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Nitric oxide in cancer metastasis

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Abstract

COX-2, cyclooxygenase; CXCR4, CXC chemokine receptor 4; ECM, extracellular matrix; ELAM-1, endothelial leukocyte adhesion molecule 1; eNOS, endothelial nitric oxide synthase; GC, guanylyl cyclase; HIF-1, hypoxia-inducible factor-1; HUVECs, human umbilical vascular endothelial cells; IL-33, cytokine interleukin-33; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; nNOS, neuronal nitric oxide synthase; NO•, nitric oxide; NO•-NSAIDs, nitric oxide-releasing non-steroidal anti-inflammatory drugs; NOS, nitric oxide synthase; PCNA, proliferating cell nuclear antigen; PKC, protein kinase C; QUER, quercetin; TPA, 12-O-tetradecanoylphorbol 13-acetate; t-PTER, trans-pterostilbene; VEGF-C, vascular endothelial growth factor-C

Keywords

iNOS; NSAIDs; tumor; signaling; hypoxia

1. Introduction

Cancer metastasis is the spread and growth of tumor cells through angiogenesis, invasion, colonization, and ultimately proliferation from the original neoplasm to other organs,[1] which can be extremely difficult to treat and therefore often lead to death (Fig. 1).[2, 3]

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Conflict of interest
None declared.

Nitric oxide ($NO^{\bullet}$) is a signaling molecule that plays various roles pathologically and physiologically.[4] In the last two decades, the function of $NO^{\bullet}$ in the regulation of cancer formation, progression and metastasis has been extensively investigated.[5, 6] Activation of nitric oxide synthase (NOS) and elevation of $NO^{\bullet}$ have exhibited an antitumor nature,[7–9] however, $NO^{\bullet}$ may also promote cancer formation and progression.[10–12] Therefore the effect of $NO^{\bullet}$ on metastasis cannot be easily classified as “pro-metastasis” or “anti-metastasis” as it may rely on other factors such as the cell type,[13] dosage,[14, 15] organs involved,[13] or even which step of metastasis $NO^{\bullet}$ influences. This review will summarize the current knowledge of the influence of $NO^{\bullet}$ in tumor progression and metastasis. The potential therapeutic applications of $NO^{\bullet}$ in cancer treatment will also be discussed.

$NO^{\bullet}$ was first discovered as a vasodilator in the cardiovascular system.[16] Recently, $NO^{\bullet}$ has been found to have a pleiotropic effect on platelet aggregation,[17] immune response, [18] and signaling pathways critical to tumor progression,[19] all of which affect tumor cell metastasis.[17, 20, 21] $NO^{\bullet}$ is synthesized from L-Arginine and oxygen by a family of enzymes termed nitric oxide synthases (NOS). The three isoforms of NOS include neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). Because nNOS and eNOS undergo constitutive expression, they have also been named constitutive NOS.[22] These isoforms are significant because NOS expression in tumors differ from case to case, exhibiting their heterogeneous characteristics on cancer metastasis.[23]

$NO^{\bullet}$ has a paradoxical role in certain malignancies and prognoses. A recent clinical study showed that patients with iNOS-positive tumors had a significantly lower disease-specific survival rate than those with iNOS-negative tumors in various stages of colorectal cancer, suggesting iNOS overexpression is related to increased disease-specific fatality.[24] Analysis of the relationship between angiogenesis and iNOS in primary gallbladder carcinomas has shown that the degree of malignancy is significantly affiliated with the expression level of iNOS.[25] In breast cancer, $NO^{\bullet}$ is shown to decrease aggressiveness of breast tumor cells by inhibiting cell motility and reinforcing cell adhesion, ultimately hindering the cell’s metastatic characteristics.[26] While $NO^{\bullet}$ has been clinically connected to a poor cancer prognosis, not all the effects of $NO^{\bullet}$ are clear and may be impacted by dose or organs involved.

Mice transfected with an iNOS-negative retrovirus led to the formation of multiple lung metastases and aggressive, subcutaneous tumors. However, cells infected with iNOS-positive retrovirus formed few lung metastases and slowly progressing tumors.[27] In addition, when the metastatic cells of murine M5076 were transfected with functional iNOS genes, the established hepatic lesions and tumorigenesis regressed.[8] In syngeneic C57BL/6 mice, lower levels of iNOS produced tumors in the pancreas that metastasized to the liver and formed ascites. However, higher levels of iNOS expression did not result in liver metastases or ascites.[28] These results suggest that $NO^{\bullet}$ may drastically impede or even eliminate metastatic progression. However, the effect of $NO^{\bullet}$ on tumor metastasis appears to be organ-specific. A study exploring the effects of heme oxygenase and $NO^{\bullet}$ on pulmonary or liver metastasis of colon cancer in mice found that the mice receiving NG-nitro-L-arginine methyl ester, an inhibitor of eNOS, had an increased number of tumor cells 24

hours later. Those same mice had an increased number of pulmonary metastases 18 days later, but possessed a similar number of liver metastases as untreated mice.[13] Since eNOS showed anti-metastatic effects on pulmonary metastases but no effect on liver metastases, these results suggest that rather than NO• being correctly labeled “pro-metastasis” or “anti-metastasis,” the setting and organs involved have a great effect on the manifestation of NO•. [13]

During *in vitro* and *in vivo* conditions, NO• exercises its anti-tumor nature by inducing cytotoxicity and apoptosis, affectively influencing tumor metastasis.[7, 29–31] Galectin-3 is a carbohydrate-binding protein that is important for cell-cell and cell-matrix interactions and cancer metastasis.[32] NO• is involved in the mechanism by which galectin-3 enhances metastasis. In human breast carcinoma (BT549) cells, galectin-3 improves metastatic potential and protects tumor cells from death through the iNOS cytotoxicity pathway.[33, 34] Paclitaxel, an antineoplastic drug that introduces cytotoxicity against cancer cells, was shown to accomplish its effect through stimulation of NO• production in human liver cancer cells HepG2.[35] In addition, two natural and structurally similar polyphenols, trans-pterostilbene (t-PTER) and quercetin (QUER) were administered to mice and found to impede the metastasis of B16F10 melanoma cells by causing NO• to be released from the vascular endothelium, resulting in the cytotoxicity and death of B16F10 cells (Fig. 2).[36]

In addition to anti-cancer effects through cytotoxicity, there is sufficient evidence suggesting anti-cancer effects of NO• through apoptosis.[14, 37] Many cancers show resistance to apoptosis by suppressing the genes that promote apoptosis. This resistance largely contributes to poor prognosis by affecting tissue homeostasis and causing failure of treatments.[38, 39] Through the use of a series of adenoviral vectors that expressed different levels of iNOS activity, Xie *et al.* reported that although NO• has some protumor activity such as mediated gene transfer and up-regulated angiogenetic molecules, the antitumor actions including loss of malignancy due to apoptosis outweigh the protumor factors and result in an overall deregulation of malignancy.[14] These dichotomous effects on cancer progression arise from NO• regulations on specific signaling pathways.[40] Successful cancer metastasis consists of several complex, consecutive, and very particular steps.[1] Numerous evidence suggests that NO• plays important roles in nearly all steps of cancer metastasis.[6]

2. Invasion

Invasion consists of alteration in tumor cell adhesion to the extracellular matrix (ECM), proteolytic degradation of encompassing tissue, and migration of tumor cells (Fig. 1).[1, 41–43] During invasion, the matrix metalloproteinase (MMP) family is responsible for the essential degradation of the ECM[44, 45] while integrins likely assist in locomotion, the forward migration synchronized by operations of actin cytoskeleton filaments.[46]

MMPs exist at a high level in malignant cells, but are expressed at basic levels in normal cells. NO• was found to modulate MMP expression and therefore affects tumor cell invasion.[47–50] The invasion-inhibiting effects of NO• on the 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced MMP-9 expression was examined in human breast cancer cell

line MCF-7. It was observed that a supplement of NO• donor leads to a decrease of MMP-9 mRNA level and reduction of MMP-9 translation. A 0.67 kb fragment from a 5′-promoter region of the MMP-9 gene is primarily responsible for the inhibition of MMP-9 by NO•.[50] Furthermore, the TPA-triggered protein kinase C (PKC) activity was significantly inhibited by NO• in MCF-7 cells, indicating that NO• attenuates TPA-induced MMP-9 expression mediated by PKC pathway[50] and therefore avoids invasion. Conversely, an examination of human melanoma cell line C32TG found NO• enhanced MMP-1, -3, -10 and -13 expression transcriptionally via the mitogen-activated protein kinase (MAPK) (ERK/p38) pathway,[49] thereby assisting tumor metastasis (Fig. 2). This difference also suggests a potential cell-type dependent effect of NO• on MMP expression and invasion.

Integrins are a family of cell surface proteins that mediate cell-to-cell and cell-to-matrix attachment, control the adherence of tumor cells to and in the ECM, and likely aid in locomotion, thereby impacting tumor cell invasion.[46, 51–53] NO• specifically inhibits α2β1 integrin-mediated platelet adhesion to immobilized collagen.[52] Activation of integrin α9β1 enhances cell migration through the induction of iNOS expression triggering cGMP generation via guanylyl cyclase (GC).[46] NO• from human umbilical vascular endothelial cells (HUVECs) promote cell adhesion of human fibrosarcoma HT1080 to HUVEC monolayer by enhancing endothelial leukocyte adhesion molecule 1 (ELAM-1) expression on HUVECs. This further increased tumor cell invasion through the HUVEC monolayer (Fig. 2).[54] Therefore, in addition to evidence of pro- and anti-invasion effects, NO• reduces adhesion and promotes migration, and therefore invasion, through inhibition of integrin expression.[46, 51–53]

3. Angiogenesis

Angiogenesis is the growth of new blood vessels from the original vascular bed and is an essential step in tumor progression and metastasis.[55] An analysis using samples from normal liver tissue and hepatocellular carcinoma patients demonstrates that iNOS positively modulate MMP-9 expression, facilitating tumor cell angiogenesis, invasion, and metastasis.[56] Compared to normal tissue, tumor specimens from head and neck cancer showed higher NOS activity, which is associated with a higher level of angiogenesis. When the production of NO• is obstructed, tumor angiogenesis is suppressed.[57, 58] Cytokine interleukin-33 (IL-33), a regulator of vasculature, was also shown to promote proliferation, migration, angiogenesis and vascular permeability by stimulating endothelial NO• generation through the ST2/TRAF6-Akt-eNOS signaling pathway.[59] In addition, the reduction of NO• formation achieved by knockdown eNOS using siRNA markedly decreased endothelial cells migration in shear stress; however, a supplement of external NO• donor led to a 2-fold recovery in angiogenesis (Fig. 2).[60]

4. Intravasation

Although less documented, evidence suggests that NO• is also involved in the process of intravasation, which occurs when tumor cells invade the blood vessels. Leukocytes are circulated throughout the body to defend against tumor cell invasion in blood vessels. Significant to intravasation, the rolling and adhesion, and therefore effectiveness, of

leukocytes on the endothelium in tumor vessels is largely reduced in an NO• and ICAM-1 dependent manner. In addition, as shown in Fig. 2, inhibition of NO• partially reversed the lack of adhesion of leukocytes in tumor microvessels, suggesting that NO• has pro-intravasative effects.[61]

5. Extravasation and Colonization

During extravasation and colonization, the tumor cells prompt endothelial retraction that leads to the adhesion of tumor cells to the subendothelial ECM and the reformation of the capillary (Fig. 1). When a tumor cell attaches to coagulation factors such as fibrin, thrombin, or fibrinogen, an embolus is created and, with the assistance of E- and P-selectins, it causes cell arrest in the capillary beds. NO• has been shown to decrease the expression of E-selectins through inactivation of NF-kappaB, thereby resulting in an anti-metastatic outcome.[62] However, a selective iNOS inhibitor (e.g., 1400W dihydrochloride) attenuated the mRNA transcription of E-selectins in pulmonary artery endothelial cells.[63]

6. Lymphatic metastasis

Lymphatic metastasis is a common and critical determinant of cancer pathogenesis.[64] The lymph nodes are the primary metastasis location for most malignancies and they are a critical determinate in the prognosis of the patient. Two important factors in promoting lymphatic metastasis are vascular endothelial growth factor-C (VEGF-C), by which lymphangiogenesis is stimulated,[65] and the CXC chemokine receptor 4 (CXCR4), which is shown to be a contributor to lymph node metastasis.[66] With the treatment of the NO• donor DETA NONOate, the production of VEGF-C increases. Furthermore, when treated with an NO•-synthesis impeder N-nitro-L-arginine, additional VEGF-C production is halted.[67] In addition to VEGF-C, NO• also stimulates cytoplasmic CXCR4 expression and therefore may also be characterized as pro-metastatic due to its possible aiding of lymphatic metastasis in human breast carcinoma cells.[66]

7. Hypoxia evasion

Hypoxia occurs when the entire or part of the body is deprived of oxygen.[68] When this occurs in a tumor, it may lead to further tumor metastasis due to therapy resistance and accelerated invasion, and is not characterized with a promising prognosis for patients with many types of cancer.[69, 70]

NO• evokes hypoxia-inducible factor-1 (HIF-1), a transcription factor that enhances many hypoxia-inducible genes, via PI3k/Akt pathway.[71, 72] As mentioned previously, an open-label 24-month clinical trial shows that NO• annihilates prostate cancer metastasis by weakening hypoxia-induced progression.[73] In B16F10 cells, the presence of low-concentration NO•-mimetics caused an inhibition in hypoxia-induced lung nodule formation. When NOS was repressed, it caused lung nodule formation in a hypoxia-similar fashion. This regulatory effect of NO• involves the cGMP-dependent pathway.[70]

The effect of NO• also relies on the presence of additional substances. Together, expression of iNOS and cyclooxygenase (COX-2) has been found to be responsible for enhanced

vascular invasion, lymphatic metastasis and angiogenesis in various cancers. Although we cannot define both iNOS and COX-2 as exclusive pro- or anti-metastatic in gastric adenocarcinoma patients, the combined production of NO• and prostaglandins via iNOS and COX-2 expression can be associated with cancer progression, tumor angiogenesis, and a graver prognosis.[74] Besides COX-2, the Src kinase family, a key regulator of cell invasiveness, is shown to phosphorylate iNOS Tyr (1055) and stabilize iNOS half-life, thereby producing a constantly large amount of NO• to regulate tumor metastasis.[75]

8. Nitric oxide therapy

It is clear that NO• plays a significant role in tumor progression and metastasis. The bimodal effects of NO• have been established through studies focusing on variables affecting NO• impact. These factors include duration of NO• exposure, cellular microenvironment, NO• flux, tumor cell cycle stage, as well as prevalence of oxidizing and reducing agents.[76] Since the influence of NO• is also dependent on the concentration, angiogenesis, proliferation and metastasis can normally be stimulated by lower levels of NO• (<100nM). However, higher concentrations of NO• (>400–500 nM) do not facilitate tumor progression but instead promote cytotoxicity and cell apoptosis.[40, 76] Accordingly, the inhibition of cell survival and anti-apoptosis pathways by NO• donors has been proposed as a novel therapy to various cancers.[4, 77] Most of the research has been in the area of nitric oxide-releasing non-steroidal anti-inflammatory drugs (NO•-NSAIDs), which has been particularly promising because in many cases, NO• appears to exhibit both combinatory and enhancing effects on pre-existing anticancer effects of the parent NSAIDs. Several studies have found a link between NSAIDs use and decreased risk of colorectal cancer[78–80], suggesting a possible chemopreventive role. NO•-NSAIDs have been shown to be more effective in the inhibition of cancer cell growth and metastasis than the parent drug alone.[77, 81]

Evidence suggests NO•-NSAIDs act on cancer cells in several ways. First, they inhibit cell proliferation by decreasing expression of proliferating cell nuclear antigen (PCNA).[82] PCNA is a protein that acts as a processivity factor for DNA polymerase delta in eukaryotic cells. It plays an important role in DNA synthesis, and as such, decreased PCNA expression leads to decreased DNA synthesis and reduced cell proliferation. However, this decrease in PCNA expression has not been shown to correspond linearly to the decrease in cell proliferation, so it appears that other factors are involved.[82] NO•-aspirin exhibits greater pro-apoptotic effects than aspirin itself. Interestingly, NO•-aspirin also induced a large population of “atypical” cells.[82, 83] These “atypical” cells exhibit diminished or no DNA while retaining their basic cellular structure. NO•-NSAIDs also appear to block cell cycle transitions from G_0-G_1 to S, further inhibiting growth (Fig. 3).[82]

Accordingly, NO•-NSAIDs may likely augment cellular pathways favorable to apoptosis and diminish metastasis in certain cell types. For example, the anti-cancer effects of NO•-NSAIDs may not help reduce metastasis in hepatocytes, endothelial cells, B-cell lymphomas, and other cell lines, where NO• has been shown to obstruct pro-apoptotic mechanisms.[84–86] However, other tumor cells such as breast carcinomas have been shown to undergo apoptosis following prolonged exposure to elevated NO• concentrations

(1 mM).[40] In colon adenocarcinomas, NO•-NSAIDs repress tumor proliferation through epigenetic contributions such as DNA methylation and histone modifications.[76, 82]

Another study found that nitric oxide-donating pro-drugs significantly enhanced the efficacy of the chemotherapy drug fludarabine against chronic lymphocytic leukemia cells.[87] Three-dimensional analysis of the combination of fludarabine and DETA-NO• showed that at the optimum combination, cell death of 80–90% should be expected. As shown in Fig. 3, NO• also appears to preferentially sensitize cells that exhibit fludarabine resistance.[87]

9. Conclusion and perspective

In this review, we have identified the underlying biological factors influencing the pro- and anti-metastatic effects of NO•. We discussed the various ways NO• can regulate tumor metastasis, ultimately providing justification for utilization of NO•-NSAIDs against carcinomas due to their ability to hinder cell-to-cell attachment and invasion. Several important aspects of NO• must be considered when assessing the biological outcome of NO• on cancer, such as the duration of NO• exposure, tumor microenvironment that NO• encounters, as well as specific signaling pathways that NO• regulates including MMP, integrin, cGMP-dependent, and COX-2 pathways. The double-edged nature of NO• action facilitates utilization of NO•-NSAIDs against carcinomas complex. Multiple factors including NO• pathways, tumor redox microenvironments, and NO•-mediated signaling cascades, must be considered *in vitro* and *in vivo* for potential therapies. Thus, NO•-NSAIDs can be important drugs for anti-cancer treatment if the effect of NO•-NSAIDs is optimized. Although NO• can assist in cancer progression by inducing angiogenesis and cell invasion, we conclude that nitric oxide-donating prodrugs, such as NO•-NSAIDs, are a promising development for further research.

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Highlights

- This review summarizes the dichotomous effects of $NO^{\bullet}$ in tumor metastasis.
- Biological factors influence the pro- and anti-metastatic impact of $NO^{\bullet}$.
- $NO^{\bullet}$ induces cytotoxicity and apoptosis to hinder cancer cell growth.
- $NO^{\bullet}$ promotes intravasation and angiogenesis to enhance cancer cell growth.
- The bimodal nature of $NO^{\bullet}$ facilitates the anti-cancer potential of $NO^{\bullet}$-NSAIDs.

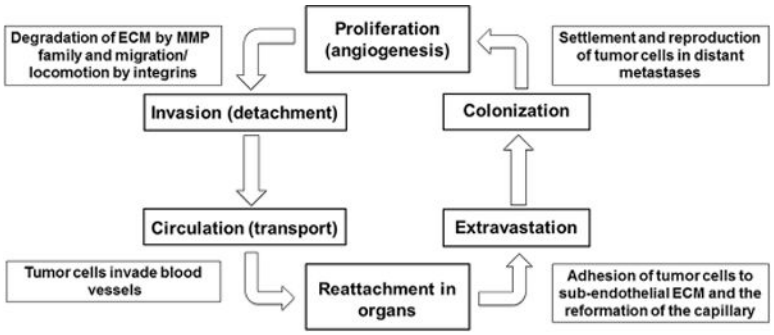

Fig. 1.

This schematic demonstrates the typical progression of tumor cell metastasis. Abbreviations: extracellular matrix (ECM), matrix metalloproteinase (MMP).

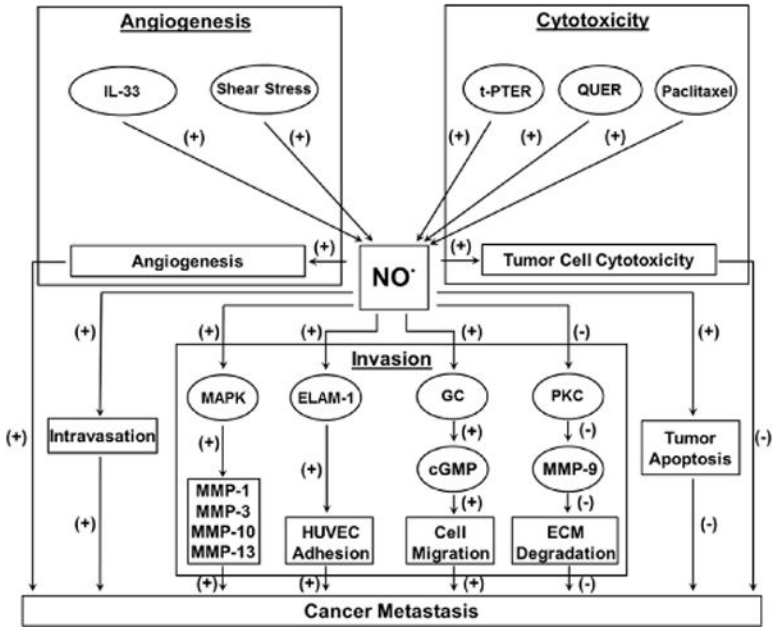

Fig. 2.

This schematic demonstrates the multitude of mechanisms through which nitric oxide increases or decreases the activation of and prevalence of cancer metastasis. Abbreviations: extracellular matrix (ECM), endothelial leukocyte adhesion molecule 1 (ELAM-1), guanylyl cyclase (GC), human umbilical vascular endothelial cells (HUVECs), Cytokine interleukin-33 (IL-33), mitogen-activated protein kinase (MAPK), matrix metalloproteinase (MMP), Nitric oxide ($NO^{\bullet}$), protein kinase C (PKC), quercetin (QUER), trans-pterostilbene (t-PTER).

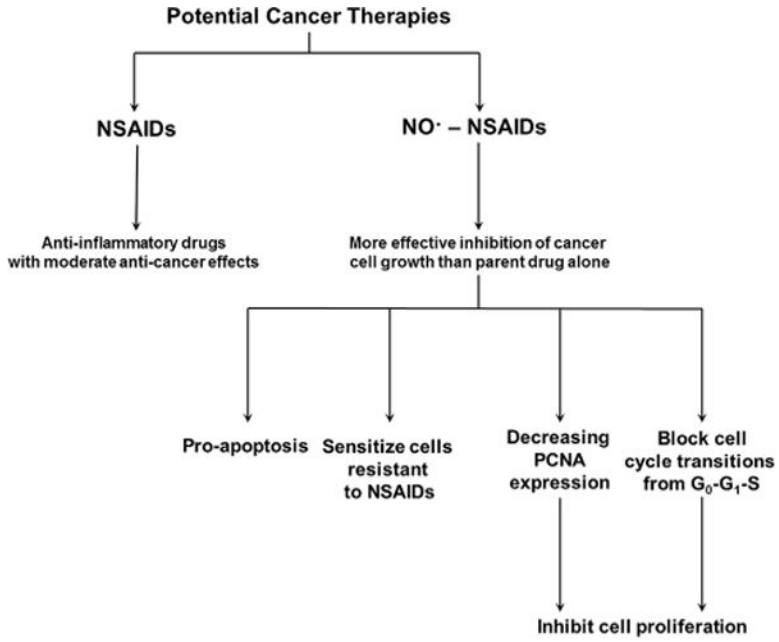

Fig. 3.

This flowchart highlights the increased effectiveness of NSAIDs contrasted to NO•-NSAIDs in blocking cancer development. Abbreviations: nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NO•-NSAIDs), proliferating cell nuclear antigen (PCNA).