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Free Radical: Nitric Oxide in Cancer Therapy

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Abstract

Nitric oxide (NO) is a free radical that is involved in many biological processes within the body, including angiogenesis, cell signaling, and the killing of cancerous cells. At low concentrations, NO can promote tumor growth in ways such as promoting angiogenesis. At high concentrations, NO becomes very toxic to cancer cells and it suppresses tumor growth. This quality of being potentially toxic to cancer cells makes NO the subject of much research in the medical treatment of cancer. Currently, many site-directed delivery methods are in development to accurately deliver and activate NO within cancerous tumors and nanoparticle delivery methods in particular have been shown to be effective in the treatment of cancer. This article highlights the role of nitric oxide in cancer and its application in the medical treatment of cancer.

Keywords: Nitric Oxide: Cancer: Nanomedicine: Radio Sensitizer: Circumvent Drug Resistance

Introduction

Nitric Oxide (NO) is a reactive nitrogen species (RNS), which makes it highly reactive with metal ions and biomacromolecules. As such, it is often involved in the deactivation of proteins, lipids, and deoxyribonucleic acid (DNA) within cells [1]. NO can also act as a signaling molecule to regulate the development of body systems such as the vascular and the nervous systems [2]. As a free radical gas, NO is highly reactive with reactive oxygen species (ROS), particularly during periods of oxidative stress [1]. Free NO mediates numerous biological processes, within the human body. In biological systems, nitric oxide is produced by enzymes known as nitric oxide synthases, which exist in three isoforms: inducible NOS (iNOS), neuronal NOS (nNOS), and endothelial NOS (eNOS) [3]. iNOS generates large amounts of NO in tumors, which suppresses the growth of tumors sensitive to NO but promotes the growth of tumors which are resistant to NO, often depending on the concentration of NO existing in the cellular environment [4]. eNOS generates NO that stimulates angiogenesis, the formation of blood vessels [2]. NO and NOSs are found in abundance in cancerous tumors, where depending on their concentration, [5] can promote or suppress tumor progression [6]. This quality of NO where it can both promote and repress the proliferation of cancer cells based primarily on its concentration is put under consideration in many studies. The exact concentrations of NO can vary based on other factors such as the donor or delivery method, but in general, the concentration of NO that is needed to influence cancer cell proliferation is very small. Lower concentrations tend to promote the proliferation of cancer cells, while higher concentrations tend to inhibit the proliferation of cancer cells. One study demonstrated that low concentrations of SNAP/Deta-NONOate, NO donor, around 20-2000 nM, promoted the growth of cancer, while high concentrations of greater than 20 μM of the NO donor suppressed the growth of the cancer [7]. Studies have shown that NO promotes tumor growth in ovarian cancer cells by regulating the Warburg effect, which increases glycolysis and decreases mitochondrial activity, allowing the cancer cells to sustain their ATP production under hypoxic condition of the tumor cell [7]. Additionally, NO degrades the effectiveness of leukocytes and promotes hypoxia inducible factors, which keep tumor cells from invading blood vessels and promote tumor growth respectively [5].

Nitric oxide mediates the oxidation of lipids and lipoproteins in many oxidative pathways [8]. NO also induces cell death by activating the ASK1/JNK1 (apoptosis signal-regulating kinase 1/c-Jun N-terminal kinase 1) pathway, which mediates the degradation of the anti-apoptotic protein, MCL^{-1} (induced myeloid leukemia cell differentiation protein), and activates BAK and BAX, leading to intrinsic cell apoptosis [9]. The high reactivity of NO with reactive oxygen species (ROS) often triggers cytotoxic pathways with higher concentration of ROS being more susceptible to NO mediated cell death [1]. Nitric oxide reacts with ROS to produce peroxynitrite, which is highly toxic to cells [1]. Whether a cancer cell undergoes NO-mediated cell death depends on whether the concentration of NO is high enough. However, it has also been shown that the cancer cell's position in the cell cycle is important. A study showed that apoptotic colon cancer cells exposed to NO accumulated in the G2-M phase [10]. Nitric oxide production has been shown to activate cytotoxic bone marrow-derived dendritic cells as well, which has cancer killing properties [11]. Therefore, cytotoxicity of NO towards various cancerous cells makes NO the subject of much study in the medical treatment of cancer [5].

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Nitric Oxide Delivery to Cancer Cells

Nitric oxide has been the subject of research throughout the past two decades for its use in site-directed delivery to treat cancer. Site-directed delivery of NO is accomplished mostly through the prodrug strategy, where NO is bonded to a triggering group, creating a non-toxic molecule, which is consequently activated by enzymes that are over expressed in cancerous cells, releasing NO into the cancer cell [1]. Some examples of drugs that NO has been conjugated within the prodrug strategy include, esterase activated diazeniumdiolates, glutathione, and doxorubicin [1]. Studies have shown that NO donors conjugated with doxorubicin (DOX) increase the cancer cell killing efficiency of DOX by as much as 30% [12]. When NO reacts with DOX superoxide, it forms peroxynitrate much quicker, which increases cancer cell death [12]. Several studies have utilized the prodrug delivery strategy and found success in inhibiting cancer growth [13, 14].

Nitric Oxide to Circumvent Drug Resistance

One major challenge in the medical treatment of cancer is multi-drug resistance (MDR), which can occur when cancer drugs alter the chemical environment of the cancer cells, such as by changing the pH, thereby influencing the permeability of cancer cell membranes and making them less receptive to the drugs [15]. MDR is largely mediated by transmembrane efflux pump proteins, such as ATP-binding cassette (ABC) transporters and multidrug resistance-associated proteins (MRP) [15]. Hypoxia in tumor cells increases the expression of the efflux pumps [15] the focus of recent researches has been to deliver NO in conjunction with other cancer fighting drugs to study its secondary effects in cancer cells such as, its inhibition of the transmembrane efflux pumps. Nitric oxide has been found to be effective in deactivating transmembrane efflux pumps by conjugating with heme groups, those when bound with NO, assume an oxy conformation in the hypoxic environment of tumor cells, thereby deactivating the expression of the transmembrane efflux pumps [15]. Moreover, Nitric oxide has been recently investigated for its potential in combating the multi-drug resistance of certain cancers by inhibiting DNA repair and production of proteins in cancer cells, proving to be effective in multi-drug chemotherapy treatment [12].

Nitric Oxide as a Radio sensitizer

In recent study nitric oxide has also proven to be an effective radio sensitizer, an agent that increases a cell's vulnerability to be destroyed by radiation. In cancer radiotherapy, a challenge is that hypoxic cells, cancer cells with minimal levels of oxygen, in the tumors are minimally radiosensitive [16]. Studies have shown that NO is capable of radio sensitizing hypoxic cells by increasing their level of oxygenation through NO-mediated pathways that alter blood flow and increase intake of oxygen by the cell [17]. Studies have demonstrated that increasing the rate of nitric oxide production in cancer cells in conjunction with radiotherapy can lead to as much as 3.4 fold increase in tumor decay when compared to treating with radiation alone [18]. Nitric oxide and ionizing radiation have been shown to induce apoptotic cell death in conjunction with one another by phosphorylating p53 which induces the apoptotic pathway [19]. It was found in one study that treating colorectal cancer cells with NO donors led to a significant increase in the radiosensitivity of the cancer cells [18].

Nitric Oxide based Nanomedicine Platform

Recently, nanoparticle-based delivery systems have made the site-selective delivery of exogenous NO sources more effective to tumor cells. It has also shown to counteract the extremely indiscriminate toxic effects of NO and allow its cytotoxic effects be more focused on the target cancer cells [20]. In one study, nanoparticle platforms have shown to be superior to small molecule chemo sensitizers in that they allow cancer fighting drugs, such as NO, to accumulate within the body system by extending their blood circulation, improving their effectiveness and increasing successful, accurate tumor penetration rate [21]. It may also be an effective tool to counter MDR, as specifically demonstrated in a study with NO bound to BNN6 and enclosed in mPEG-PGLA copolymer nanoparticle. Results showed that the nanoparticles are capable of precisely delivering NO carriers to tumor cells and successfully initiating the NO-mediated reversal of MDR and radio sensitization in the tumor cells [12].

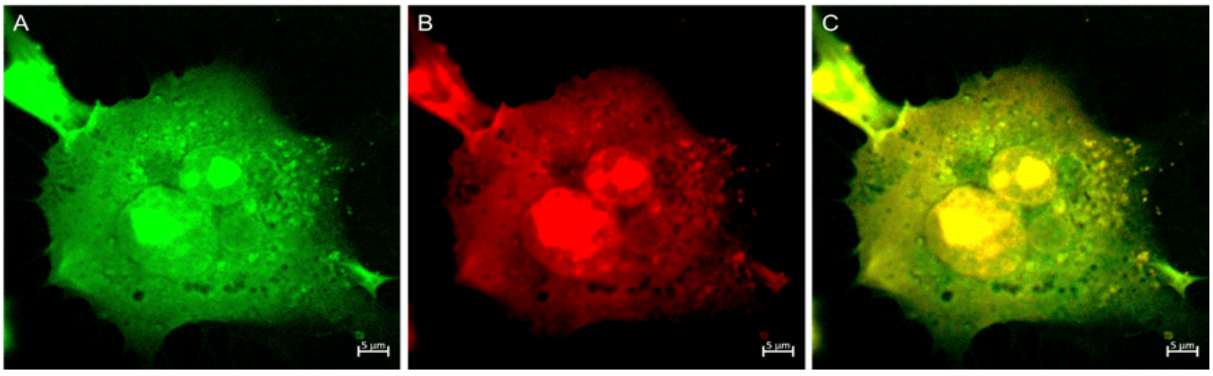

Figure 1: Effects of nitric oxide in cancer treatment: (A) Cancer cells treated with nitric oxide releasing nanoparticles platform and cells were irradiated with X-ray. NO production after X-ray exposure nitric oxide was labeled with labeling dye DAF-FM (green). (B) Resulting NO production enhanced the radio sensitizing effects and increased the DNA damage (red), cells stained with thidium Homodimer III (EthD-III) dye for DNA damage staining. (C) The merge image shows the localization of both signals in the cellular compartment, which shows the effects of nitric oxide in DNA damage for cancer treatment.

During preliminary reach study, we tried to investigate how nitric oxide-based delivery systems play a significant role in the treatment of cancer. In this study we designed a nanoparticle-based platform for delivery of nitric oxide to the cellular compartment of cancer cells and to investigate their radio sensitizing properties after exposure to low dose of X-Ray radiation. We observed that nitric oxide slowly releases to the cellular compartment and also produces more free radicals like nitric oxide (NO) (Fig-1, green chancel). After irradiation with the X-ray, it resulted in enhanced DNA damage of the cancer cells (Fig-1, red chancel) and suppressed growth of the tumor, effectively due to radio sensitizing properties (data not published).

Limitations of Nitric Oxide Delivery

The most significant limitation of nitric oxide is that an accurate delivery method is needed for effective utilization. This is because NO, as a free radical, is extremely toxic to all cells, not just the targeted cancer cells. Therefore, most research has focused on developing effective delivery methods for NO, whether it is by delivering it conjugated with other drugs or through a nanomedicine platform. The concentration of NO delivered is also something that has to be finely tuned, since concentrations that are too low will actually promote the growth of cancer cells by increasing the rate of glycolysis [7].

In addition, despite many NO donors, such as organic nitrates, diasenium diolates, S-nitrosothiols, metal-NO complexes, and furoxans showing anti-cancer effects on certain types of cancer cells, it is difficult to apply treatment with NO donors because NO donors can have serious negative, toxic side effects if they are not accurately, site-specifically delivered and activated.

Conclusion and Future Perspective

Nitric oxide is a gaseous free radical molecule that is produced in living cells by nitric oxide synthesis, which are produced in large amounts within cells that have become cancerous. NO can promote tumor growth in cancer, as angiogenesis, stimulated by NO, is essential for tumor growth. However, high concentrations of NO are extremely toxic to cancer cells and so tumor growth can also be repressed. Due to its toxicity to cancer cells, NO has been studied for its potential usage in the medicinal science for cancer treatment. It has been found that site-directed delivery of NO deactivates the protein efflux pumps causing multidrug resistance in cancer. NO is also effective in radio sensitizing the tumor cells by increasing the cell oxygen level, thereby increasing the effectiveness of radiotherapy. Furthermore, Nanoparticle-based delivery systems of NO have been developed to increase the cancer-fighting capabilities of NO by increasing the accuracy of delivery, mitigating negative side effects of NO, and increasing tumor penetration.

NO has already been shown to overcome many of the problems faced by other cancer-fighting agents and treatment methods, such as MDR and hypoxia of cancer cells. Effective site-delivery and activation of NO is the focus of many future researches and, utilizing the "prodrug" strategy and nanoparticle-based drug delivery are promising approaches for NO delivery in cancer treatment. The use of nanoparticle NO delivery systems might be an effective platform for site-directed delivery to increase delivery accuracy, tumor penetration, efficiency against MDR, and cancer killing efficiency. In future studies, nanoparticle-based NO delivery platforms will likely have a significant role in improving the effectiveness of radiation therapy for the treatment and management of many types of cancer.

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