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Mini-review

Nitric oxide in cancer metastasis

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ABSTRACT

Cancer metastasis is the spread and growth of tumor cells from the original neoplasm to further organs. This review analyzes the role of nitric oxide (NO·), a signaling molecule, in the regulation of cancer formation, progression, and metastasis. The action of NO· on cancer relies on multiple factors including cell type, metastasis stage, and organs involved. Various chemotherapy drugs cause cells to release NO·, which in turn induces cytotoxic death of breast, liver, and skin tumors. However, NO· has also been clinically connected to a poor cancer prognosis because of its role in angiogenesis and intravasation. This supports the claim that NO· can be characterized as both pro-metastatic and anti-metastatic, depending on specific factors. The inhibition of cell proliferation and anti-apoptosis pathways by NO· donors has been proposed as a novel therapy to various cancers. Studies suggest that NO·-releasing non-steroidal anti-inflammatory drugs act on cancer cells in several ways that may make them ideal for cancer therapy. This review summarizes the biological significance of NO· in each step of cancer metastasis, its controversial effects for cancer progression, and its therapeutic potential.

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]. Nitric oxide (NO·) is a signaling molecule that plays various roles pathologically and physiologically [4]. In the last two decades, the function of NO· 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· have exhibited an antitumor nature [7–9]; however, NO· may also promote cancer formation and progression [10–12]. Therefore the effect of NO· 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· influences. This review will summarize the current knowledge on the influence of NO· in tumor progression and metastasis. The potential therapeutic applications of NO· in cancer treatment will also be discussed.

NO· was first discovered as a vasodilator in the cardiovascular system [16]. Recently, NO· 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· is synthesized from L-Arginine and oxygen by a family of enzymes termed nitric oxide synthases (NOSs). 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 in cancer metastasis [23].

Abbreviations: 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.

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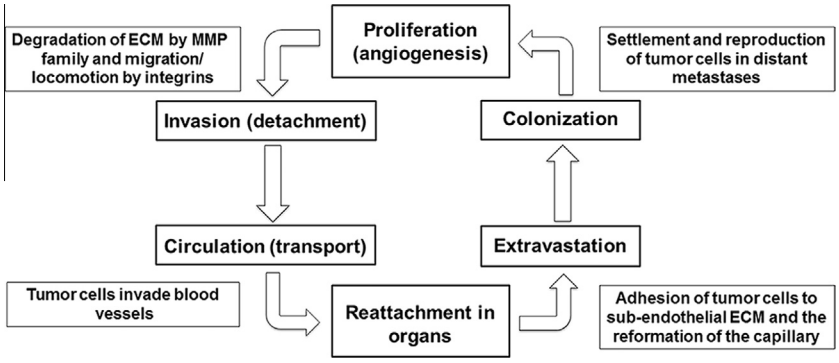

Fig. 1. This schematic demonstrates the typical progression of tumor cell metastasis. Abbreviations: extracellular matrix (ECM), matrix metalloproteinase (MMP).

NO· 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· 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· has been clinically connected to a poor cancer prognosis, not all the effects of NO· 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· may drastically impede or even eliminate metastatic progression. Yet, the effect of NO· on tumor metastasis appears to be organ-specific. A study exploring the effects of heme oxygenase and NO· 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 h 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, effectively 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 pro-tumor activity such as mediated gene transfer and up-regulated angiogenetic molecules, the antitumor actions including loss of malignancy due to apoptosis outweigh the pro-tumor 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 particular steps [1]. Numerous evidence suggests that NO· plays important roles in nearly all steps of cancer metastasis [6].

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· donors lead to a decrease of MMP-9 mRNA levels and reduction of MMP-9 translations. A 0.67 kb fragment from the 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 the 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