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# Nitric oxide and reactive oxygen species: Clues to target oxidative damage repair defective breast cancers

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ABSTRACT

The identification of various biomolecules in cancer progression and therapy has led to the exploration of the roles of two cardinal players, namely Nitric Oxide (NO) and Reactive Oxygen Species (ROS) in cancer. Both ROS and NO display bimodal fashions of functional activity in a concentration dependent manner, by inducing either pro- or anti- tumorigenic signals. Researchers have identified the potential capability of NO and ROS in therapies owing to their role in eliciting pro-apoptotic signals at higher concentrations and their ability to sensitize cancer cells to one another as well as to other therapeutics. We review the prospects of NO and ROS in cancer progression and therapy, and analyze the role of a combinatorial therapy wherein an NO donor (SNAP) is used to sensitize the oxidative damage repair defective, triple negative breast cancer cells (HCC 1937) to a potent ROS inducer. Preliminary findings support the potential to employ various combinatorial regimes for anti-cancer therapies with regard to exploiting the chemo-sensitization property of NO donors.



## 1. Introduction

Discovery of the miracle molecule Nitric oxide (NO) and its identification as an endothelium derived relaxing factor, has been tremendously revolutionizing bio-molecular research (Ignarro et al., 1987; Palmer et al., 1987). This short-lived gas which is

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endogenously produced in the cells is highly unstable with an average half-life of just 1–5 s (Gladwin et al., 2003). NO acts *via* cGMP dependent and independent pathways in creating a concentration dependent effect and inducing either its pro-tumorigenic or its anti-tumorigenic role (Mocellin et al., 2007). In mammalian cells, NO is synthesized by the enzyme nitric oxide synthase (NOS), which has three isoforms namely NOS1/nNOS (neuronal NOS), NOS2/iNOS (inducible NOS) and NOS3/eNOS (endothelial NOS), all of which play varied roles in cancers. nNOS and eNOS synthesize NO in nanomolar concentrations for a short span of time while iNOS generates micromolar concentrations of NO lasting for hours or days (Michel and Feron, 1997). NO brings about concentration dependent triggering of either the pro-apoptotic or anti-apoptotic pathways which determine the cellular status of proliferation or death; hence undoubtedly this miracle molecule is also a double edged sword as reported earlier (Mocellin et al., 2007).

Reactive oxygen species (ROS) in-turn defines the group of oxygen containing chemical species including the super oxide anion ($O_2^-$) and hydroxyl free radicals ($HO^\cdot$) and hydrogen peroxide ($H_2O_2$). Within the cells, mitochondria, endoplasmic reticulum and peroxisomes are the major sites of generation of ROS especially the superoxide anion and hydrogen peroxide. Sources of ROS can be either non-enzymatic which includes the mitochondrial electron transport chain or enzymatic including the reactions catalyzed by any of the following enzymes: NADPH oxidase (NOX), xanthine oxidase, uncoupled eNOS, lipoxygenase, cyclooxygenase (COX) etc. (Gorrini et al., 2013b). Superoxide anion is converted to hydrogen peroxide by the enzyme super oxide dismutase (SOD), which in turn produces hydroxyl radicals *via* Fenton's reaction (Halliwell et al., 1993). Pathologic conditions due to ROS arise when there is a disruption in the equilibrium between the ROS production and degradation, either by the abrupt increase in ROS levels due to metabolic defects or as a result of decrease in the levels of ROS scavengers. ROS was initially considered to be just a metabolic by-product, but now it has become evident that it acts as a signaling molecule and also plays significant roles in human diseases especially cancers. Similar to NO, ROS also exhibits concentration dependent triggering of either the pro- or the anti-tumorigenic pathways within cells.

Under situations of surplus ROS within the cells, NO can react with oxygen or superoxide to form Reactive nitrogen oxide species (RNOS), which in turn can induce nitrosative or oxidative stress through nitrosamines or S-nitrosothiols. The scientific understanding of the role of these nitrogen oxides in cancer progression or therapy is continually expanding. Additionally, ROS and NO could work independently albeit complementarily to ensure cancer cell death. Interestingly, a number of studies in the 1990s have found that NO, at high concentration, could hinder DNA repair through multiple mechanisms involving direct chemical alterations of DNA by RNOS or indirect inhibition of DNA repair enzymes such as Fpg protein and $O^6$-methylguanine-DNA-methyltransferase (Graziewicz et al., 1996; Laval and Wink, 1994; Laval et al., 1997; Wink and Laval, 1994). BRCA1 is a DNA repair protein that is reported to be mutated in majority of Hereditary Breast–Ovarian Cancers (HBOCs). In ROS signaling, BRCA1 is an important intermediate which acts downstream of the induction step and upstream of Nrf2 to protect cells against oxidative damage and apoptosis. Hence, BRCA1 plays a critical role in protection from oxidative damage *via* induction of anti-oxidant signaling (Gorrini et al., 2013b). More recently a few studies have mentioned conflicting roles for NO in contextually activating pathways involving BRCA1 or down regulating BRCA1 expression (Singh et al., 2013; Van de Wouwer et al., 2012; Yakovlev, 2013). Here, we review the prospects of NO and ROS inducers in cancer therapy with respect to the role of NO donors in sensitizing cells to ROS inducers. The link between ROS and NO induction in BRCA defective condition is not well understood, but the two could potentially act in synergy to control cell growth in such conditions. Given the unique role of BRCA1 in ROS signaling, we discuss the potential role of NO to act as a sensitizing agent to therapeutic ROS inducers in BRCA1 defective breast cancers. We also share our views on ROS in the context of cancer stem cells.

## 2. Hallmark effects of NO in cancer

Biological functions in the human body could be modulated by NO in a concentration dependent manner. At low concentrations, NO plays various inductive as well as inhibitive roles in signal transduction (Bult et al., 1990; Garthwaite et al., 1989) resulting in regulation of the cardiovascular and circulatory systems (Hirst and Robson, 2011), control of the nervous and respiratory systems (Garthwaite et al., 1989) and immune regulation (Shi et al., 2000), while being toxic to both pathogenic micro-organisms and the tumor cells at higher concentrations (Burke et al., 2013; Ignarro, 2000).

Apart from diatomic NO, the biological effects are also mediated through numerous intermediates like nitrite, nitrate, S-nitrosothiols, nitrosamines, nitrous oxide and S-nitroso glutathione, to name a few. All of these are dependent on factors such as concentration of NO, duration of the exposure, NO flux, cellular status of chemical redox potential, the stages of cell cycle, the microenvironment and the type of cells (normal versus tumor) (Burke et al., 2013; Ridnour et al., 2006; Villalobo, 2007). NO, mostly at low concentration, is associated with numerous pathways involved in cancer progression and metastasis either *via* soluble guanylyl cyclase (contributing to increased angiogenesis), activation of HIF-1 α (which controls MMP-2, uPA and VEGF), activation of ERK 1/2 (leading to phosphorylation of c-Myc and Elk-1), PI3K-AKT activation or EGFR signaling (induce CD44 and c-Myc), S-nitrosylation of Src and Ras (leading to Raf-MEK-ERK 1/2 pathway) and β-catenin activation (*via* Wnt, EGFR, Src and PI3K/AKT pathways) (Benita et al., 2009; Glynn et al., 2010; Mujoo et al., 2010; Switzer et al., 2011; Tam et al., 2007; Thomas et al., 2008; Wink et al., 2011). In both ER positive and negative breast cancers, elevated levels of NO induces AKT phosphorylation leading to the suppression of Bad and Caspase-9, thus mediating tumorigenesis (Glynn et al., 2010; Lim et al., 2008; Tam et al., 2007). Reaction between ROS and NO can promote the formation of S-nitroguanine in inflammation associated cancers (Burke et al., 2013; Kundu and Surh, 2012). Thus, NO could be tumorigenic or anti-tumorigenic in a concentration dependent fashion also depending upon the various factors mentioned above. In this review, we lay emphasis on the effects of high levels of NO/ROS in targeting breast cancer cells including the cancer stem cells (CSCs).

## 3. ROS and cancer

As with NO, the cellular effects of ROS are also concentration dependent. Low to moderate levels of ROS induce proliferation, differentiation and migration of cells while high concentrations of ROS damage the cellular macromolecules (Cairns et al., 2011; Gorrini et al., 2013b; Janssen-Heininger et al., 2008; Perry et al., 2000; Sena and Chandel, 2012; Trachootham et al., 2009). Malignant cells under varied contexts show elevated ROS levels as well as ROS scavenging enzymes as compared to the normal cells, indicating a strong oxidative stress within them, both in culture and *in vivo* (Kumar et al., 2008; Martinez-Sanchez and Giuliani, 2007; Patel et al., 2007; Trachootham et al., 2009; Tsao et al., 2007). Expression of various oncogenes is found to be associated with elevated ROS levels both from increased ROS production and decreased antioxidant enzymes (Brandon et al., 2006; Halliwell, 2007; Horn and