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ASPECTS OF NITRIC OXIDE SYNTHASE IN PLATELETS OF KAPOSI'S SARCOMA

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ABSTRACT
Nitric oxide synthases are a family of enzymes catalyzing the production of nitric oxide from L-arginine. Nitric oxide (NO) is an important cellular signaling molecule. It helps modulate vascular tone, insulin secretion, airway tone, and peristalsis, and is involved in angiogenesis and neural development. The enzyme nitric oxide synthase (NOS) of which there are three isoforms. Neuronal (nNOS, NOS1) and endothelial (eNOS, NOS3) are constitutive calcium-dependent forms of the enzyme that regulate neural and vascular function respectively. The third isoform (iNOS, NOS2), is calcium-independent and is inducible. Importantly, various studies have shown that all three isoforms of NOS, (iNOS, eNOS and nNOS), have been detected in tumour cells from a wide range of isolates. NOS activity has been observed in human tumour cell lines and cells from tumour biopsies. However, the precise function(s) of NO in tumour biology remains unclear, and several lines of research have indicated that NO may have dual effects in cancer. The various aspects of nitric oxide syanthase in different types of Kaposi's sarcoma are summarized under this review as well as some general immune response of NO.

KEYWORDS: Nitric oxide, nitric oxide syanthase, kaposis sarcoma, iNOS.

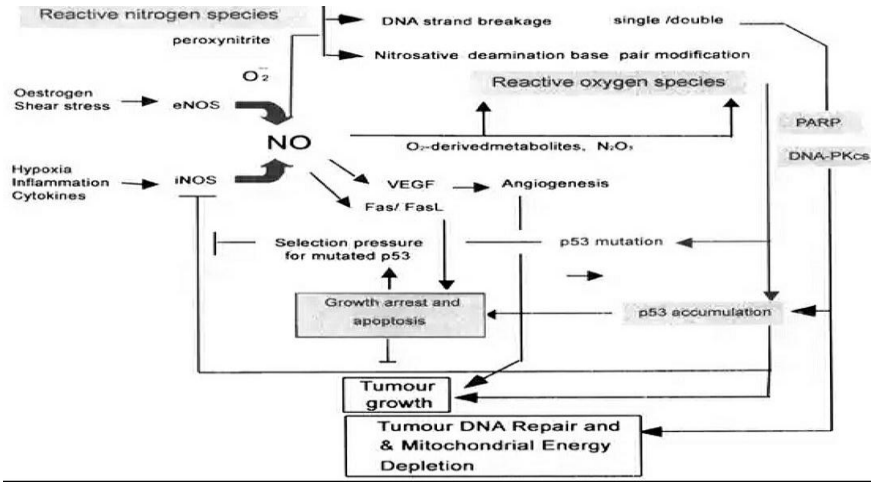
INTRODUCTION
Kaposi's sarcoma is a type of cancer that forms in the lining of blood and lymph vessels. The tumors (lesions) of Kaposi's sarcoma typically appear as painless purplish spots on the legs, feet or face. Lesions can also appear in the genital area, mouth or lymph nodes. In severe Kaposi's sarcoma, lesions may develop in the digestive tract and lungs. The underlying cause of Kaposi's sarcoma is infection with a virus called human herpesvirus 8 (HHV-8). In healthy people, HHV-8 infection usually causes no symptoms because the immune system keeps it under control. In people with weakened immune systems, however, HHV-8 has the potential to trigger Kaposi's sarcoma.People infected with human immunodeficiency virus (HIV) — the virus that causes AIDS — have the highest risk of Kaposi's sarcoma. The immune system damage caused by HIV allows cells harboring HHV-8 to multiply. Through unknown mechanisms, the characteristic lesions form. Recipients of organ transplants who take immune system-suppressing drugs to prevent transplant rejection also are at risk of Kaposi's sarcoma. In this population, though, the disease tends to be milder and easier to control than it is in people with AIDS.Another type of Kaposi's sarcoma occurs in older men of Eastern European, Mediterranean and Middle Eastern descent. Known as classic Kaposi's sarcoma, this cancer progresses slowly and typically causes few serious problems.A fourth type of Kaposi's sarcoma that affects people of all ages occurs in equatorial Africa.

GENERAL IMMUNE RESPONSE OF NITRIC OXIDE SYNTHASE
Nitric oxide is generated by phagocytes (monocyte, macrophages, and neutrophils) as part of the human immune response. Phagocytes are armed with inducible nitric oxide synthase (iNOS), which is activated by interferon-gamma (IFN-γ) as a single signal or by tumor necrosis factor (TNF) along with a second signal. On the other hand, transforming growth factor-beta (TGF-β) provides a strong inhibitory signal to iNOS, whereas interleukin-4 (IL-4) and IL-10 provide weak inhibitory signals. In this way, the immune system may regulate the armamentarium of phagocytes that play a role in inflammation and immune responses.Nitric oxide is secreted as free radicals in an immune response and is toxic to bacteria and intracellular parasites, including *Leishmania* and malaria the mechanism for this includes DNA damage and degradation of iron sulfur centers into iron ions and iron-nitrosyl compounds.The inducible pathway (iNOS) of nitrogen oxide synthesis in phagocytes can generate large amounts of NO that trigger apoptosis and kill other cells. In vitro studies indicate that phagocyte-dependent generation of NO at

concentrations greater than 400-500 nM triggers apoptosis in nearby cells and that this effect may act in a manner similar to Specialized pro-resolving mediators to dampen and reverse inflammatory responses by neutralizing and then speeding the clearance of pro-inflammatory cells from inflamed tissues. However, the role of NO in inflammation is complex with model studies involving viral infection suggesting that this gaseous mediator can also promote inflammation.

NITRIC OXIDE AS A THERAPEUTIC AGENT IN TREATMENT OF CANCER

NO-based therapeutics can be traced back for more than a centrury when Willaim Murell proposed the sublingual application of nitroglycerin as a remedy for angina pectoris. From the time of discovery of the vasodilatory properties of the organic nitrates and nitrites, it took more than hundred years to elucidate their mode of action at the molecular level. For example, it was not until 1987 that NO gas was identified both as the endogenous endothelium-derived relaxing factor and as being involved as a primary defence mechanism against tumour cells and intracellular microorganisms.Several laboratories have demonstarted that NO-releasing agents can kill tumour cells, and as a consequence there have been attempts deliver NO to cells. While NO-releasing drugs are under developement, an attractive alternative mechanism for delivery would be to transfer NOS-encoding cDNA sequences into cancer cells for gene therapy purposes. Several studies have shown that this approach may work. For example, using a mouse model it was demonstarted that transfection of K-1735 melanoma cells with an iNOS cDNA expression cassette suppressed tumourogenicity and abrogated metastasis. Transfection of human renal carcinoma cells with a retroviral iNOS cassette showed similar results. A problem with current approaches however is that constitutive expression of NOS can quickly result in death of the transfectant, shortening the time that NO can be generated, and potentially limiting the utility of the approach. NOS transfectants often have to be cultured under conditions that reduce toxicity (for example in the presence of a NOS inhibitor), and transfection attempts may result in cells that are capable of relatively low levels of NO-generation ·As discussed above, this may result in concentrations of NO that promote tumour growth rather than cell killing. Another significant point is that NOS enzyme activity requires a panel of substrates and co-factors for full activity, and these may be missing from the target cell type. For example synthesis of the important co-factor tetrahydrobiopterin (BH4), requires transcriptional regulation of the rate-limiting enzyme GTP-cyclohydrolase, which may not be induced in all target cells. Lastly, both retroviral and adenoviral vector maybe hazardous to the host and pose a major health and safety risk.A potential strategy to overcome the problems associated with gene therapy is to use a cell-based approach. Cell-based approaches utilise the delivery of recombinant cells (rather than genes) to the target site, with the advantage that the expression of the gene of interest can be optimised prior to delivery. For example, we have recently shown the utility of two novel iNOS-expressing human cell lines that can generate high concentrations of NO following treatment with analogues of either the insect hormone ecdysone or tetracycline. In order to make the NO-generating cells suitable for therapeutic delivery they have been encapsulated within a semipermeable alginate-poly-L-lysine membrane. Encapsulated cells are protected from environmental stresses encountered in the host (such as the host immune response) and can be delivered to tumour site(s) in a nude mouse model. Following delivery, high concentrations of NO and reactive nitrogen species can be generated by administration of the appropriate inducer. This approach has been very successful, and we have used it in a tumour model showing 100% killing of SKOV-3 tumours and 54% killing of DLD-1 tumours Importantly, this strategy allowed the mechanism of tumour killing to be determined as it was shown that tumour killing was associated with concomitant up-regulation of the Fas/FasL proteins. Overall we believe that the cell-delivery approach addresses some of the shortcomings of competing strategies and has the potential to inhibit or kill many different types of tumours from various histological origins.



Representation of dual action of NO

EFFECT OF NOS IN KAPOSIS SARCOMA

Epidemic Kaposi Sarcoma (HIV-Associated Kaposi Sarcoma)

- Patients with human immunodeficiency virus (HIV) are at risk of developing epidemic Kaposi sarcoma (HIV-associated Kaposi sarcoma).
- The use of drug therapy called highly active antiretroviral therapy (HAART) reduces the risk of epidemic Kaposi sarcoma in patients with HIV.
- Signs of epidemic Kaposi sarcoma can include lesions that form in many parts of the body.

Signs of epidemic Kaposi sarcoma can include lesions that form in many parts of the body.

The signs of epidemic Kaposi sarcoma can include lesions in different parts of the body, including any of the following:

- Skin
- Lining of the mouth
- Lymph nodes
- Stomach and intestines
- Lungs and lining of the chest
- Liver
- Spleen

1. Skin

NO is produced by one of three nitric oxide synthase (NOS) isoforms present in nearly all types of skin cells and functions in establishing the skin's protective barrier, circulation and UV-stimulated melanogenesis and erythema. *In vitro* skin irradiation with UVA leads to NO release from dermal NO stores, which may act to lower blood pressure, although other mediators and processes could be involved. Supporting NO's role is the finding that systemic inhibition of NO formation leads to immediate increases in blood pressure.NO's role in the skin's response to UV radiation and skin pigmentation may pave the way for future use as a cosmeceutical and/or novel non-UV-based tanning agent capable of reducing photoaging and skin cancer risk.

2. Lymph node

Reduction of peritumor lymphatic hyperplasia by NOS-blockade could thus lead to decreased delivery of tumor cells to lymph node and, thus, fewer lymph node metastases. Inhibiting eNOS activity either genetically or pharmacologically significantly reduces the number of tumor cells arriving to the tumor draining lymph node and the subsequent formation of macroscopic metastasis. Because NOS inhibition reduced the number of cancer cells delivered to the draining lymph nodes, NO participates in the earliest steps of lymphatic metastasis. As NOS inhibition can also decelerate tumor growth, likely through an antiangiogenic mechanism, the use of NOS inhibition may prove a useful adjuvant therapy for metastatic disease. Both the further spread of cancer cells from the tumor bed to the lymph node and the growth of those cells in lymph nodes could be reduced by NOS inhibition.

3. Stomach and intestine

Having witnessed abundant advances in the field of therapeutics, some diseases still remain as a global threat to life: one among them is gastric cancer. It is well established that the carcinogenesis of the gastric tissues is positively attributed to infection of Helicobacter pylori and excessive intake of salt4 whereas there has been a debate on whether alcohol consumption and tobacco smoking are associated with the neoplastic transformation. Interestingly, all the three isoforms of NOS (nNOS, iNOS, and eNOS) express in the gastric canal. High expression levels of iNOS and eNOS have been observed in human colorectal cancers.Tumor angiogenesis plays a vital role in progression of the cancerous cells and result in metastasis. Cell proliferation promotes by supplying blood vessels to the tumor by VEGF, which aids in the formation new blood vessels. The levels of VEGF correlate with the levels of NO. High serum VEGF levels were noted in gastric cancer patients in comparison to the healthy control group, which directly indicates that NO plays an important role in the progression of cancer by providing means of better blood supply and ways for cancer cells to establish new centers via metastasis.

4. Lungs

Lung cancer is one of the leading causes of mortality and morbidity at present.The primary cause for this devastating disease is tobacco smoke, which triggers chronic inflammation of the airway and activates leukocytes that produce ROS and NO at higher levels. An increased level of nitrite, nitrotyrosine, and NO are noted in lung cancer patients.Substantially higher levels of iNOS are observed in lung tissues of smokers in comparison to the non-smokers.It is observed that cigarette smoke has about 10^{14} ROS and 700 ppm NO in a single puff.A high level of NO is present in the exhaled air from the lung cancer patients in comparison to healthy controls. A significantly high level of nitrated proteins were found in the serum correlating to the presence of nitrosative and oxidative stress.

It was also demonstrated that cells treated with NO over a long period of time increased the number of filopodia per cell and cell division cycle 42 protein was also found to be in higher concentration. This shows that long term exposure of cells to NO has a unique effect on migratory potential of the cells via caveolin-1-dependent mechanism.NO works towards carcinogenesis by nitrating proteins. Nitration of proteins takes place by the reaction of NO and its metabolites with ROS which lead to production of high caliber nitrating agents. One of the main chemical modifications occurring due to oxidative/nitrosative stress is the formation of 3-nitrotyrosine in proteins. NO at high levels deactivates p53 which consequently aids in the progression of cancer and it stands true in 90% of lung cancer cases, which are found to be p53 defective.

5. Lining of mouth

One of the major causes of morbidity and mortality and the sixth most common cancer type is the oral squamous cell carcinoma. Its high occurrence is affiliated with the use of tobacco and tobacco-related products such as cigarettes. Tobacco components act as initiators of inflammation which consequently are responsible for the production of RNS. RNS hamper the antioxidant protective system and induce lipid peroxidation in tobacco users. Head and neck cancer (HNC) shows an increased level of NOS activity which is correlated with high levels of cGMP levels and vascularization in the tumor mass. Lymph node metastases are associated with microvessel density as well as high NOS activity, which is indicative of high metastatic nature.Mutations in p53 have been correlated with overexpression of iNOS as well as oxidative stress. Patients with oral pre-cancer displayed high levels of NO_2 and NO_3 as well individuals with tobacco usage showed similar results. This is indicative of the injuries caused by the RNS in patients with pre-cancer as well healthy individual with tobacco usage. This aspect has a clinical relevance in acting as a biomarker of inflammation and can be utilized for the estimation of cancer risk. Ethanol stimulates NO generation and is important to the etiology of a few cancer including HNC.

6. Liver

In the liver, both viral and helminth infections have been closely associated with the development of tumors. In humans, NO levels are elevated in patients with chronic hepatitis and this has been linked with the predisposition to develop liver cancer.

The process of carcinogenesis is thought to be a step-wise process involving the inactivation of tumor suppressor genes and the activation of oncogenes by either mutations or deletions of the DNA. Subsequent to DNA damage, cell division must occur for a tumor to develop. NO and its reactive derivatives may play an active role in the multistage process of carcinogenesis by direct cellular cytotoxicity and mutagenicity. The mechanisms of NO-induced cytotoxicity and mutagenicity are numerous and include nitrosative deamination, DNA strand breakage by NO_2^-, oxidative deamination by peroxynitrite, and DNA modification by metabolically activated N-nitrosamines. The formation of N_2O_3 leads to the formation of N-nitroso compounds which deaminate and crosslink DNA. N_2O_3 can also inactivate important enzymes involved in DNA repair mechanisms. As discussed earlier, NO reacts with superoxide anion to form the cytotoxic radical peroxynitrite which is also capable of decomposing to hydroxyl radicals and nitrogen dioxide. The generation of peroxynitrite and other oxidizing agents has also been shown to be genotoxic . Interestingly, DNA deamination results in specific mutations including GC to AT. Sequence data on gene mutations from HBV isolated from chronically infected patients reveal the same GC to AT mutation. This same mutation is evident in the p53 gene from patients with liver cancer. The DNA repair molecule p53 plays an important role in the cellular response to DNA damage from ionizing radiation, UV light, and both exogenous and endogenous chemical mutagens. Forrester et al. demonstrated that endogenous NO induces DNA damage, and results in p53 accumulation, which through a negative feedback mechanism inhibits iNOS gene expression. This implies that p53 expression is upregulated in the presence of excessive NO production to prevent NO-induced DNA damage. These data suggest that hepatocytes possess a mechanism to prevent the deleterious effects of NO in the development of liver tumors.

In addition to its carcinogenic potential, it has been shown that NO (from activated macrophages) can kill tumor cells. The target for NO-mediated anti-tumor actions are iron and sulfur containing enzymes involved in mitochondrial respiration. However, whether NO acts as a tumor surveillance mechanism in the liver is unclear. Data from Stadler et al. , showed that exogenous NO markedly suppressed mitochondrial aconitase as well as complex I and complex II enzymes, two components of the electron transport chain, while endogenously generated NO was less effective. Fukumura and coworkers demonstrated in an *in vivo* and *ex vivo* model using a co-culture system with a hepatoma cell line and Kupffer cells, that induced NO from Kupffer cells produced mitochondrial dysfunction followed by cell membrane disruption leading to tumor cell death. These results suggest that the liver, via Kupffer cell interactions, possesses a mechanism of tumor cell recognition and eradication.

Nitric oxide synthase role other than kaposis sarcoma

1. Endometrial cancer

Increased NO levels are manifested in the progression of endometrial carcinogenesis. Previous reports indicate the interaction between iNOS, Bcl-2, and p53 to be a prognostic factor associated with the progression of endometrial carcinoma. Molecular studies have shown that the susceptibility to gynecological cancers such as endometrial and cervical cancers increases by inflammatory markers including NOS and COX-2. There is a positive correlation between elevated iNOS, COX-2, and angiogenesis in endometrial tumors.A recent case study on the Turkish women identified the G894T and variable number tandem repeat (VNTR) intron 4 polymorphisms in the eNOS gene to instigate endometrial cancer in such women. eNOS expression has also been correlated with endometrial invasion. Various therapeutic agents administered for endometrial cancer emphasize on their anti-inflammatory potential, especially with respect to the downregulation of NOS expression.

2. Prostate cancer

There has been strident investigation to associate the risk of prostate cancer with NO synthase levels.A plethora of meta-analyses has identified NOS3 gene polymorphisms

as strong susceptibility factors for the progression towards prostate cancer. A meta-analysis conducted by Zhao suggested the eNOS gene 894G > T polymorphism to aggravate the onset of prostate cancer in males. Nikolić et al. also corroborated the involvement of eNOS or NOS3 gene in the pathogenesis of prostate cancer. NOS3 rs1799983 polymorphism augmented the risk of prostate cancer in various populations.As one of the possible mechanisms, the involvement of the NO receptor component, sGCα1, in mediating the proliferation of prostate carcinogenesis, has been surmised. Anti-cancer agents such as gold lotion have successfully demonstrated their anti-carcinogenic potential through the downregulation of iNOS also elucidated the significance of eNOS as a seemingly promising strategy for targeting anti-androgen resistant prostate cancer. Arginine-releasing compounds such as carboxypeptidase-D increased NO production, which correlated with survival and progression of prostate cancer.

3. Cervical cancer

Cervical cancer takes the second position as one of the most common female cancers recording > 200,000 deaths per year. Its risk factors include chronic inflammation, long use of oral contraception, smoking, multiparity, and sexually transmitted diseases such as chlamydia, herpes. Cervical cells, such as squamous, glandular epithelial, and stromal cells, generate NO, which is a key signaling molecule shown to play an eminent role in various physiological processes (preparing the cervix for pregnancy, etc.). However, elevated levels of NO have been reported in cervical cancer patients, thereby indicating its mutagenic and carcinogenic potential in cervical cancer progression. NO plays a crucial role in the progression of cervical carcinogenesis through human papilloma virus (HPV).It was concluded that NO is a pragmatic cofactor that promotes HPV-induced cervical carcinogenesis. Moreover, susceptibility to HPV infection as observed through the cytological changes in the cervix correlates with an elevated expression of both eNOS and iNOS. Of these, iNOS, plays a prominent role in tumor progression, by promoting angiogenesis and thus regulating the level of VEGF.This explanation was corroborated by an iNOS knockdown experiment in HeLa cells that reduced their proliferation.High NO concentrations impair the migration of neutrophil migration, which express receptors necessary for extricating tumor cells in cervical neoplasia. NO also encumbers the success rate of conventional treatment therapy. In a study by Chen et al., overexpression of iNOS along with COX-2 correlated with diminished survival rates of cervical cancer patients on radiotherapy regime.

4. Breast cancer

Breast cancer is the predominant cause of mortality among women worldwide. Tumorigenesis in the mammary gland has been reported to correlate with VNTR 4 a/b polymorphism that regulate eNOS expression.NO can modulate tumor aggression in breast carcinoma through the inhibition of enzymes linked to DNA repair machinery. Moreover, iNOS inhibits DKK glycoprotein, which is crucial to regulating the Wnt/β-catenin pathway. Abrogation of this pathway is activated in several tumors, including breast cancer.A diagnostic evaluation of inflammatory markers associated with breast cancer prognosis includes NO as well.Apoptosis of triple negative breast cancer cells have been attained through the modulation of iNOS activity. Knockdown of genes regulated by NO signaling pathway ameliorated breast tumor metastasis.Augsten et al. demonstrated an upregulation of NOS levels in CXCL14-expressing cancer-associated fibroblasts which triggers tumor growth and metastasis. The manifestation of chemo-resistance as well as metastasis in breast cancer cells are rendered through NO elevation, which therefore results in poor survival of patients.Importantly, NO induces tamoxifen-resistance in ER positive breast cancer.

5. Brain tumors

Many vital physiological activities, such as vasculature and neurotransmission, are influenced by NO. Many disorders of the central nervous system portray NO as an important mediator of neurotoxicity. nNOS expression is an indicator for differentiation of brain tumor and malignancy. Unexpected high NO levels are observed in central nervous system malignancies and this suggests that there is some correlation between NO production and pathophysiological processes of tumor progression.

CONCLUSION

NO is a relatively stable, free radical gas that readily diffuses into cells and cell membranes where it reacts with molecular targets. The precise reactions of NO depend on the concentration of NO achieved and on subtle variations in the composition of the intra- and extracellular milieu. NO seems to play a part in various stages of carcinogenesis from initiation to progression. Expression of NOS have been detected in various human cancers. As nitric oxide synthase isoform used in many different tumours, it also has its specific role in kaposis sarcoma we conclude the various parts of epidemic type of kaposis sarcoma containing skin, liver lining of mouth, lungs, stomach and intestine, lymph node has role of nitric oxide synthase linking with other receptor. Nitric oxide can be the new therapeutic tool for the treatment of sarcomas/ cancers. NO can act as a novel potential therapeutic agent in patients with refractory cancer by sensitizing tumor cells to chemotherapy, radiotherapy or immunotherapy. Nevertheless, further validation and experimental/clinical trials are required to develop NO based strategies for cancer prevention and treatment.

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