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REVIEW ARTICLE

Possible role of nitric oxide in the biology of breast carcinoma: review of the available literature

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Summary Nitric oxide was studied to investigate its possible involvement in the promotion of breast carcinoma: both the development of the primary tumour and the process of metastasis seem to be influenced by the presence and the amount of nitric oxide. We review the available literature on this topic, which seems to suggest an influence of nitric oxide on the cancer cell biology in breast carcinoma, but the argument is still controversial. More studies are needed to clarify the sequence of events and the real impact of nitric oxide on the behaviour of the disease.

Introduction

Nitric oxide (NO) is a molecule with a short half-life and a range of bioactivity, which is responsible for numerous functions such as neurotransmission, vascular homeostasis, immune regulation (mediation of injury by macrophages to bacteria and tumour cells) and host defence. It is generated from a guanido nitrogen of L-arginine.

Large amounts of NO are cytotoxic for various pathogens and tumour cells: the cytotoxic effect against tumour cells is associated with apoptosis (programmed cell death), which involves the inhibition of mitochondrial respiration and DNA synthesis in cell targets, including tumour cells. NO may not only mediate apoptotic cell death, but also provides protection against apoptosis induced by other agents; NO and its derivatives produced in inflamed tissues could contribute to the progress of carcinogenesis. High concentrations of NO in malignancies are demonstrably mutagenic and several studies have shown that after reaction with either oxygen or super oxide, NO forms genotoxic species.[1–5]

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This paper will focus on current knowledge on the role of NO as a possible co-promoter of breast cancer.

NO in oncology

NO seems to have an important role in maintaining the blood flow and the nutrient/oxygen supply to tumours. It has been demonstrated that tumour blood flow decreases after both regional and systemic treatment with an NO synthase (NOS) inhibitor and is correlated with the amount of NO produced in the tumour. It is not clear how NO might inhibit angiogenesis. It is possible that endogenous NO mediates angiogenesis directly or as a second messenger of other growth factors.[3,6–9] The induction of inducible NPS (iNOS) can inhibit the proliferation of endothelial cells and vascular smooth muscle cells. NO may reversibly inhibit vascular smooth muscle cell migration independent by proliferation or cytotoxicity, a novel mechanism by which both endogenous and pharmacological NO may alter vascular pathology.

NO-mediated antiangiogenesis may also be involved in the suppression of angiogenesis-related gene expression.[6,7,9,10] As a result, the vascular response to NOS inhibitor is likely to be heterogeneous and tumour dependent. Tumour cells and/or host cells influenced by a tumour produce relatively high levels of NO.[6–8] Although an important role of macrophage-derived NO as an antineoplastic effector molecule has been established, it is also true that the generation of NO exerts significant negative effects on the macrophage producing it.[10–12] There is evidence to show that tumour cell-host cell interactions have a decisive role in tumour development or regression in various types of cancer: both primary tumour lesions and metastases are often infiltrated by different host cells, and a tumour microenvironment includes not only organ parenchymal cells but also T-lymphocytes with the potential for mediating specific antitumour immune reactions and antigen-nonspecific host cells. As an example, a large proportion of stroma cells are macrophages, and together with endothelial cells these can produce in situ cytotoxic amounts of NO after appropriate activation with an important role in a highly effective adoptive cellular immunotherapy (ADI) of Esb T lymphoma in DBA/2 mice. In addition to cytoxicity, NO can modulate several steps in the metastatic process, including suppression of platelet aggregation, down-regulation of the expression of adhesion molecules, such as VCAM and ICAM, and inhibition of angiogenesis.[8,11–13]

Stimulation of iNOS in host cells in the tumour microenvironment with appropriate inflammatory cytokines could be of importance for effective treatment of cancer patients by the induction of apoptosis in tumour lesions and in circulating tumour cells. In addition, NO donors might be promising new agents in therapeutic protocols. Prolonged exposure to high-level NO may result in apoptotic cell death, whereas a short incubation with low-level NO may cause a cell-protective effect.[3,7,8,10,14–16]

Discussion

The exact consequences of NO production by tumour cells have not yet been precisely defined, but NO may affect tumour growth, differentiation, metastatic capability, and chemosensitivity and radiosensitivity.

Because NO has a wide range of bioactivity and is involved in different steps of many physiological functions, it is difficult to identify a clear indication that NO has a direct role in cancer cell metabolism. Another critical point is that NO is a labile substance with a short half-life and that direct measurement of it has proved difficult.

The production or inhibition of NO synthase (NOS) influences blood flow in the tumour environment and may have a direct role in cancer progression or regression. There are three isoforms of the NO synthase (NOS). Endothelial NOS (eNOS) is one of the isoforms of NOS: the presence of eNOS in breast apocrine metaplastic cells of fibrocystic disease in the human seems to promote the progression of metaplastic epithelium into carcinoma.[1,17] This observation could mean that NO is involved in the carcinogenesis in some cases of breast carcinoma.

While the functional implications of elevated serum NO levels observed in many cancer patients remain unexplored, a number of reports indicate a contributory role of NO in tumour progression.[10,14,18,19] Several human breast cancer cell lines express eNOS mRNA: this eNOS expression is closely correlated with the ER status of these lines.[20] Mapping of NOS expression within tumour tissue from breast and gastric cancers shows that iNOS is expressed predominantly in stromal (macrophage and endothelial) cells, although the level of NOS activity is at least 1,2 orders of magnitude lower than the enzyme activity associated with cytotoxicity and apoptosis. The correlation between NOS activity and grade for breast cancer suggests that NO may provide a positive growth signal within the tumour microenvironment: in vivo

studies showing increased growth rate, vascular density and microinvasiveness of a human tumour cell line transfected for constitutive expression of iNOS support this observation. Furthermore, in vivo administration of a highly selective inhibitor of iNOS limited invasion and growth rate of iNOS-transfected tumours and other murine tumours expressing this isoform. Inhibition of NO generation in the intratumoural microenvironment may be an important point, preventing angiogenesis, invasion and metastasis. The previously published data examining NOS activity in benign and malignant tissue in cases of breast, gastric, cervical and ovarian cancer show that NOS activity is below the level of detection for normal tissue, but is frequently elevated in malignant tissue.[10,11,13–15,17,18,21]

In in situ lesions of the breast there were significantly more cases with iNOS-positive tumour cells in high-grade than in low-grade tumours; iNOS positivity in tumour cells increased in ductal lesions from in situ to invasive carcinoma. There were also more cases with a very high number of iNOS-positive stromal cells among invasive than among in situ carcinomas. These results suggest that iNOS positivity is up-regulated as the biological aggressiveness of breast lesions increases. NO produced by iNOS in breast tumour cells and in stromal cells could be an additional factor in the enhancement of apoptosis. Local NO production by iNOS in breast carcinoma cells can modulate angiogenesis; NO production by stromal cells enhances this effect even more.[19]

Data on human tumour models have been reviewed by Thomsen and Miles and data on experimental tumour models, by Lala and Orucevic: a positive correlation of NOS expression with tumour progression has been shown in several human tumour models, such as central nervous system tumours, gynaecological cancers and breast cancers. Thomsen and Miles analysed the association of iNOS expression in stromal (macrophage and endothelial) cells within the tumour with tumour grade in human breast and gastric cancer specimens. They detected eNOS expression in tumour cells from eight gynaecological tumours and found a positive correlation with tumour differentiation. The same group reported on increased nitrite/nitrate accumulation in a small series of 15 in situ and invasive breast carcinoma tissues compared with benign breast tissue. The level of NOS activity was far below the enzyme activity associated with cytotoxicity and apoptosis.[10,14,15]

Loibl et al. found that eNOS positive tumours were more frequently highly differentiated or moderately differentiated than were eNOS negative tumours; they did not find any correlation between eNOS expression and tumour size, oestrogen and progesterone receptor status, or DNA index. In contrast, the detection of e-NOS in benign breast lesions is very rare.[9]

The paper by Thamrongwittawatpong et al. examined the capacity of oestrogen, of progesterone, and of both together to elicit the release of NO from T47D breast cancer cells in vitro. Breast cancer cells were stimulated by oestrogen, or progesterone, or both, with or without inhibition of NO or tamoxifen, to see what the effect on cell proliferation would be. The results are consistent with these Authors' hypothesis that NO expression may be increased in breast cancer, with associated raised oestrogen and progesterone concentrations; there was no inhibitory effect on NO production when the cells were exposed to tamoxifen alone, suggesting that tamoxifen does not exert a direct effect on NO production. They concluded that establishment of the mechanistic basis for hormone-mediated changes in NOS activity in tumours would be an important development, as would an understanding of how these changes influence the rate of tumour growth and assessment of its therapeutic consequences.[20]

Simeone et al. found a relationship between tamoxifen and NO production: they demonstrated, in particular, that 4-HRP, N-(4-hydroxyphenyl) retinamide, an apoptotic inducer in breast cancer cells, induced NO production in breast cancer cells and that NO production was essential for 4-HRP-induced inhibition. Tamoxifen has been found to enhance the potency of 4-HRP in ER-positive breast cancer cells, but the mechanism is not well understood.[4]

Another study, from Reveneau et al., investigated 40 malignant lesions and 38 benign lesions of the breast and found a correlation between NOS expression and the progesterone receptor, although no correlation with either oestrogen receptor status or progesterone receptor status was confirmed. However, premenopausal patients significantly more often had eNOS positive tumours; this may be due to the higher oestrogen levels in premenopausal women.[21]

Guha et al. made an interesting observation on the status of insulin-activated NOS (IANOS) activity in human breast cancer cells known to possess both nuclear oestrogen receptor and protein synthesis machinery: their results indicated that, while subphysiological concentrations of oestrogen (1.4 nM) would be expected to inhibit the IANOS activity, a physiological amount of the steroid hormone (16–64 nM) resulted in stimulation of the enzymatic activity. One implication is that some of

the beneficial effects of tamoxifen in breast cancer might be mediated through the systemic increase in NO levels by way of the activation of IANOS, independent by of the nuclear oestrogen–receptor interaction.[22]

From these studies we can derive the hypothesis that tamoxifen may have two different properties that work against malignancies, especially breast tumours: it may function as both an antioestrogen agent and a modulator of NO production, but the latter role is not clear yet.

Studies on cytokines-chemokines show that in the tumour microenvironment they may provide appropriate signals for activation of macrophages that are capable of maintaining a steady low level of NO production, which facilitates tumour progression: they show that administration of a selective iNOS inhibitor limits the in vivo growth of an iNOS-transduced human colonic adenocarcinoma in nude mice and an iNOS-expressing EMT6 mammary adenocarcinoma in syngeneic mice.[7,10,14,15]

Metastatic cells exhibit higher sensitivity to exogenous NO derived from NO-releasing compounds than do nonmetastatic cells.[7,8,10,11] Many clinical and experimental data suggest a promoting role of NO and its metabolites in carcinogenesis. Both stimulatory and inhibitory effects of NO have been reported in relation to breast cancer, and its role in the development of malignancies and metastasis remains uncertain. NO, with its lipophilicity, can rapidly cross cell membranes and enter intracellular compartments to exert its action. Thus, it can mediate interactions between tumour cells and host cells.[17,18,23–31]

Jadeski et al. studied the role of endogenous NO, via eNOS expression in tumour progression/metastasis, using a murine mammary tumour model which included spontaneously arising mammary adenocarcinomas and two clonal derivatives of a spontaneous tumour differing in metastatic capacity. They conclude that in this tumour model tumour-derived NO has a stimulatory role in tumour progression and metastasis, which is exerted by multiple mechanisms: promotion of migration, matrix degradation and angiogenesis.[18] Thus NOS inhibitors may be important components of combination therapy protocols in certain human tumours, including breast cancer, which exhibits a positive association of NOS activity with tumour grade.[17,18]

Ellies et al. studied a polyomavirus middle T antigen (PyV-mT) targeted to the mouse mammary gland model and bred into an iNOS-deficient C57Bl/6 strain to examine a possible role of NO in modulating tumours that develop in the complex environment of the whole animal. They found that the development of hyperplasias was delayed, to the extent that the earliest palpable tumours arose 2,4 weeks later in PyV-mT/iNOS minus/minus mice than in PyV-mT/iNOS $+/+$ mice, identifying a role of iNOS in early events in mammary tumour formation. They also found that the metastatic potential was retained by PyV-mT-transformed epithelium in the absence of iNOS, indicating that NO production by iNOS is not essential for this process. They concluded that further analysis of the mechanisms underlying the promotion of tumourigenesis by iNOS may lead to the identification of therapeutic targets.[27]

Song et al.[30] and Moon et al.[29] investigated the role of galectin-3 in metastasis from human breast carcinoma in the hepatic ischaemia/reperfusion metastasis model: galectin-3 is a 31 kDa carbohydrate-binding protein with an affinity for β-galactoside. This protein is involved in such processes as cell/cell and cell/matrix interactions, induction of pre-mRNA splicing, cell proliferation, cell-cycle regulation, angiogenesis and, more importantly, tumourigenesis and metastasis. These Authors concluded that galectin-3 enhances the metastatic potential of human breast carcinoma by increasing resistance to the reactive nitrogen and oxygen species, such as NO and ONOminus and that galectin-3 is a critical determinant of anchorage-independent and free radical-resistant cell survival during metastasis.

Interleukin-18 (IL-18) is an important structure for metastatic breast cancers. Gunel et al. studied the levels of IL-18 in breast cancer patients and found that breast carcinoma patients had higher serum IL-18 and nitrate and nitrite levels than control subjects. Serum IL-18 levels were significantly higher in metastatic patients than in non-metastatic patients, and among the metastatic patients, those with bone metastases had higher serum IL-18 and lower nitrate and nitrite levels than did patients with visceral and local metastases. They conclude with the suggestion that the serum IL-18 and NO activity can be used to evaluate breast cancer, especially in patients with bone metastases.[28]

Lim et al. investigated cyclosporin A and 4HPR: the latter is under investigation as a breast cancer chemopreventive and therapeutic agent, because it accumulates selectively in breast tissue. Their findings support the notion that cyclosporin A significantly enhances the growth inhibitory and apoptotic effects of 4HPR in both ER-positive and ER-negative breast cancer cells, and that this is correlated with increased production of NO.[32]

Samoszu et al. observed an association between nitrotyrosine levels and microvascular density in human breast cancer. Nitrotyrosine (NO2Y) is a

global marker of protein modification by reactive nitrogen species, such as peroxynitrite derived from NO. In the samples tested, the NO2Y levels were generally low. Breast cancers with a high microvascular density, however, had a significantly higher average level of NO2Y than tumours with a low microvascular density. NO2Y was generally not evident within the tumour cells or inflammatory cells in the stroma. These Authors conclude that low levels of reactive nitrogen species are located predominantly within inflammatory cells near blood vessels of breast cancer and higher NO2Y levels are associated with an increased density of blood vessels, and that these findings could support a possible association between inflammatory cells and reactive nitrogen species in modulating the microvascular density at the edges of breast cancer.[33] Activity of iNOS is important for invasive carcinomas. The studies in human breast and gastric cancer indicate that the stromal macrophages and endothelial cells are a predominant source of NOS and it is possible that focal iNOS activity in breast and gastric tumour tissue architecture may lead to high concentrations of NO approaching cytotoxic activity in localised ''hot spots''. In some cases, a low ratio of hot spots to total tissue volume could mean that NOS activity is below biochemically detectable levels. Studies are ongoing about the long-term delivery of iNOS inhibitors and indicate a of successfully reducing tumour progression.[8,10,11,13,17,34–37]

It is necessary to see whether there is any relationship between angiogenesis and tumour-histo-pathological features. Davel et al. were the first to demonstrate a reduction in tumour size and tumour-induced angiogenesis which they did by blocking NOS activity in vivo.[38]

A study published by Khalkhali-Ellis et al. deals with NO regulation of maspin expression in normal mammary epithelial and breast cancer cells. Their data reveal that NO induces maspin expression in MCF-7 cells, and that the maspin induced results in diminished cell motility and invasion concomitant with an increase in the apoptotic index. This novel finding provides new information on the molecular role of maspin in regulating mammary epithelial growth, remodelling, tumour progression, and the metastatic process. Targeted delivery of NO within the tumour microenvironment could provide a feasible noninvasive approach for effective breast cancer treatment.[39]

The winged helix/forkhead transcriptional factor FKHRL1 (FOXO3a) triggers apoptosis and ROCK kinase is an effector molecule in human breast carcinoma cell apoptosis and the human breast carcinoma T47D cell line releases a large amount of NO. Radisavljevic investigated the signalling of FKHRL1 to ROCK kinase during NO suppression. Their study demonstrates that NO suppression promotes FKHRL1 thr-32-enhanced phosphorylation, which that triggers apoptosis by way of the FKHRL1/ROCK kinase pathway in the human breast carcinoma cell line T47D. This study demonstrates that the concentration of NO, which was significantly below the basal level, could trigger apoptosis through a novel signalling mechanism involving FKHRL1 and ROCK kinase.[40]

The role of NO in tumour biology is extremely complex. Some data on the relationship between breast carcinoma and NO seem promising, but before we can understand the correlation between breast cancer/NO and assess of NOS expression and activity in breast cancer further validation and experimental/clinical trials will be needed.

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