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Review Article

Nitric oxide-mediated sensitization of resistant tumor cells to apoptosis by chemo-immunotherapeutics ☆

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ABSTRACT

The generation of NO by the various NO synthases in normal and malignant tissues is manifested by various biological effects that are involved in the regulation of cell survival, differentiation and cell death. The role of NO in the cytotoxic immune response was first revealed by demonstrating the induction of iNOS in target cells by immune cytokines (e.g. IFN-γ, IL-1, TNF-α, etc.) and resulting in the sensitization of resistant tumor cells to death ligands-induced apoptosis. Endogenous/exogenous NO mediated its immune sensitizing effect by inhibiting NF-κB activity and downstream, inactivating the repressor transcription factor YY1, which inhibited both Fas and DR5 expressions. In addition, NO-mediated inhibition of NF-κB activity and inhibition downstream of its anti-apoptotic gene targets sensitized the tumor cells to apoptosis by chemotherapeutic drugs. We have identified in tumor cells a dysregulated pro-survival/anti-apoptotic loop consisting of NF-κB/Snail/YY1/RKIP/PTEN and its modification by NO was responsible, in large, for the reversal of chemo and immune resistance and sensitization to apoptotic mechanisms by cytotoxic agents. Moreover, tumor cells treated with exogenous NO donors resulted in the inhibition of NF-κB activity via S-nitrosylation of p50 and p65, inhibition of Snail (NF-κB target gene), inhibition of transcription repression by S-nitrosylation of YY1 and subsequent inhibition of epithelial–mesenchymal transition (EMT), induction of RKIP (inhibition of the transcription repressor Snail), and induction of PTEN (inhibition of the repressors Snail and YY1). Further, each gene product modified by NO in the loop was involved in chemo-immunosensitization. These above findings demonstrated that NO donors interference in the regulatory circuitry result in chemo-immunosensitization and inhibition of EMT. Overall, these observations suggest the potential anti-tumor therapeutic effect of NO donors in combination with subtoxic chemo-immuno drugs. This combination acts on multiple facets including reversal of chemo-immune resistance, and inhibition of both EMT and metastasis.

Contents

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1. Introduction

Since the early introduction of various nonspecific chemotherapeutic drugs and, subsequently, radiotherapeutics, hormonal drugs and immunotherapies, there have been significant clinical and objective responses in patients with various malignant diseases. While these therapeutic strategies continue to improve and are still currently in use in oncology, there remains a major problem in that a subset of cancer patients does not initially respond and another responding subset develops resistance to further treatments and both subsets succumb to the disease. Clearly, the non-responding patients underlie the rapid and successful development of new classes of targeted therapeutics based on the various molecular mechanisms that govern resistance. Noteworthy, it is recognized to date that many of the cytotoxic agents (drugs, hormones, immunotherapies) used in cancer therapy mediate their effects by inducing programmed cell death, or apoptosis, in the responding sensitive tumor cells. Hence, a major, but not the only mechanism of resistance, is the mechanism in acquisition of tumor cells of several molecular and genetic means to evade cell death by apoptosis.

The inhibition of pro-apoptotic pathways in cancer cells is the result of tumor cells exhibiting constitutively hyperactivated survival and proliferative signal pathways that counteract the pro-apoptotic pathways. A good example is the constitutively hyperactivated NF-κB pathway in the majority of cancers that was shown to play a pivotal role in tumorigenesis, angiogenesis, resistance, inflammation, immunomodulation, and metastasis. Further, other survival/proliferative/anti-apoptotic pathways are also upregulated in cancer cells such as the PI3K/AKT and MAPK pathways. These various pathways also crosstalk and synergize in their anti-apoptotic effects [1,2].

There have been several strategies to therapeutically inhibit anti-apoptotic pathways in cancer. Among these, many earlier and recent studies suggested the potential cytotoxic and sensitizing effects of NO in examined various cancer cell models, *in vitro* and *in vivo* [3–10]. Recently, Reynolds el al. [11] reviewed the use of NO in the inhibition of tumor cell proliferation and its consideration as a novel therapeutic.

2. Nitric oxide properties and applications

Nitric Oxide is a versatile and pleiotropic molecule. It has been reported to be involved in cell signaling and it regulates a variety of physiological functions in mammals [12,13]. NO is a lipophilic free radical gas that can readily diffuse through cell membranes and chemically reacts with metal containing proteins and other effector molecules. NO is synthesized from arginine by the nitric oxide synthases (NOS). Three types of NOS have been discovered, namely, eNOS (endothelial nitric oxide synthase or type III NOS), nNOS (neuronal nitric oxide synthase or type I NOS), and iNOS (inducible nitric oxide synthase or type II) [14]. Zinc finger proteins have been reported to be important targets of NO [15] and NO can also mediate nitrosation and nitrosylation of various proteins and affects their functions and activities [16–19].

The guanylyl cyclase (sGC) α-1 and α-2 heterodimers are found in many tissues and are the receptors for NO [20,21]. The generation of NO by nitroglycerine or nitroprusside activates soluble guanylate cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP) that, in turn, activates Protein kinase C (PKC) to phosphorylate proteins [22]. The recent findings by Bian and Murad [23] established that the NO/sGC/cGMP axis is involved in the regulation of proliferation and differentiation. They also reported that undifferentiated tumor cells and stem cells have low levels of sGC. Thus, by elevating endogenous cGMP it resulted in the inverse correlation with the proliferation and colony formation of glioma cells.

The various NOS isoforms' functions are interconnected and operate as a pivotal part of Red-OX-based molecular signaling systems that result in major impacts on the cell's physiology and behavior and include S-nitrothiols (SNOs), which are generally produced by S-nitrosation of cysteine thiols by NO. These result in the disruption or dysregulation of normal cell signaling and in the impairment of cellular functions [19,24]. Both eNOS and nNOS are calcium-dependent, but iNOs is calcium independent. Further, eNOS and nNOS release lower levels of NO whereas iNOS releases high levels of NO [25]. Of relevance, NO signaling has been reported to initiate apoptosis through its activity on the mitochondrial membrane permeability and the consequent release of cytochrome c oxidase [6, 26].

NO-mediated apoptosis consists also in the endogenous formation of reactive nitric oxide species (RNOS). These RNOS include superoxide (O_2^-) that reacts with NO to form peroxynitrite ($ONOO^-$) that strongly induces apoptosis. Peroxynitrite acts as a DNA oxidant and induces single strand breaks in DNA [27,28]. Also, NO induces changes in the mitochondrial permeability transition pore (MPTP) and its opening results in the release of cytochrome c into the cytoplasm and induces apoptosis via binding Apaf-1 that activates caspase-9 leading to the activation of caspases 7 and 3 and downstream apoptosis [29]. The release of cytochrome c also results in the significant release of intracellular calcium resulting in cell death [30].

The consideration of the potential therapeutic application of NO donors has been the subject of many reports and reviews [7–9,11,13,31–33]. In this review, we will briefly discuss (1) the roles of NO-mediated regulation/interference of gene products that form a loop, namely, the NF-κB /Snail/YY1/RKIP/PTEN loop, that