

Bladder Cancer and NOS-NO Gene Polymorphisms

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ABSTRACT

Nitric oxide (NO) is a pleiotropic molecule with a variety of functions. NO may have both pro- and antitumor functions. NO could take part in tumour development and progression. Polymorphisms in the endothelial nitric oxide synthase (eNOS) gene may influence the risk of cancer, but the results are still conflicting. Most studied eNOS gene mutations are T-786C, G894T and intron 4a/b polymorphisms in the literature. Environmental factors and genetic susceptibility may contribute to cancer development. Numerous clinical and molecular studies have shown that bladder cancer is a heterogeneous disease. Different chemical carcinogens are associated with similar levels of risk for developing varying grades and stages of bladder cancer, and therefore, carcinogen exposure alone cannot explain the heterogeneity of the disease. Molecular alterations may lead to heterogeneity and are influenced by environmental exposure. There are many genetic polymorphisms in metabolic enzymes, and other genes have been found to be bladder cancer risk factors. But a few studies have evaluated the role of eNOS polymorphisms in a this cancer. The objective of this review is to evaluate the role of the eNOS polymorphisms in cancer susceptibility, especially in bladder cancer which is the second most common malignancy of urinary tract.

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Key words: Nitric oxide; Nitric oxide syntase polymorphism; Bladder cancer

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INTRODUCTION

Bladder cancer is one of the most common cancers in the world. This cancer is the second most common malignancy of urinary tract cancer and fourth most frequent cancer diagnosed in men accounting for 7% of total cancers and the eighth most common cancer in women in the United States^[1]. Bladder cancer is a multi-factorial disease that is mediated by both genetic and environmental factors such as occupational chemical exposure, tobacco use, some microorganisms and genetic factors^[2].

Nitric oxide (NO) is an important biological molecule with a variety of functions. Nitric oxide synthase (NOS) is the responsible enzyme in NO production from L-arginine. Three main isoforms, derived from separate genes, have been described and named after the cells in which they were first found^[3]. These are neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3)^[4]. In the last three decades, the role of NO in tumor cell biology and tumor angiogenesis has been firmly established. NOS isoforms are reported to be present in human solid tumors and tumor cell lines (Table 1). Importance of NOS gene polymorphisms relies on the contradictory role of NO in carcinogenesis. At the present time, several polymorphisms identified in human eNOS gene but a few variations are assumed to have functional importance^[5,6]. The G894T variation (rs1799983) may alter eNOS activity or regulation. Several studies reported that some of the eNOS gene polymorphisms are significantly associated with development of several types of cancer^[7,8].

Table 1 Inducible NOS expression in various cancers^[10].

Organ	Expression of inducible NOS
Colon	Aberrant crypt foci Adenoma Adenocarcinoma
Breast	Invasive lesions Tumors
Prostate	High-grade prostatic intraepithelial neoplasia Cancer
Bladder	Dysplastic lesions Carcinoma
Head and neck	Carcinoma

NO has multiple physiological functions, including vasodilatation, neuronal transmission, smooth muscle relaxation and immunity and is released by endothelial cells^[3,5]. The cellular effect of NO is complex and can be cytoprotective or cytotoxic, depending on its concentration and cellular microenvironment^[11]. At low concentrations, NO may exert cytoprotective function through cell homeostasis, muscle relaxation, neurotransmission and platelet function^[4,12,13]. Low levels of NO may also protect endothelial cells from exposure to risk factors such as reactive oxygen species (ROS) and tumor necrosis factor- α ^[14-16]. The specific actions whereby NO protects against ROS may include interacting with metals to prevent the formation of oxygen species, scavenging oxidants, preventing the degradation of metalloproteins and preventing lipid peroxidation^[16]. At high concentrations, NO and its derivatives can cause DNA strand breaks and impair the tumor suppressor function of p53, which may lead to carcinogenesis^[17-20].

NITRIC OXIDE SYNTASE

eNOS is one of three isoforms of NOS that generates NO in vascular endothelium and is encoded by the eNOS gene which is localized to chromosome 7q35-36^[21]. nNOS is located on chromosome 12 and iNOS on chromosome 17^[22,23]. The eNOS gene have about 1,500 base pairs of upstream promoter sequence similar to other NOS and include transcription factor-binding sites that mediate regulation by estrogens, shear stress^[24]. eNOS generates low levels of the endogenous free radical nitric oxide during the transformation of L-arginine to L-citrulline at the presence of tetrahydrobiopterin (BH4), an essential eNOS cofactor^[3,25]. It has been shown that eNOS regulates blood pressure, platelet aggregation, leukocyte adherence, and vascular smooth muscle cell mitogenesis and angiogenesis^[26]. More than 168 polymorphisms have been identified in the eNOS gene^[27]. Only three of these polymorphisms, namely 894G>T, intron 4a/b, and -786T>C, seem to be functional, and they have been investigated for their association with the risk of several diseases, predominantly cardiovascular diseases^[28,29]. Several polymorphisms in eNOS gene have been identified, including a common missense variant at nucleotide position 894 (G894T) in exon 7, resulting in a substitution of glutamic acid to aspartic acid at amino acid position 298. This polymorphism is associated with differing susceptibility to cleavage in eNOS enzyme in transfected cells and human endothelial cells, related to reduced basal NO production^[30,31]. Previous studies have shown increased risk of coronary artery disease and hypertension associated with eNOS Glu298Asp genotypes^[32,33].

NITRIC OXIDE

NO is a very important intercellular messenger molecule. However, previous studies have indicated that NO may have both pro- and antitumor functions, under varied conditions in the course of tumor

initiation, development, and progression^[34,35] (Figure 1). The pro-tumor property may be related to DNA damage, mutation of tumor suppressor gene p53, promotion of angiogenesis and stimulation of tumor cell blood flow whereas the antitumor effects may be associated with the induction of apoptosis, activation of antioxidant defense, cytostatic effect and immunity^[34,35]. The effective NO functions depend, to a great extent, on its concentration, local microenvironment and timing of production. Lancaster *et al.*^[11] noted that heterogeneity in the chemical process, biological production of NO and cellular responses to NO, among other factors, might render different biological outcomes related to NO. Elevated levels of all three isoforms of nitric oxide synthase have been found in human solid tumors, indicating that NO is a mediator of tumor progression^[36]. The production of NO is part of the macrophages' antitumor immune response mechanism, yet an over-expression of NO levels in tumor sites has been found to correlate with poor clinical outcome^[37]. Table 1 summarizes evidence in support of iNOS association during tumor development in humans and animals^[10].

**Figure 1** The dual role of nitric oxide in cancer biology^[39].

NITRIC OXIDE SYNTASE ISOFORMS AND POLYMORPHISMS

NOS isoforms and polymorphisms are reported to be present in human solid tumors and tumor cell lines^[38]. Yang *et al.*^[4] did not observe a significant relationship between NOS3 Glu298Asp and breast cancer risk, but we did find that this polymorphism was associated with the risk of post-menopausal breast cancer among subgroups of women defined by smoking status or intensity of smoking. Ozturk *et al.*^[40] reported that Glu298Asp and VNTR intron 4 polymorphisms in the eNOS gene could be an intriguing factor that modulates an individual's risk of endometrial carcinoma in Turkish population. Unal *et al.*^[41] showed that neither 984G>T nor intron 4a/b polymorphisms were related with gastric cancer. Some studies have found that NOS2A and NOS3 genetic polymorphisms are associated with prostate cancer risk^[4,42].

Evidence for NO involvement in tumour biology has been reported from several different forms of cancer both *in vitro* and *in vivo*^[43]. However, there are conflicting reports whether NO promotes or inhibits tumour growth. Thomae *et al.*, described that low concentrations of NO stimulated endothelial cell growth, whereas high concentrations acted in an inhibitory fashion^[45]. This dual effect of NO on cell growth has also been demonstrated in bladder cancer cell lines by Morcos *et al.*^[46]. In a study, iNOS expression was found in biopsies from bladder tumours and they found no correlation between the levels of iNOS and tumour grade or stage^[9]. NO levels are higher in patients with CIS lesions than in patients with papillary bladder tumours and this increase is also likely due to an elevated expression of iNOS^[9]. Furthermore, Several studies propose that NO also actively mediate some of the anti tumour effects seen after BCG treatment^[46,47]. NO levels are higher in the bladder after BCG treatment and are likely to reflect an increased expression

of iNOS in bladder urothelial cells and immune competent cells in the submucosa and these findings are in line with previous results implicating that BCG may act through NO/NOS pathways, which is further supported that polymorphisms (eNOS -786T>C polymorphism, eNOS Glu298Asp polymorphism) in the iNOS and eNOS genes may influence treatment outcome for BCG^[9]. eNOS expression has been demonstrated in the endothelium of bladder tumour vessels and endothelial derived NO, produced by eNOS, has been proposed to promote angiogenesis and cancer invasiveness (48). *In vitro* studies on bladder cancer cells have suggested a similar role for NO in bladder cancer, promoting cell growth when produced at low concentrations whereas high concentrations result in cytostatic and cytotoxic effects^[46,47].

Increased iNOS expression also has been found consistently in bladder cancers^[49-52]. In one study, all 94 transitional cell carcinomas examined exhibited some immunostaining for iNOS. All dysplastic lesions adjacent to carcinomas exhibited staining patterns similar to malignant tissue, suggesting that upregulation of iNOS is an early event during bladder carcinogenesis^[49]. iNOS expression has been reported in several cancers including bladder cancer^[48]. The iNOS (CCTTT)n promoter microsatellite polymorphism at -2.6 kb has been suggested to be associated with gastric cancer and bladder cancer^[53-55]. In a recent study by Ryk *et al.*, a long set (13 or more) of (CCTTT)n repeats were associated with a lower risk for developing bladder cancer but also with a higher risk for disease progression and cancer specific death once cancer had emerged^[56]. In addition, eNOS polymorphisms have been associated to cancer risk and progression^[56]. Ryk *et al.*, has recently found a correlation between bladder cancer and both the eNOS promoter polymorphism -786T>C and the intragenic eNOS polymorphism Glu298Asp^[55]. iNOS promoter (-2.6 Kb) microsatellite (CCTTT)n polymorphism has been correlated to the development and aggressiveness of bladder cancer, patients with a long set of repeats of the (CCTTT)n polymorphism had a lower risk of developing bladder cancer but a higher risk for stage progression and cancer specific death once tumour had developed^[55]. The presence of elevated NO concentration and iNOS expression in the urinary bladder from BCG treated patients and patients with CIS further supports the notion that NO may be an important factor in bladder cancer biology and that the BCG effect on superficial bladder cancer may partly be due to stimulation of local NO formation^[57].

Ryk *et al.* reported that the eNOS is expressed in the bladder tumor vessels, suggesting the possible relation between eNOS and the bladder cancer. They found no increased risk of bladder cancer with Glu298Asp polymorphism. However there was an association between the Glu298Asp and bladder tumor grade^[5]. In a study by Verim *et al.* also suggested an increased risk role of eNOS GT genotype in bladder cancer susceptibility in our Turkish population^[58]. Koskela *et al.* reported that the iNOS is found to be localized to the urothelium and macrophages underlying it and their study also confirms elevated levels of endogenously formed NO and increased mRNA expression and protein levels for iNOS in patients with BCG treated bladder cancer^[59]. *In vivo* studies results provide evidence for frequent iNOS protein expression in TCC. In addition, Their observations indicate that overexpression of iNOS expression may be one of the early events in the carcinogenesis of TCC^[50]. Some results suggest that various urinary bladder lesions alter the normal differentiation pathway of urothelial superficial cells, which induces the expression of NOS in mitochondria of partially differentiated cells^[60-61].

Recently published study showed a strong relationship between the eNOS4a/b polymorphism and superficial bladder cancer. Univariate

analyses revealed that the eNOS4a/b polymorphism predicted recurrence and progression. The authors suggested that a genotype containing the 'a' allele of the eNOS4a/b polymorphism may be a risk factor for bladder cancer. Furthermore, patients harboring the 'aa' plus 'ab' genotype are more likely to experience tumor recurrence and progression. In addition, compared to patients with the 'b' allele, those with the eNOS4a/b gene polymorphism 'a' allele or at least 1 mutated 'a' allele (aa or ab) were more susceptible to high grade disease ($p=0.007$) and bladder tumor invasiveness^[62].

CONCLUSION

Nitric oxide (NO) is an important biological molecule with a variety of functions. NO may have both pro- and antitumor functions. NO could take part in tumour development and progression. However, for researchers, the problems of NO chemistry in the tumor environment, NO output, NO protumor activity, and the target cell type remain to be further investigated by moving from *in vitro* biology to more relevant animal models. NOS select inhibitors and their possible use for the prevention and treatment of various cancers. NO may be involved in the anti-tumor mechanism that BCG exerts on bladder cancer cells.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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