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iNOS-selective inhibitors for cancer prevention: promise and progress

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Abstract

Nitric oxide (NO) is involved in various physiological functions and its role in tumorigenesis has been well studied. A large majority of human and experimental tumors appear to progress owing to NO resulting from iNOS, further stimulated by proinflammatory cytokines. Conversely, in some cases, NO is associated with induction of apoptosis and tumor regression. This dichotomy of NO is largely explained by the complexity of signaling pathways in tumor cells, which respond to NO very differently depending on its concentration. In addition, NO alters many signaling pathways through chemical modifications, such as the addition of *S*-nitrosothiols and nitrosotyrosine to target proteins altering various biological pathways. Hence, iNOS inhibitors are designed and developed to inhibit various organ site cancers including the colon. Here, we review iNOS expression, generation of NO, involvement of NO in altering signaling pathways, and iNOS select inhibitors and their possible use for the prevention and treatment of various cancers.

Nitric oxide (NO) is one of the smallest signaling molecules that can diffuse into the cell [1]. It is present in almost all cells in the body, synthesized through several enzymatic and non-enzymatic pathways. As a free radical with complex redox chemistry, NO can modify all biological molecules and is, therefore, implicated in all biological functions in living systems. These pleiotropic biological actions are reflected in the publication of more than 117,000 articles on NO in the past 20 years. Out of this number, over 16,000 have been published on the role of NO in cancer. In spite of this extensive research in cancer, the role of NO in cancer is still ambiguous because NO is reported to be both anti- and pro-tumorigenic in animals and humans. The reason for these seemingly opposite effects is that: different types of cells respond differently to NO exposure the presence of varied levels of different forms of NOS in different tissues; and exogenous NO coming from the diet adds to the complexity of NO signaling [2].

NO is an important bioregulatory mediator involved in a variety of biological processes in both normal and pathophysiological conditions. NOS, particularly inducible NOS (iNOS or NOSII) and endothelial forms are overexpressed in various cancers in both humans and rodents (Table 1) [3–6]. NO can exert its effects directly by forming reactive nitrogen–oxygen species and indirectly by post-translational modifications of proteins via *S*-nitrosylation or tyrosine nitration [7,8]. Nitrosylation of proteins in key signaling pathways causes dysregulation of these pathways leading to disease formation [7,9]. Significant efforts

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have been made to address the iNOS/NO in tumor production and its effect on tumor biology. Before logically designing an effective preventive and therapeutic strategy to target tumor-associated iNOS/NO, one must clearly understand the mechanisms of iNOS expression and NO production and their actions in the tumor microenvironment, which have yet to be fully defined. There is growing interest in the cancer field to understand and target the effects of NO that play a key role in mediating tumorigenesis and metastases. In this review, the authors present some recent evidence on both the pro- and anti-tumor activities through direct and indirect effects of NO and will also discuss the implications of these data on the use of iNOS inhibitors in prevention of colorectal and other cancers.

NO generation & synthesis

NO is a free diatomic molecule formed from the nitrogen in the guanidine present in L-arginine, under the catalytic action of the NOS enzymes, generating equimolar concentrations of L-citrulline [10–12]. NO synthesis occurs from the activation of NOS, which exists as two isoforms: the constitutive and the inducible isoforms [13–15]. The constitutive isoforms (cNOS) were originally found in the endothelium and in neurons, thus, they were initially called endothelial NOS (eNOS) and neuronal NOS (nNOS), respectively. Both isoforms (eNOS and nNOS) are stimulated by a complex signaling pathway that is dependent or independent of Ca^{2+}. Both eNOS and nNOS require an electron donor, the $NADP^{+}$ and co-factors such as flavine-adenine-dinucleotide (FAD), flavine mononucleotide (FMN) and tetrahydrobiopterin (BH4) [15–17]. In humans and, presumably, in most other species, these isoforms are encoded by three different genes located in three different chromosomes [18]. iNOS is induced by pathological stimuli, such as bacterial lipopolysaccharide (LPS), cytokines, including IL-1, endotoxins and TNF, which is Ca^{2+}-independent, this isoform can be expressed in a large variety of cell types, including monocytes, macrophages, lymphocytes, neutrophils, eosinophils, Kupffer cells, hepatocytes and epithelial cells. One of the main differences between cNOS and iNOS is that during inflammatory response iNOS is able to release large amounts of NO for relatively long periods of time in a sustained manner, which can generate some exaggerated effects, producing toxic, tumorigenic responses in the body, whereas cNOS produces small amounts of NO within seconds and is short acting [19–21]. Irrespective of these differences in NOS isoforms, all of them act as catalysts in the oxidation of the terminal nitrogen atom in L-arginine forming equimolar amounts of NO and L-citrulline.

Non-enzymatic generation of NO

Dietary sources of nitrate and nitrite are physio-logically recycled in blood and tissues forming NO and other bioactive nitrogen oxides. Therefore, they are now viewed as storage pools for NO-like bioactivity, thereby complementing the NOS-dependent pathway. Nitrate is further reduced to NO by various pathways under hypoxic and acidic conditions. Nitrate from dietary sources is reduced to nitrite with the help of commensal bacteria present in the intestines. It is absorbed by into the bloodstream and picked up by the salivary glands. It is then released into mouth through saliva and oral bacteria use it as a food source to generate nitrite [22]. Nitrite is reabsorbed into the bloodstream [23].

Some of the animal studies suggested that nitrate itself is not carcinogenic. Maekawa *et al.* administered sodium nitrite in the drinking-water for 2 years at levels of 0.125 or 0.25% and sodium nitrate in the diet at levels 2.5 or 5% to F344 rats and observed no carcinogenic effect of nitrate or nitrite [24]. However, it has been reported that exposure to higher levels of nitrates and nitrites has been associated with increased incidence of cancer in adults [25–31]. Thus, the evidence that nitrate can cause diseases is controversial. There is even an emerging evidence of a possible benefit of nitrate in cardiovascular health. Although nitrates

as such may not be carcinogenic, in processed meat foods, due to the formation of *N*-nitroso compounds, they are carcinogenic. Individuals with increased rates of endogenous formation of carcinogenic *N*-nitroso compounds are likely to be susceptible to the development of cancers in the digestive system.

Association of iNOS with various cancers

Studies from humans and laboratory rodents show that iNOS has been associated with the development of cancers. Table 1 summarizes evidence in support of iNOS association during tumor development in humans and animals. Most of the studies emphasize the positive association with aggressive tumor promotional activities in almost all epithelial cancers.

Colon

Studies in both carcinogen-induced and genetic models support a role for iNOS in the promotion of colon carcinogenesis [5,6]. Increased iNOS expression and activity were observed in carcinogen-induced rat dysplastic aberrant crypt foci (ACF), adenomas and adenocarcinomas, but not in hyperplastic ACF [32]. The tumor-enhancing effects of iNOS in the colon may be associated with the ability of NO to increase the expression/activity of the enzyme COX-2 [33], likely via cross-talk between the iNOS and COX-2 signaling pathways [6]. In support of a role for iNOS in colon tumor promotion, mice from a $Apc^{Min/+}$-iNOS-knockout genetic background showed decreased intestinal tumor formation [34]. In humans, iNOS expression is up-regulated in carcinomas compared with patient's normal-appearing colonic mucosa (Table 1) [35,36].

Breast

Studies largely support a role for iNOS in the promotion of breast neoplasia. Vakkala *et al.* reported that iNOS expression increases with ductal carcinoma *in situ* (DCIS) grade and further increases in invasive lesions; increased expression also correlates with increased tumor vascularization and apoptotic index (Table 1) [37–39].

Prostate

Upregulation of iNOS expression has consistently been reported in cancerous prostatic tissue compared with normal and adjacent normal-appearing tissue [40–43]. Expression in precancerous high-grade prostate intraepithelial neoplasia (PIN) and cancerous is also more intense than that in low-grade PIN and benign lesions, suggesting that up-regulation is associated with progression to malignancy (Table 1) [44].

Bladder

Increased iNOS expression also has been found consistently in bladder cancers [45–48]. 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 (Table 1) [46].

Skin

iNOS also is upregulated during progression of malignant melanoma. Although iNOS is absent from benign melanocytic nevi, its expression increases during progression from cutaneous melanoma *in situ*, to invasive melanomas, to subcutaneous metastases. Also, iNOS overexpression is reported in human skin squamous cell carcinoma [49].

Similarly, overexpression of iNOS was reported in head and neck [50], esophageal [51] and other cancers. Overall, there is strong evidence that most cancers overexpress iNOS and upregulate iNOS activity, justifying it as valid target for chemoprevention (Table 1).

NO-induced *S*-nitrosylation & its tumorigenic effects

NO diffuses easily into surrounding tissues and affects targets at different locations through covalent binding, resulting in direct chemical modifications such as addition of *S*-nitrosothiols and nitrosotyrosine residues. *S*-nitrosylation regulates numerous signaling pathways in intact cellular systems, and recent genetic evidence supports a diversity of regulatory roles for this protein-modification reaction [52–54]. Ions (Ca^{2+}, Mg^{2+}, H^{+}) and O_2-based modifications, which can cause changes in protein structure, have been shown to promote *S*-nitrosylation and/or denitrosylation [55–58], and they also play a significant role in specifying the sites of *S*-nitrosylation. *S*-nitrosylation has been reported to regulate the activity of a number of metabolic enzymes, oxidoreductases, proteases, protein kinases and phosphatases, both *in vivo* and *in vitro*, as well as respiratory proteins, receptors, ion channels and transporters, cytoskeletal and structural components, transcription factors and regulatory elements, including G proteins [59].

NO induces *S*-nitrosylation of the active site cysteines in caspases and other related proteins leading to inhibition of apoptosis and the formation of *S*-nitrosothiols leading to the oxidation of thiol proteins, which may act as switches in cells survival and apoptotic signaling pathways [60–62]. Nitrosylation of nucleic acid bases leads to conversion of cytosine to uracil and guanine [61]. NO also can inhibit DNA repair by nitrosylation of DNA repair enzymes [63–66]. This might be one of the key elements favoring the carcinogenesis process during inflammatory conditions. NO is known to stimulate the anti-apoptotic Akt pathway via activation of Ras [67]. NO can protect human colon cancer cells from apoptosis *in vitro* by scavenging superoxide radicals in mitochondria [68]. This results in the formation of peroxynitrate (ONOO-), which can inactivate iron–sulphur proteins and cause DNA damage. Furthermore, the oxidant products of peroxide can initiate NF-κB and AP1 activation leading to tumor cell proliferation and development. NO in nanomolar concentrations has been reported to activate Ras post-translational modification via *S*-nitrosylation of the critical CYS118 residue, which stimulates guanine nucleotide exchange [69]. Ras is overexpressed in many cancers including pancreas and colon cancers. It is possible that low concentrations of NO could amplify Ras signaling by inducing conformational changes of membrane-bound Ras proteins. The authors have previously shown that by using iNOS and COX-2 inhibitors, there is molecular synergism between NO and COX-2 pathways in colon cancer [5–6,70]. Furthermore, Kim *et al.* reported that an increase in PGE2 formation by iNOS activation is due to binding of iNOS to COX-2 to deliver NO in appropriate proximity for *S*-nitrosylation of COX-2, which, in turn, activates COX-2 formation of PGE2 [71]. This may create favorable inflammatory conditions for tumor development.

Estrogen stimulation of endothelial NO production is well established [72–74]. Estrogens are reported to stimulate dynamic endothelial protein *S*-NO via mechanisms linked to specific estrogen receptors (ERs; β/α), possibly on the plasma membrane, and endogenous production of NO. The authors have observed more iNOS expression in human colon cancer cell lines expressing ER-β [Janakiram NB, Rao CV *et al.*, Unpublished Data]. Estrogen induced *S*-NO of proteins was blocked by both ERβ and ERα antagonists, suggests a potential role of estrogens in enhancing *S*-nitrosylation of proteins in cancer [75]. This observation of the authors and others suggest that estrogen may play a role in altering signal mechanisms through NO. These studies open a novel avenue for investigations on the

effects of estrogens and their receptors on nitrosylation and the effects on colon cancer cell proliferation.

Anti-tumorigenic properties of NO

Several reports suggest that NO can exert pro-apoptotic effects. A positive role of *S*-nitration in killing the cancer cells was reported by Leon-Bollotte *et al*. [76], post-translational modifications (*S*-nitrosylation of cysteine residues 199 and 304) in the cytoplasmic domain of Fas occurs in colon and mammary cancer cells and this leads to enhanced apoptosis; *S*-nitrosylation at CYS-304 promotes redistribution of Fas to lipid rafts, formation of the death-inducing signal complex, and induction of cell death in these cancer cells. Hussain *et al*. provided genetic and mechanistic evidence that NO can suppress tumorigenesis in transgenic mice [77].

Hussain *et al*. reported that p53, a tumor suppressor protein, accumulates upon NO mediated DNA damage and growth arrest [78]. NO inhibits the activity of NF-κB by stabilizing its inhibitor, Iκ-Bα. NF-κB is a constituent of one of the anti-apoptotic pathways observed to be overactivated during tumorigenesis [79]. NO is also reported to inhibit NF-κB by nitrosylating its p50 subunit, thus, inhibiting its binding to DNA [80]. In the ApcMin/+ colon cancer mouse model, deletion of iNOS (iNOS$^{-/-}$ mice) has been found to promote intestinal tumorigenesis, thereby substantiating a role for iNOS in host defense mechanisms [81]. This report is contradictory to the finding by Ahn *et al*. [34]. The reasons for opposite results in these animal models may be due to the type of diets given and the conditions in which the mice were harbored. These results simulate the conditions seen with human patients and further confirm the dual role of NO in a given situation. Evidence also suggests that NO regulates cell proliferation at the translational level. NO causes a cell cycle arrest through inhibition of cyclin D1 protein. Ambs *et al*. reported an increased concentration of p21 in tumors of iNOS expressing LoVo cells [82]. Ropponen *et al*. suggest that high iNOS staining intensity and percentage distribution in tumor epithelium may have a protective role against colorectal cancer progression in humans [83]. They reported that iNOS expression correlated positively with cell cycle regulators p21 and AP-2.

Concentration-dependent effects of NO on tumor growth

NO affects diverse signaling pathways and has opposing biological effects under different contexts and at concentrations (Figure 1). Low physio logical concentrations of NO are implicated in various different processes of tumorigenesis. Low concentrations of NO can stimulate cell growth and protect many cell types from apoptosis, whereas high concentrations of NO can inhibit cell growth and induce apoptosis [84]. Clinical and preclinical reports by the authors and others have shown a positive relationship between high expression of iNOS and tumorigenesis in colon tumors [5,6]. Solid tumors exhibited sustained levels of NO that may be produced by infiltrating cells such as monocytes, macrophages and fibroblasts. Some clinical studies have shown that iNOS increases significantly in colon adenoma and carcinoma with little or no expression in normal colon tissue [3,35,36], whereas other studies report that iNOS expression is decreased in colon cancer compared with high expression in normal colon tissue [85–88]. In animal models, induction of iNOS is correlated with colorectal cancer regression (both *in situ* and metastatic) [89,90], whereas the authors found that NOS inhibitors prevent colonic aberrant crypt foci formation and adenocarcinomas in azoxy methane (AOM)-induced rats (Table 2) [5,6,70,91]. NO also is shown either, to promote or to inhibit growth of colorectal cancer cells [92,93] and the effect was suggested to be concentration dependent [94]. Two separate groups recently reported opposite effects of iNOS gene knockouts in mice on intestinal carcinogenesis [3,95].

Liu *et al*. observed growth inhibitory activity at higher concentrations of GSNO, an NO-releasing compound. At low doses, NO exposure for 8 days with drug replenishment every other day caused cytostasis of Caco-2 cells by inhibition of ODC activity [97]. ODC is an important enzyme in polyamine synthesis, which is expressed highly in colon cancer and polyamines help in increased proliferation of tumor cells. This report suggests that a low dose of NO is an inhibitor of ODC and proliferation of tumor cells. In non-tumorigenic, non-transformed colon cells, the NO donors SNAP and NOR-1 increased both COX-2 mRNA transcription and protein synthesis [98]. Liu *et al*. reported that GSNO induced both COX-1 and -2 protein expression and stimulated PGE2 production in a dose- and time-dependent manner in three colon cancer cell lines [96]. Jenkins *et al*. reported that NO slowed down the growth of DLD-1, a colon adeno carcinoma cell line, *in vitro* but accelerated its cell growth *in vivo* [99]. However, micro encapsulated iNOS-expressing cells (human fetal kidney cell line EcR293) could inhibit DLD-1 cell growth *in vivo* (xenografts) and this was ascribed to the difference in iNOS activity in the cells [94,93]. It is possible that high levels of iNOS expression may be cytostatic/cytotoxic for tumor cells; lower activity can have the opposite effect, promoting tumor growth and neovascularization (Figure 1).

iNOS selective inhibitors & NO-releasing donors in cancer

NOS activity was increased in AOM-induced colonic tumors in rats [5,6,100] and NO and NOS are increased in Crohn's disease [101] and ulcerative colitis [102]. NO is overexpressed in preneoplastic lesions of the colon [103] and in human adenocarcinomas of the colon [104–106]. Wan *et al*. found high iNOS levels in the colon when animals were fed a high-fat diet and it is well known that a high-fat diet is associated with colon cancer in humans [107]. The many reports indicating high NOS activity and a positive role of NO in colon cancer support the testing of structural analogues of iNOS substrates and iNOS inhibitors to suppress/inhibit the induction of iNOS and to reduce its tumorigenic effects.

L-arginine structural analogues (Table 3) have been in use to inhibit tumor growth and proliferation. L-nitroarginine methyl ester (L-NAME) is an irreversible inhibitor of brain constitutive NOS (both nNOS and eNOS) with reversible effects on macrophage iNOS [108]. Kawamori *et al*. examined its influence on the development of ACF induced by AOM at a dose of 15 mg/kg once a week for 2 weeks in rats [91]. Dietary administration of 100 ppm of L-NAME for 11 weeks inhibited the development of ACF by 32% in terms of multiplicity (Table 2) [91]. The results are in line with the authors' study, which showed that *S,S*-1,4-phenylene-*bis*(1,2-ethanediyl)*bis*-isothiourea, a selective iNOS inhibitor, suppressed the development of ACF induced by AOM in rats and reduced protein levels of COX-2 and iNOS in colonic mucosa (Table 2) [5]. A similar observation was observed with Se-PBIT on ACFs induced by AOM in rats (Table 2). We have extensively studied the role of iNOS in molecular mechanisms of colon carcinogenesis [6,109].

Previously, we reported that low-dose combination of a COX-2 inhibitor (celecoxib) and an iNOS inhibitor (SC-51) inhibited AOM-induced crypt formation in rats (Table2) [6]. Cianchi *et al*. also reported that iNOS and COX-2 are co-expressed within same cancer cells and iNOS levels and PGE2 production correlative [110]. These data indicate that iNOS expression may correlate with increased COX-2 expression in cancer cells, which is similar to the observation in inflammatory conditions, hence, this effect may play a pivotal role in colon tumorigenesis [111]. It is likely that iNOS is associated with the modulation of COX-2 activity in colon cancer. NO enhances the activity and expression of COX-2 in a variety of cell types [112]. Overall, these data suggest that the rate of development of adenoma, adenocarcinoma [91,113] and adenomatous polyps [34] is significantly decreased with L-NAME and iNOS-specific inhibitors (Table 2).

NO has been reported to be involved in invasion and metastatic spread of tumors. The signaling cascades involved in the initiation of migratory behavior have been noted to be NO dependent. For example, colon tumors that are exposed to an iNOS inhibitor showed decreased potential for invasion, with decreased expression of VEGF [82]. Ridnour *et al.* reported the MMP9 is a key physiologic mediator of the effects of NO. MMPs are associated with cancer progression and usually high expressions of MMPs are observed in cancers [114]. Table 3 summarises various iNOS inhibitors and their IC_{50} values in human and animal cells [115]. Conversely, as discussed above, NO can also be anti-tumorigenic. Hence, chemopreventive interventions were created to develop NO-release compounds in colon cancer. The NO-releasing agent DETONONOate sensitized SW620 metastatic colon cancer cells to pro-apoptotic treatments [116]. The NO donor sodium nitroprusside induced caspases and reduced Bcl2 expression. Hence, these NO donors are being developed to sensitize or enhance apoptosis of cancer cells. NO drug molecules tagged to known nonsteroidal anti-inflammatory drugs (NSAIDs) as NO–NSAIDs, such as NO–aspirin, NO–sulindac, NO–naproxen, NO–ibuprofen are well studied. These agents are being tested *in vitro* and *in vivo* for growth inhibition in colon cancer [117]. The mode of action of some of these agents is reported to be through *S*-nitrosylation and tyrosine nitration of important signaling proteins, such as p53, NF-κB and Wnt signaling proteins in human colon cancer cells [118].

iNOS inhibitors are studied in detail in colon cancer, whereas very little studies are available on use of iNOS inhibitors in other cancers. The following available studies have suggested the usefulness of iNOS inhibitors in other cancers. NOS inhibitors (l-NAME, l-NMMA, cavtratin) showed decreased angiogenesis and tumor growth:

- In murine melanomas;
- By inhibition of eNOS in murine lungs;
- By inhibition of iNOS in breast tumors;
- By NOS inhibition in human head and neck tumors;
- By inhibition of e-NOS and iNOS in hepatoma by xenografts (Table 2) [119–121].

In another mouse study, iNOS deficiency decreased pulmonary metastases, impaired angiogenesis, and suppressed pleural effusion of injected murine melanoma cells [122]. Aminoguanidine caused statistically significant reduced proliferation and increased apoptosis in a xenograft model with injected gastric cancer cells compared with untreated animals [123]. *S,S′*-1,4-phenylenebis(1,2-ethanediyl)bis-isothiourea PBIT reduced the production of NO in nitrosomethylbenzylamine (NMBA)-induced preneoplastic and papillomatous esophageal lesions when compared with comparable lesions in rats treated with NMBA only [124]. PBIT caused a statistically significant reduction in tumor incidence and multiplicity in rats fed PBIT in an NMBA-induced rat model of esophageal cancer (Table 2) [124].

Conclusion & future perspective

NO plays an important role in growth, immunity and development. The role of NO in cancer is multifactorial based on its temporal and spatial concentrations. Various iNOS inhibitors have been under development for colon cancer, individually or in combination with COX-2 inhibitors for better efficacy. Simultaneous inhibition of external and internal sources of NO can prove beneficial in suppressing tumor formation. The number of factors affecting NO generation, including types of NOS present and the complex concentration-dependent actions, make the design of specific inhibitors for use in colon cancer challenging. Post-

translational modification of proteins by NO, such as protein nitrosylation, has emerged as an important mechanism for regulation of the activity and function of key proteins in various signaling pathways. Identification and targeting of specific nitrosylated proteins in key pathways affecting tumor initiation, growth and metastasis may provide a better approach to tackle in colon and other cancers. Although the most desirable animal models in colon cancer, such as AOM-induced colon cancer in F344 rats, ApcMin/+ mice are available, opt preclinical models that simulate initiation and development of other human cancers, as well as methods to evaluate the role of NO and its inhibition during initiation, or development of cancer are needed. Continuous investigation into the biology of NO at initiation or early development of cancers will improve our knowledge on its signaling functions and may help in development of more appropriate agents to prevent colon and other cancers.

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Executive summary

Background

- NO is synthesized by NOS. Specifically, inducible NOS (iNOS) is implicated in various cancers. NO can cause protein post-translational modifications leading to deregulation of signaling pathways causing diseases. Both pro- and anti-tumorigenic effects of NO are reported.

NO generation & synthesis

- NO is generated by the action of NOS enzymes on L-arginine.

Non-enzymatic generation of NO

- Inorganic nitrate from dietary sources is another major source of NO, independent of the arginine-dependent NOS pathway.

Association of iNOS with various cancers

- High iNOS expression is reported in almost all epithelial cancers, suggesting its tumorigenic potential. iNOS expressions were observed at preneoplastic or low grade lesion stages in the mentioned epithelial cancers justifying it as a valid target for chemoprevention.

NO induced S-nitrosylation & its tumorigenic effects

- NO can produce reactive nitrogen species that cause post-translational modification of proteins, such as enzymes, receptors, respiratory proteins and ion channels, altering their functions. This leads to anti-apoptotic signaling and helps in tumor development.

Anti-tumorigenic properties of NO

- NO mediates growth arrest on tumor cells. Also, *S*-nitrosylation of proteins causes pro-apoptotic effects of tumor cells.

Concentration-dependent effects of NO on tumor growth

- NO is observed to possess both pro- and anti-tumorigenic properties. This biphasic nature of NO is attributed to its concentrations effects on tumor growth or inhibition.

iNOS-selective inhibitors & NO-releasing donors in cancer

- As it is evident that iNOS is expressed at preneoplastic lesion stages, use of specific iNOS inhibitors at early stages in epithelial cancers such as colon cancer inhibited these early lesions and also invasive adenocarcinomas. Also, NO-releasing donors have shown anti-tumorigenic efficacy in *in vivo* and *in vitro* cancer models.

Conclusion & future perspective

- Expression of iNOS, concentrations of NO, time of NO exposure, region of exposure, are important factors that determine NO functions. Also, understanding the above effects of NO and role of nitrosylated proteins in a tumor cell or in tumor environment may help in better designing of preventive inhibitors for colon and other epithelial cancers.

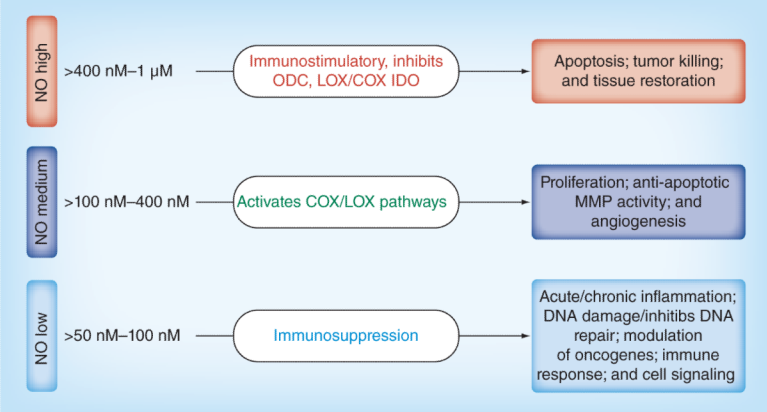

Figure 1. Pleiotropic effects of different levels of nitric oxide on inflammation and tissue response

NO: Nitric oxide.

Table 1

Inducible NOS expression in various cancers.

Organ	Expression of inducible NOS in	Ref.
Colon	Aberrant crypt foci	[5,6,32]
	Adenoma	
	Adenocarcinoma	
Breast	Invasive lesions	[38,39]
	Tumors	
Prostate	High-grade prostatic intraepithelial neoplasia	[40–44,50]
	Cancer	
Bladder	Dysplastic lesions	[45–48]
	Carcinoma	
Skin	Preneoplastic	[50]
	Papillilomatous lesions	
Esophageal	Malignant melanoma	[51]
Head and neck	Carcinoma	[49]

Table 2

Effect of inducible NOS inhibitors on aberrant crypt foci/tumors inhibition in different animal cancer models.

Organ site	iNOS inhibitor	% inhibition	Ref.
Colon cancer			
Rat (AOM)- aberrant crypt foci	PBIT	78 (4/ > crypts Foci)	[5]
	PBIT-Se	47 (4/ > crypts Foci)	[125]
	AG1(high dose)	48 (4/ > crypts Foci)	[6]
	SC51 (high dose)	52 (4/ > crypts Foci)	[6]
	SC51+celecoxib (low-dose combination)	41 (4/ > crypts Foci)	[6]
Rat (AOM) adenocarcinoma	PBIT	43 – incidence	[126]
		60 – multiplicity	
		70 – incidence	[126]
		83 – multiplicity	
	PBIT and celecoxib	40 – incidence	[126]
		46 – multiplicity	
	NILT	40 – incidence	[70]
	Adenocarcinoma	60 – multiplicity	[70]
	Invasive adenocarcinoma	67 – multiplicity	[70]
	NILT + Celecoxib	70 – incidence	[70]
	Adenocarcinomas	77 – multiplicity	
Mouse ($Apc^{Min/+}$-$iNOS^{-/-}$)	AG	29 – incidence	[34]
		7 – multiplicity	
Mouse ($Apc^{Min/+}$) high-fat diet	PBIT	>80	[127]
Mouse (xenograft with iNOS-expressing human colon tumor)	1400W	Promoting effect	[128]
Mouse (iNOS-expressing intra-tumoral-macrophages)	1400W	No effect	[128]
Gastric cancer			
Mice (Xenografts)	Aminoguanidine	35–47	[129]
Mammary cancer			
Mouse (iNOS-expressing adenocarcinoma)	1400W	49–59	[128]
Liver cancer			
Mice (HepG2 tumors)	Cavtratin	52	[119]
Lung cancer			
Mice (Lewis lung cancer tumors)	Cavtratin	38	[119]
Esophageal cancer			
Rat (nitrosomethylbenzylamine)	PBIT	77–83 – incidence	[124]
		50–59 – multiplicity	
Head and neck			
Rabbit (human tumor specimens, A-431 cells)	L-NMMA	Reduced angiogenesis/invasion	[129]
Melanoma			

Organ site	iNOS inhibitor	% inhibition	Ref.
Mice (xenografts with B16F1 and B16F10)	L-NMMA	Reduced angiogenesis/invasion	[122]

AG: Aminoguanidine; AOM: Azoxymethane; iNOS: Inducible NOS; NILT: N6-iminoethyl-lysine tetrazoleamide; NMMA: N-Monomethyl-L-arginine; PBIT: S,S'-1,4-phenylenebis(1,2-ethanediyl)bis-isothiourea.

Table 3

List of induced NOS inhibitors and their selectivity at a particular concentration for different NOS.

Arginine analogues	Neuronal NOS	Found in
L-arginine	1.6 μM	Rat
L-canavanine	60 μM	Murine
L-NAMA	0.18 μM	Rat
L-NNA	15 nM	Bovine
N6, NG-dimethyl-L-arginine		
L-NIL	35 μM	Human
	92 μM	Rat
NILT	1850 μM	Human
L-NIO	1.7 μM	Rat
Vinyl-L-NIO	100 nM	Rat
AMT	34 μM	Rat
L-thiocitrulline	0.06 μM	Rat
S-methyl-L-thiocitrulline	50 nM	Rat
	1.2 nM	Human
N-propyl-L-arginine	57 nM	Bovine
1400W	2 μM	Human
Urea/guanidine derivatives		
A-guanidinoglutaric acid	2.7 μM	Rat
S-(2-aminoethyl)isothiourea	1.8 μM	Human
EIT hydrobromide		
AMT	34 nM	Rat
Mercaptoethylguanidine	60 μm	Rat
S-ethylisothiourea	29 nM	Human
	250 nM	Rat
S-ethyl-*N*-[4-(trifuromethyl)phenyl] isothiourea	0.32 μM	Human
S-isopropylisothiourea	37 nM	Human
S-methylisothiourea	0.16 μM	Human
1,3-PBIT	0.25 μM	Human
1,4-PBIT	16 nM	Human

Arginine analogues	Neuronal NOS	Found in
Aminoguanidine	160 μM	Rat
Other induced NOS inhibitors		
2-imi no-4-methylpiperidine	0.2 μM	
TRIM	28.2 μM	Murine
7-nitroindazole	0.7 μM	Murine
3-bromo-7-nitroindazole	0.17 μM	Rat
BYK 191023 dihydrochloride	17000 nM	

AMT: 2-amino-5,6-dihydro-6-methyl-4H-l,3-thiazine; L-NIL: L-N^6-(1-iminoethyl)lysine dihydrochloride; L-NIO: N(5)-(-iminoethyl)-L-ornithine; L-NNA: Nω-nitro-L-arginine; NILT: N6-iminoethyl-lysine tetrazoleamide; PBIT: S,S'-1,4-phenylenebis(1,2-ethanediyl)bis-isothiourea; TRIM: 1-[2-trifluoromethylphenyl] imidazole.