

Nitric oxide synthases in cancer genetics; focus on nasopharyngeal carcinoma

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Abstract

Nitric oxide (NO) is an interesting lipophilic molecule that can be synthesized in most tissues and has important physiological and pathological functions. It is a highly diffusive free radical and can therefore affect all biological systems in the body. Described for the first time as an endothelium-derived relaxing factor, NO emerged over past decades, as a key signaling molecule with prominent role in inflammation and cancer. Angiogenesis, apoptosis, genomic instability, mutation, oncogene activation, cell cycle, and metastasis have been reported to be affected by NO which exhibits opposite effects depending on its concentrations. In carcinogenesis, NO can be either antitumor or tumor promoting factor. The dysregulation of NOS gene expression induced by genetic, epigenetic and environmental factors has been reported to be associated with cancer risk and progression. Here we discuss the contribution of NOS/NO system in cancer genetics focusing on nasopharyngeal carcinoma (NPC).

Introduction

The tumorigenesis is a complex pathophysiological process involving several inflammatory signalling and molecular mechanisms leading to the progressive cell transformation and formation of cancer. Several lines of evidence showed that inflammation is involved in cancer development and progression and chronic inflammatory diseases can promote cell transformation and tumorigenesis [1-4]. In response to endogenous and exogenous stimuli, activated inflammatory cells such as macrophages, sentinel dendritic cells, endothelial cells and neutrophils are able to synthesize and release a plethora of inflammatory factors including lipid mediators, cytokines, reactive oxygen species (ROS), matrix metalloproteases, and NO [5-7]. It has been demonstrated that NO is one of the most multifunctional gaseous molecule involved in inflammation-driven diseases such as cancer [8,9]. Since its discovery and the historic Nobel Prize in Physiology and Medicine 1998 awarded to Ferid Murad, Robert Furchgott and Louis J. Ignarro, NO has sparked a lot of scientific research in all the fields of biology and medical sciences with fascinating results and an exponential number of scientific publications. Despite its short half-life, NO participates in various biological and pathological functions. NO is a very fascinating and attractive molecule in that it by itself exhibits opposite effects depending on its variable production and its heterogeneous chemistry. It has been reported that NO has pro and anti-inflammatory activities due to the biphasic regulation of NF- κ B [10]. NO is distinctly known as an intracellular signaling molecule with complex and dichotomous effects. The dual role of NO in cancer biology demonstrate its dynamic involvement in tumor development and progression. In cancer, the heterogeneous effects of NO are dependent on many factors such as the activity and localization of NOS isoforms, concentration and duration of NO exposure, and cellular sensitivity to NO [11]. The well-known dual effects of NO are closely linked to its concentration which is under the control of several factors primarily genetic variations affecting its bioavailability. In carcinogenesis, it is known that at low concentrations (less than 100 nM), NO acts as a pro-tumorigenic factor [12]. However,

high concentrations of NO (more than 500 nM) were known to be pro-apoptotic causing cytotoxic and anti-tumorigenic effects [13].

NO is endogenously synthesized from L-arginine by the action of three different isoforms of NOS: neuronal NOS (nNOS or NOS1) which is the first isoform to be purified and cloned; inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). The constitutively expressed NOS1 and NOS3 are calcium-dependent and post-translationally regulated isoforms of NOS [14]. They are involved in the regulation of neuronal and vascular functions, respectively. Because their activities require an increase in calcium ion concentrations, NOS1 and NOS3 catalyse the production of small amounts of NO (nanomolar) for short duration. NOS2 can be induced by several inflammatory factors including cytokines and other proinflammatory mediators. In contrast to the constitutive isoform of NOS (NOS1 and NOS3), NOS2 is calcium-independent and can therefore produce higher amounts of NO (micromolar) for longer duration [14]. For these reasons, NOS2 gene was generally considered as the main isoform of NOS involved in immunity, inflammation and cancer.

It is now widely accepted that NOS3 isoform acted as a potential endogenous mediator involved in inflammation-driven cancer because of its ability to induce the expression of various inflammatory factors including NF- κ B and cyclooxygenase-2 [15]. In addition, NOS3 enzyme is involved in the intracellular sustained generation of NO and many cancer-related events have been reported to be modulated

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by NOS3-derived NO. [10]. The dysregulation of the NOS3 gene has been reported to be associated with the development of several cancers [16]. Although an exponential number of research papers have been published on NO, few of them were interested on the head and neck cancers, especially NPC.

Nasopharyngeal carcinoma

Among head and neck cancers, NPC is a complex epithelial malignancy characterised by its unique clinical, etiological and biological parameters [17,18]. Despite its multifactorial etiology in relation to the Epstein-Barr virus (EBV), the influence of genetic and environmental factors, NPC differ from other head and neck cancers because of its geographical distribution and its radiosensitivity [19,20]. The prevalence of NPC is limited to specific regions of the world. Particularly higher in Southern China and Southeast Asia (20–30/100,000), NPC is rare in Europe and North America (less than 1/100,000 persons/year). An intermediate prevalence for NPC was found in North Africa (~3.5 per/100,000 persons/year) [20]. In Tunisia, NPC is the first cancer of the aerodigestive tract in women and the second after laryngeal cancer in men [21]. Because of its deep location, NPC is only detected at advanced stages. Moreover, NPC has a high rate of lymph node and visceral metastasis which partly explains the therapeutic failures despite the marked radiosensitivity that allows controlling the primary tumor with a relatively high frequency. In its classification, the World Health Organization (WHO) distinguishes three histologically different types of NPC; WHO type I (keratinizing squamous cell carcinoma), WHO type II (non-keratinizing carcinoma) and the undifferentiated NPC, WHO Type III, which is the most frequent NPC type the endemic regions.

The development of NPC resulted from the accumulation of genetic and epigenetic alterations caused by many factors including, EBV infection, environmental carcinogens, dietary, and genetic deregulations. Several inflammatory constitutive and inducible genes have been reported to be involved in the development of NPC [22]. In the following sections, we discuss the contribution of the association studies to the oncogenetic role of NOS2 and NOS3 genes focusing on NPC.

NOS genes and association studies

The effects of NO on tumorigenesis are both complicated and multifaceted. The expression and activity of the NOS enzymes have been found in various tumor pathologies including head and neck, thyroid, breast, stomach and bladder cancers [8]. A significant correlation between the increased production of NO and tumor growth has been clearly reported in NPC [23]. Because of its ability to induce nitrative and oxidative stress leading to the DNA damage and cancer development, the production of NO is considered as a blood biomarker of inflammation and can be used in monitoring tumor growth. The contribution of each isoform of NOS (NOS2 and NOS3) to the endogenous production of NO needs more investigations and discussion.

The inducible NOS: NOS2

NOS2 enzyme generates more NO than the constitutive isoforms NOS1 and NOS3. This is not surprising since the expression and activity of NOS2 can be up-regulated by proinflammatory cytokines including IL-1 β and TNF α and IL-6 known to be released during inflammation by activated inflammatory cells. Other proinflammatory factors such as lipopolysaccharide endotoxin and oxidative stress are also able to

induce NOS2 gene expression. Besides its expression in stromal and infiltrating cells such as macrophages, NOS2 has been detected in tumor cells and plays crucial roles in inflammation and angiogenesis [24]. NOS2-derived NO is known to up-regulate the expression of the lymphangiogenic factors VEGF-C and D in various cancer types especially NPC and many evidences showed that NO is involved in inducing lymph node metastasis [25]. In addition, NO reduces the proliferation of T cells and therefore inhibits the antitumor immune response. Collectively, these data showed that NOS2-derived NO plays crucial roles in cancer biology.

The human NOS2 gene is located on chromosome 17q11.2-q12 and contains 27 exons [26]. Because of its inducible expression and the presence of many binding sites for several transcription factors, NOS2 is a tightly regulated gene [27]. NOS2 enzyme is regarded as a unique oncoprotein since its aberrant expression activates many oncogenic pathways [28]. Many functional polymorphisms have been found in the coding and regulatory regions of the human NOS2 gene. The major and relevant variants described in NOS2 gene, are summarized in the Table 1. Among these polymorphisms, the G-954C mutation has been reported to be associated with gastric cancer and its impact on clinicopathological characteristics remain unknown [29]. As far as we know there is no study investigating the impact of the NOS2 G-954C variant on NPC susceptibility. Functionally, the G-954C variant described in the promoter region of the NOS2 gene has been shown to increase the expression of NOS2 enzyme, resulting in higher production of NO [30]. Indeed, the increased expression of NOS2 gene has been reported to be associated with the angiogenesis process [31]. Another mutation described in the human NOS2 promoter region is the penta nucleotide repeat variant (CCTTT)₅ which has been correlated with severity of various tumoral pathologies such as gastric cancer and urothelial carcinoma [32,33]. However, to date there is no study investigating the clinical impact of this polymorphism in NPC.

Table 1. Major variants in the NOS2 gene involved in cancer risk and progression

Variants	Cancer risk and/or lymph node involvement	References
(CCTTT) _n	Association with gastric cancer	[32]
	Clinicopathological characteristics	–
	Association with Urothelial cancer	[33]
	No significant association with pathologic grade	[33]
G-954C	Association with bladder cancer	[48]
	Clinicopathological characteristics	–
C150T	Association with gastric cancer	[37]
	No Association with gastric cancer	[29]
	Clinicopathological characteristics	–
	Association with Non hodgking lymphoma	[34]
	Clinicopathological characteristics	–
	Association with bladder cancer	[35]
	Tumor stage	[35]
	Association with colon cancer	[35]
	Clinicopathological characteristics	–
	No association with rectal cancer	[36]
	Clinicopathological characteristics	–
	No association with colon cancer	[36]
A-277G	Clinicopathological characteristics	–
	No association with rectal cancer	[36]
	Clinicopathological characteristics	–
	Association with NPC risk	[39]
	No association with Clinicopathological characteristics	[39]

The C150T mutation, also known as Ser⁶⁰⁸Leu, rs2297518, in the exon 16 is the most frequent variant found in the NOS2 gene. It resulted in a two-fold risk increase for B and T cell non-Hodgkin lymphoma [34]. In addition, the NOS2 Ser⁶⁰⁸Leu polymorphism has been shown to be associated with various tumor pathologies including bladder and colon cancers [35,36]. However, conflicting results were observed regarding this variant and gastric cancer [37]. Another relevant mutation found in the NOS2 gene is the A-277G (rs2779248) change which is known to be associated with higher production of NO [38]. A significant association was found between this polymorphism and NPC risk in Tunisian population with no impact on clinicopathological characteristics [39].

Overall, association studies addressing the impact of NOS2 polymorphisms and their clinical relevance in cancer showed conflicting results.

The endothelial NOS: NOS3

Although, NOS2 gene remains a potential actor in inflammation-driven cancer, the dysregulation of the NOS3 gene and enzyme activity may drive tumorigenesis in human. NOS3 gene has been shown to play a key role in NO bioavailability and its involvement in carcinogenesis has been clearly documented [40]. In addition, NOS3 gene represents the major origin of circulating levels of NO.

The NOS3 enzyme is a peripheral membrane protein that is localized to the caveolae of plasma membranes and Golgi complex. The gene encoding human NOS3 enzyme is located on chromosome 7q36, comprises 26 exons harboring various polymorphisms. The clinically relevant and the most studied polymorphisms described in NOS3 gene were summarized in Table 2. Although several SNPs have been

identified in NOS3 gene, a few of them are functional and are associated with altered NO bioavailability. Among these variants, the T-786C and G894T polymorphisms have been shown to be associated with various tumor pathologies including gastric, breast and prostate cancers [41-43]. Association studies reported that NOS3 G894T polymorphism has been reported to be associated with various cancer risk and development and have functional and clinical impacts in malignancies [44]. Our recently published study showed that the T-786C and G894T polymorphisms of the NOS3 gene differently contribute to NPC risk and development in the Tunisian population [45]. Indeed, a significant association was found between NOS3 G894T polymorphism and NPC risk. However, no association was observed between NOS3 T-786C mutation and the risk of NPC. It should be important to note that association studies regarding the T-786C variant and cancers, especially NPC showed conflicting results [46]. The complex interactions between epigenetic, genetic, viral, environmental and dietary factors could explain these conflicting data as was previously suggested in NPC [22]. Functionally, we have demonstrated that the T-786C mutation reduced the levels of plasma NO and decreased risk of lymph node metastasis in NPC patients [45]. Given the fact that NPC has a high preponderance for regional lymph node metastasis that can be induced by NO, the T-786C mutation can be considered as a protective factor against NPC development.

In contrast to the T-786C mutation, the NOS3 G894T variant appeared to have no significant effects on the levels of NO. The final message of the study regarding the impact of NOS3 G894T and T-786C variants on NPC relies on the association between a “biological” evidence and a “clinical” event; (higher probability to develop NPC, higher probability of nodal localization). Overall, data regarding NOS3 polymorphisms showed that the changes in the expression and/or activity of NOS3 enzyme determined by genetic variations may play an important role in the tumor pathogenesis and NOS3 gene could be carefully considered in the evaluation of the role of NO in cancer risk and progression.

Conclusions

The involvement of the NOS-derived NO in the genetics of cancers appeared to be a complex evolving topic. The role of NOS2; the inducible isoform of NOS has been extensively studied in inflammation and carcinogenesis. This isoform of NOS remains a crucial target in the development of NO-based strategies for cancer prevention and treatment. Over the past decades, NOS3 gene emerged as a potential actor with pivotal roles in inflammation-driven cancer. It supported the constitutive production and the circulating levels of NO which is involved in angiogenesis and cancer progression. NOS3 gene is also known to reduce the lymphatic resistance in normal and tumors tissues *via* collecting lymphatic vessels. Pharmacogenetic targeting of NOS3 signaling will help in developing novel NO-based therapies for the prevention and the treatment of several lymphatic system diseases, such as NPC. Although, tremendous efforts have been made to address the exact role of endogenous NO in cancer biology, several processes remain unclear and need more investigations. This is not surprising since the levels of endogenous NO is under the control of several epigenetic, genetic and environmental factors. The combined effects of these factors should be considered in the development of NO-based therapy for the prevention and treatment of cancer pathologies. The general consensus is that the effects of NO are closely linked to its concentrations. For these reasons, the use of NO-releasing compounds could be crucial in reducing the controversy regarding the dichotomous effects of NO, especially in tumor biology. Moreover, the development

Table 2. Relevant NOS3 gene variants and their roles in cancer risk and progression

Variants	Cancer risk and/or lymph node involvement	References
G894T	Association with breast cancer risk	[43]
	Lymph nodal localization	[46]
	Association with gastric cancer risk	[42]
	Lymph nodal localization	–
	Association with NPC risk	[45]
	No lymph nodal localization	[45]
	No association with prostate cancer risk	[46]
	Lymph nodal localization	–
	Association with colorectal cancer risk	[48]
T-786C	Lymph nodal localization	–
	Association with breast cancer risk	[41]
	No association with breast cancer risk	[49]
	Lymph nodal localization	[49]
	Association gastric cancer risk	[42]
	Lymph nodal localization	–
	No association with NPC risk	[45]
	Lymph nodal localization	[45]
	Association with colorectal cancer risk	[48]
VNTR (4a/b)	Lymph nodal localization	–
	No association with prostate cancer risk	[44]
	Clinicopathological characteristics	–
	Association with colorectal cancer risk	[48]
	No association with clinicopathological characteristics	[48]
	No association with breast cancer risk	[50]
	Lymph nodal localization and metastasis	[51]
	Association with superficial bladder cancer risk	[47]
	Association with tumor grade	[52]

of new selective inhibitors targeting each isoform of NOS enzyme may be crucial in modulating the levels and the effects of NO. Finally, further investigations are needed to understand how genetic contribution involving NOS2 and NOS3 genes may be relevant to tumor pathologies and to optimize cancer drug therapy.

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