/S transition has been shown to be mostly a cGMP-independent process (7,9), although dual mechanisms were found to mediate the antimitogenic effects in VSMCs (30).

A mechanism for cGMP-independent proliferative arrest is S-nitrosylation, which can reversibly inhibit the catalytic activity of the 26S proteasome and enhance Ras guanine nucleotide exchange under nitrosative stress (31,32). A recent study has also demonstrated that NO-mediated Ras nitrosylation in different subcellular compartments regulates downstream pathways stimulating cell proliferation (33). In addition, the EGFR trans(auto)phosphorylation inhibition was found to be independent of cGMP and directly inhibited by NO (19). However, an increase in NO-mediated cGMP enables the disruption of the downstream mitogenic signal through Raf1 phosphorylation (25).

eNOS S-glutathionylation is a pivotal redox regulator of endothelial function and vascular tone. Indeed, in endothelial cells, this modification reversibly decreases eNOS activity with increased production of superoxide anions. Moreover, in hypertensive vessels, eNOS S-glutathionylation is increased with impaired vasodilation (34). The impact of another mechanism, tyrosine nitration, was demonstrated in a recent study that identified several nitrated proteins involved in the different checkpoints toward the cell cycle (35).

Another cGMP-independent mechanism involves mitochondria that influence several pathways modulating cell proliferation, cell cycle arrest, and apoptosis through oxidative and nitrosative reactions that are mediated by the NOS mitochondrial isoform (23,36). Moreover, peroxynitrite can also change calcium homeostasis, allowing the opening of the mitochondrial permeability transition pore that promotes mitochondrial signaling of cell death (37). In this context, NO instead exerts a protective action by directly inhibiting the opening of the mitochondrial permeability transition pore. Furthermore, cytochrome *c* oxidase is one of the most important targets for NO signaling, leading to inhibition of mitochondrial oxidative phosphorylation, the control of apoptosis, and ROS generation (37,38).

Cell Cycle NO-Dependent Effects in the Cardiovascular System

Cell proliferation, inhibition, and apoptosis. NO is able to inhibit cell growth and proliferation and to induce apoptosis in a dose-dependent manner (7,12,20,28,29,39,40) (Table 1). Indeed, an adequate concentration of NO is required to induce inhibition of cell proliferation. There is a lack of correlation between increased NOS expression and inhibition of cell proliferation in some cellular systems, probably due to the short life of NO that is transformed into inactive compounds (6,8,28).

NO donors and drugs affecting the NO pathway through different mechanisms have been used to inhibit cell proliferation of several cell types. Indeed, it is known that endogenously produced NO can negatively regulate cell proliferation and/or the proliferation of neighboring cells. The increase of endogenous NO depends on the availability of L-arginine and/or inducible NOS expression mediated by several cytokines (41–43).

A proliferative arrest induced by endogenous NO production has been found in different cell types, including VSMCs (8). Proliferation of these cells has been accepted as a common event in the pathophysiology of many vascular diseases. Delivery of L-arginine, pharmacological NO