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Targeting nitric oxide and NMDA receptor-associated pathways in treatment of high grade glial tumors. Hypotheses for nitro-memantine and nitrones

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ABSTRACT

Glioblastoma multiforme (GBM) is a devastating brain cancer with no curative treatment. Targeting Nitric Oxide (NO) and glutamatergic pathways may help as adjunctive treatments in GBM. NO at low doses promotes tumorigenesis, while at higher levels (above 300 nM) triggers apoptosis. Gliomas actively secrete high amounts of glutamate which activates EGR signaling and mediates degradation of peritumoral tissues via excitotoxic injury. Memantine inhibits NMDA-subtype of glutamate receptors (NMDARs) and induces autophagic death of glioma cells *in vitro* and blocks glioma growth *in vivo*. Nitro-memantines may exert further benefits by limiting NMDAR signaling and by delivery of NO to the areas of excessive NMDAR activity leading NO-accumulation at tumoricidal levels within gliomas. Due to the duality of NO in tumorigenesis, agents which attenuate NO levels may also act beneficial in treatment of GBM. Nitrone compounds including *N-tert*-Butyl-α-phenylnitrone (PBN) and its disulfonyl-phenyl derivative, OKN-007 suppress free radical formation in experimental cerebral ischemia. OKN-007 failed to show clinical efficacy in stroke, but trials demonstrated its high biosafety in humans including elderly subjects. PBN inhibits the signaling pathways of NF-κB, inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX). In animal models of liver cancer and glioblastoma, OKN-007 seemed more efficient than PBN in suppression of cell proliferation, microvascular density and in induction of apoptosis. OKN-007 also inhibits SULF2 enzyme, which promotes tumor growth via versatile pathways. We assume that nitromemantines may be more beneficial concomitant with chemo-radiotherapy while nitrones alone may act useful in suppressing basal tumor growth and angiogenesis.

1. Introduction

Glioblastoma (GBM) has the highest incidence among primary brain tumors, second only to meningioma and exerts a very poor prognosis [1,2]. In high-grade glial tumors, maximum surgical resection, radiotherapy, and adjuvant temozolomide is the new standard of care. However, even if these standards are employed at the optimal conditions, the median survival of patients with GBM is around 15 months with a 5-year survival rate of < 4% from the time of diagnosis [1,2]. Hence, discovery of novel innovative approaches is an urgent need in treatment of these devastating tumors. Increasing recent evidence suggest that nitric oxide (NO), NO-synthase (NOS) enzymes, glutamate and its receptor (NMDA) play important roles in pathogenesis and treatment responses of glioma cells, which will be outlined below. As will be explained, we propose that drugs which block NO at basal conditions (such as nitrones) may block aggressive behaviour of high grade glial tumors by reducing growth, invasion and angiogenesis. As NO exerts dual and opposite functions on glioma metabolism, NO donors, such as nitromemantines may potentiate chemo-sensitivity selectively within glial tumor cells, as they may specifically accumulate in areas of NMDA-receptor (NMDAR) overreactivity, which is the case in high grade glial tumors. At the first glance, it may seem paradoxical and even discursive to propose that both NO synthesis inhibitors and NO donors can be employed in the treatment of glioblastoma. However, substantial data provides evidence that constitutive low doses of NO propagates tumor growth, while higher doses (suggested to be above 300–500 nM in various resources) of NO acted tumoricidal. Hence, NO inhibitors may block basal growth of glioblastoma while NO-donors may potentiate chemo-radiotherapy efficacy of glioblastoma via enhancing cell apoptosis and secondary necrosis. In sections below, we will provide clues for our hypotheses after explaining general biochemistry of NO and NO-derived molecules and their involvement in tumor metabolism.

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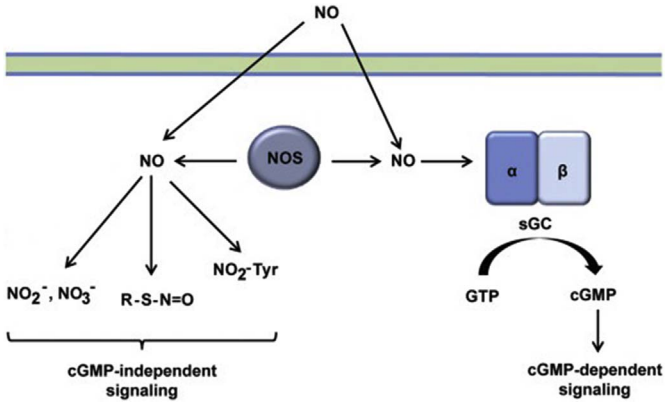

Fig. 1. cGMP dependent and independent pathways triggered by NO. Note that differing NO products may trigger cGMP-independent cascades. (Adapted from El-Sehemy et al. Nitric oxide signaling in human ovarian cancer: A potential therapeutic target. Nitric Oxide. 2016; 54:30–7. http://dx.doi.org/10.1016/j.niox.2016.02.002).

2. Nitric oxide, *S*-nitrosylation and cancer

Nitric Oxide (NO) is a relatively stable free radical that neither dimerizes nor easily reacts with reducing or oxidizing biological substances. Rather, NO is selective, which weakly reacts with diamagnetic substances like the majority of organic molecules but strongly reacts with paramagnetic substances such as transition metals (iron and copper), $O_2\cdot^-$, O_2, and nitrogen oxides [3]. NO regulates versatile physiological pathways via activating two major signaling cascades: The activation of the guanylyl cyclase to form cGMP and *S*-nitrosylation, a covalent attachment of an NO moiety to a reactive cysteine residue in peptides and proteins [4] (Fig. 1). Three NO synthases (NOS) synthesize NO from the amino acid L-arginine and the NOS1, NOS2, and NOS3 genes encode, respectively, neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) [5]. The constitutively expressed eNOS and nNOS generally release low levels of NO that are associated with cytoprotection and cell proliferation [4]. Inflammatory cytokines induce iNOS in macrophages and production of NO under these conditions is much higher compared to its synthesis by constitutive enzymes [6]. Higher NO fluxes such as those produced by iNOS stimulated by inflammatory cytokines in macrophages or generated by millimolar concentrations of NO donors are cytotoxic and stimulate apoptosis [6].

NO-mediated nonclassical signaling includes modifications in tyrosine and cysteine residues of proteins [7]. Nitration of protein tyrosine residues happens by reaction with peroxynitrite ($ONOO^-$), a powerful oxidant produced by the reaction of NO with $O_2\cdot^-$ and by NO_2 generated by neutrophil myeloperoxidase [8,9]. Both oxidants nitrate tyrosine at position 3 of the phenolic ring, producing 3-nitrotyrosine. Tyrosine nitration may modify tyrosine phosphorylation/dephosphorylation signaling cascades directly or indirectly [4]. *S*-glutathionylation is the incorporation of a GSH thiol group into a protein and the formation of a mixed disulfide bridge between a cysteine residue and the GSH thiol (Fig. 2). It can be induced by $ONOO^-$ or formed after the reaction between a thiol and a nitrosothiol, but *S*-glutathionylation can also occur without NO [7].

Lipophilic compartments like the cell membrane will concentrate NO and O_2 and facilitate the reactions between them to trigger the production of N_2O_3 [10]. In aqueous systems, N_2O_3 in the presence of a nucleophile such as thiolate or an amine group will form a hybrid of covalent and ionic resonance structures NO^+–NO_2^-. Nitrosation reactions occur between NO^+ and the thiolate or between NO^+ and the amine form RSNOs (*S*-nitrosothiols) and nitrosamines, respectively [10]. Although RSNOs are widely considered as NO donors, they can also act through *trans*-nitrosylation, without release of NO [11]. *Trans*-nitrosylation is the exchange between a thiol group and a nitrosothiol group without release of NO [4]. Nitrosylating species of biological relevance, such as *S*-nitroso-glutathione (GSNO) and *S*-nitrosocysteine, can nitrosylate/*trans*-nitrosylate signaling proteins [12].

Protein *S*-nitrosylation (*S*-nitrosation) is the addition of a nitroso group moiety to a thiol group of a cysteine residue in peptides or proteins [4]. Protein *S*-nitrosylation is considered a mechanism for signal transduction by NO and RSNOs [6] without requirement of enzymatic activities [4]. The nitrosylating agent's efficiency and the intracellular redox environment regulated by the redox couples [oxidized glutathione/reduced glutathione (GSSG/2GSH), oxidized thioredoxin/reduced thioredoxin (TrxSS/Trx)] determine *S*-nitrosylation [4,13] (Fig. 2). Specificity of *S*-nitrosylation can be associated with the accumulation of high levels of nitrosylating species in the vicinity of specific cysteine residues in subcellular compartments [14]. At least 1000 proteins modified by *S*-nitrosylation have been identified in mammalian cells [15].

The constitutive NOS isoforms have been detected in various cancers. Activation of eNOS contributes to the initiation and progression of tumor growth in pancreatic cancer cell lines [16]. Tumor progression in prostate cancer is associated with eNOS expression [17], while melanoma progression is associated with nNOS expression [18]. The iNOS isoform is ubiquitously distributed in malignant tumors [19]. The decomposition of specific chemical compounds generally termed as NO donors serves as exogenous sources for the generation of NO. Nitroglycerin (glyceryl trinitrate) is the oldest and the most well-known NO donor [4]. Today, at least 16 different classes of nitrogen–oxygen–bonded compounds were developed which decompose or to be reduced/oxidized with the production of NO and other reactive nitrogen species. Clinical studies with glyceryl trinitrate to treat prostate cancer [20] and preclinical trials with NO-donating nonsteroidal anti-inflammatory drugs to treat colon cancer [21] indicate that NO donors potentially could act as antineoplastic.

It is generally assumed that low doses of NO stimulates cancer progression, while high amounts of NO helps in killing cancer cells [4,22,23] (Fig. 3). Cancer development involves *S*-nitrosylation of specific enzymes and proteins, for instance: a) *S*-nitrosylation of specific caspases which trigger apoptosis block apoptosis of cancer cells [24]; b) *S*-nitrosylation of Bcl-2 hinders its proteosomal loss allowing it to

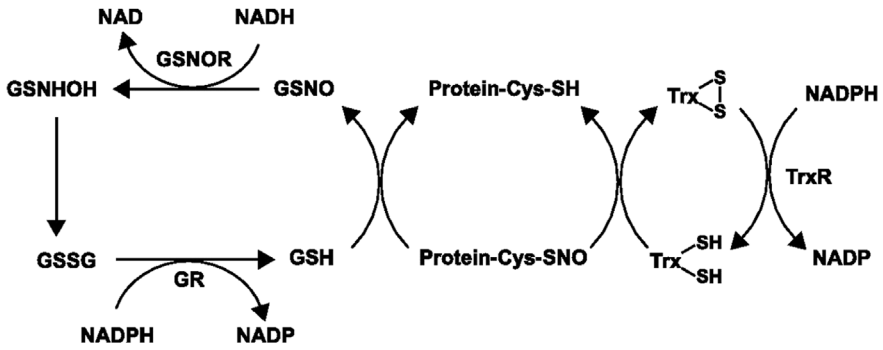

Fig. 2. Biochemical interactions of NO with glutathione (GSH) and thioredoxins (Trx). (Adapted from Lima et al. *S*-nitrosylation in cardiovascular signaling. Circ Res. 2010; 106(4):633–46. http://dx.doi.org/10.1161/CIRCRESAHA.109.207381).