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REVIEWS

Review

Nitric Oxide: The Forgotten Child of Tumor Metabolism

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Nitric oxide (NO) is a signaling molecule with pleiotropic physiological roles in normal cells and pathophysiological roles in cancer. NO synthetase expression and NO synthesis are linked to altered metabolism, neoplasticity, invasiveness, chemoresistance, immune evasion, and ultimately to poor prognosis of cancer patients. Exogenous NO in the microenvironment facilitates paracrine signaling, mediates immune responses, and triggers angiogenesis. NO regulates posttranslational protein modifications, *S*-nitrosation, and genome-wide epigenetic modifications that can have both tumor-promoting and tumor-suppressing effects. We review mechanisms that link NO to cancer hallmarks, with a perspective of co-targeting NO metabolism with first-line therapies for improved outcome. We highlight the need for quantitative flux analysis to study NO in tumors.

Trends

NO is a key messenger in the TME with pro- and antitumorigenic roles.

NO is emerging as a key regulator of cancer metabolism via *S*-nitrosation of enzymes.

NO has a wide range of control over gene expression in tumors via NO-mediated epigenetic modifications

Quantified approach to studying effects of NO synthesis in cancers should guide the design of therapies targeting NO – from *in vitro* experiments to clinical trials.

Dissecting the Links between Cancer Hallmarks and Nitric Oxide

The uncontrolled proliferation of cancer cells requires a significant shift in metabolism. To support a higher growth rate, cancer cells redirect nutrients into anabolic pathways to maintain biomass production [90]. However, to survive in a harsh **tumor microenvironment (TME)** (see Glossary), tumors need to strike a balance between anabolic demands and catabolic energy production. Rewired **energy metabolism** is, therefore, a ubiquitous hallmark of cancer [91]. Rapid formation of solid tumors is accompanied by poor vasculature leading to limited supply of nutrients and oxygen, which further contributes to the altered metabolism in tumors [1]. Therefore, there is a complex interplay between (i) cell-autonomous metabolic alterations, (ii) intercellular metabolic crosstalk, and (iii) extracellular stimuli. Nitric oxide (NO) is a metabolic product of the nitric oxide synthase (NOS) reaction that catalyzes the conversion of arginine into citrulline. NOS enzyme exists in three isoforms: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3) , all of which are functional in different contexts (Box 1). Historically, researchers have focused on the functions of NO as a signaling molecule, but have overlooked the ubiquitous interplay between NO synthesis and tumor metabolism, and the role NO plays in the TME.

Metabolic traits that make cancer cells distinct from healthy tissues present opportunities for therapy [2,92]. However, cancer cells share many metabolic features with healthy proliferating cells, making the search for metabolic targets that selectively attack cancer cells challenging. Moreover, due to redundancies in metabolic pathways, cancer cells can activate compensatory pathways that perform similar functions as the metabolic pathways being targeted by drugs. This enables cancer cells to acquire resistance to metabolic drugs. Despite advancements in developing small-molecule metabolic drugs, their efficacy as anticancer drugs has been underwhelming in the clinic. NO plays strategic roles in signaling and metabolic pathways, making NO metabolism a hub that control pathways responsible for supporting tumorigenesis or suppress tumor growth altogether (Figure 1, Key Figure). NO metabolism, thus, presents a

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Box 1. Biochemistry of Nitric Oxide and Nitric Oxide Synthetase

Nitric oxide (NO) is a lipophilic, highly diffusible, and short-lived molecule. These characteristics make it an ideal physiological messenger capable of regulating intercellular and extracellular signaling pathways in local niches. NO can be endogenously synthesized by the enzyme nitric oxide synthase (NOS) from the guanido nitrogen of L-arginine. It is known to regulate a variety of important cellular functions such as vasodilation, respiration, neurotransmission, cell migration, immune response, apoptosis, and metabolism [77]. The wide range of biological actions implicates its potential role in pathophysiological actions, especially in cancer.

The enzyme responsible for its synthesis exists in three isoforms enzymes that are different in structure and function: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3) [78]. NOS1 and NOS3 are constitutive isoforms that are modulated by calcium-calmodium concentrations. These isoforms have a lower capacity of producing NO than the inducible isoform, NOS2 [79]. NOS2 activity is independent of calcium concentrations but can be stimulated by cytokines in all cell types (Figure 1). NOS2 induction requires two signals, one from interferon gamma (IFNγ) and another trigger such as the endotoxin, tumor necrosis factor alpha (TNFα). The activation of NOS2 by TNFα occurs via stimulation of the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) which binds to a κB element in the NOS promoter [80]. In tumors with chronic hypoxia due to the lack of proper vasculature, hypoxia inducible factor-1α (HIF-1α) interacts with IFNγ and induces NOS2 expression. All isoforms convert L-arginine to citrulline and NO. This two-step reaction catalyzed by NOS is dependent on oxygen and nicotinamide-adenine-dinucleotide phosphate (NADPH). Therefore, this reaction directly affects arginine utilization and redox homeostasis in cells. In the context of tumor formation, major advances in the investigation of NO biology have been witnessed. NO acts like a double-edged sword, where its level of expression and duration of NO exposure determine the often-contradictory cellular outcomes. Higher concentrations (more than 200 nM) induce apoptosis, while low levels (less than 200 nM) have been associated with tumor progression [76] (Figure 1).

viable therapeutic target as it has a wide range of control over tumorigenic functions. Treatments targeting key regulators, such as NO, can be more effective when combined with conventional therapeutic strategies to achieve synergistic effects. Combination therapies target multiple pathways that contribute to complementary aspects of tumor pathology. Such an approach reduces the probability of tumors acquiring drug resistance by activating compensatory pathways [3]. This review enumerates the mechanisms of tumor progression that are initiated and supported by NO metabolism, which may be targetable using small-molecule drugs. More importantly, we present a novel perspective of **co-targeting** NO metabolism with conventional targets to achieve a synthetic lethal effect. Additionally, we highlight the importance of metabolic **flux analysis** (Box 2) of endogenous NO synthesis in providing a quantitative approach to help design therapies that effectively target NO metabolism and maximize the **therapeutic window**.

NO: Modulator of the TME

Cancers develop within a complex TME that provides support for sustained growth, invasion, and metastasis. Nonmalignant cells in TME often have tumor-promoting functions. NO secreted by cancer cells (Figure 2) reprograms stromal cells to support tumor progression. For example, cancer cell-derived NO induces chronic inflammation in the TME of melanoma to promote drug resistance [4]; and elevated levels of NO in the microenvironment contribute to increased migration of breast cancer via upregulation of caveolin-1 (Cav-1) expression [5]. Similarly, cell-autonomous induction of NOS in stromal cells also contributes to tumor progression. For example, in **cancer-associated fibroblasts (CAFs)** expressing chemokine ligand (CXCL14), NOS1 expression is essential for CAF-supported growth of breast and prostate cancer cells. Furthermore, suppressing NOS1 expression disrupts pro-tumorigenic functions of CXCL14-expressing CAFs and reduces tumor growth in mice. Since NOS1 expression did not increase extracellular NO, NOS1-derived NO supported CXCL14 activity within CAFs [6] (Figure 2). Increase in exogenous NO in the TME is also associated with tumorigenic functions in colon cancer patients. Colon cancer patients with high NOS2 expression have increased incidences of lymph node metastasis; and elevated NOS2 expression observed in the upper colon in colitis patients indicates higher risk of developing colon cancer [7,8]. In addition to regulating metastasis and tumor initiation, NO is also a key regulator of

Glossary

Angiogenesis: the process of formation of new blood vessels that branch out of existing blood vessels. This process is essential for wound healing in normal tissue, however, it is also essential for malignant tumor growth.
Cancer-associated fibroblasts (CAFs): a subpopulation of cells within the tumor that are transformed fibroblasts, but share properties of myofibroblasts that are found during the process of wound healing.
Co-targeting: a novel concept where genes or metabolic enzymes that are involved in a strong interplay to promote disease progression are both targeted to achieve a synergistic therapeutic effect.
Energy metabolism: it is the subset of cellular metabolic function responsible for the generation of energy currency molecule, ATP, by breaking down nutrients.
Epigenetic modifications: environmental factors can lead to modifications in the DNA strands or histones around which DNA strands are wrapped, which can regulate transcription. These are in the form of reversible attachment of methyl and/or acetyl groups on segments of the DNA or on histone tails.
Flux analysis: a computational technique that uses mass balance principles to estimate intracellular metabolic rates (or fluxes) from empirically measurable metabolic parameters. Fluxes are the closest representation of metabolic pathway activity.
***S*-nitrosation:** the process of protein post-translational modification, where NO is attached to the thiol group of a cysteine residue. It can cause repression or enhance protein activity depending on the protein and location of the active thiol group.
Therapeutic window: the range of drug dose that targets cancer cells effectively but avoids adversely affecting healthy cells to minimize side effects.
Toll-like receptors (TLRs): these protein receptors are characterized by their ability to respond to invading pathogens by recognizing conserved molecular structures. TLRs are primarily expressed by immune cells such as monocytes, macrophages, mast cells, and dendritic cells. Upregulated TLR expression has