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Nitrative DNA damage in inflammation and its possible role in carcinogenesis ☆

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Abstract

Chronic inflammation has long been recognized as a risk factor for human cancer at various sites. Examples include *Helicobacter pylori*-induced gastritis for gastric cancer, inflammatory bowel disease (ulcerative colitis and Crohn's disease) for colorectal cancer and chronic viral hepatitis for liver cancer. Here we review the role in carcinogenesis of nitrative damage to nucleic acids, DNA and RNA, which occurs during inflammation through the generation of reactive nitrogen species, such as peroxynitrite, nitroxyl, and nitrogen dioxide. Enhanced formation of 8-nitroguanine, representative of nitrative damage to nucleobases, has been detected in various inflammatory conditions. The biochemical nature of DNA damage mediated by reactive nitrogen species is discussed in relation to its possible involvement in mutations, genetic instability, and cell death. Better understanding of the mechanisms and role of such nitrative damage in chronic inflammation-associated human cancer is a necessary basis to develop new strategies for cancer prevention by modulating the process of inflammation.

Keywords: Chronic inflammation; Carcinogenesis; Reactive nitrogen species; 8-Nitroguanine; DNA damage; Inducible nitric oxide synthase; Peroxynitrite; Nitroxyl; Nitrative stress; 3-Nitrotyrosine

Chronic inflammation has long been recognized as being associated with increased risk for human cancer at various sites. Examples include *Helicobacter pylori* (*H. pylori*)-induced gastritis for gastric cancer, inflammatory bowel disease (ulcerative colitis and Crohn's disease) for colorectal cancer, chronic viral hepatitis for liver cancer, liver fluke infection for cholangiocarcinoma, and Barrett's esophagus for esophageal cancer [1–4]. Recent studies have shown that inflammation of many of these tissues is accompanied by upregulation of an inducible isoform of nitric oxide (NO) synthase (iNOS)[1] (reviewed in [5]), that is capable of producing excess NO for a prolonged period [6]. Although host defense and cytoprotective functions of NO have been demonstrated [7–11], accumulating evidence indicates that NO-derived reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$), nitroxyl (HNO), N_2O_3, and nitrogen dioxide ($^{\cdot}NO_2$) also have pathogenic potential in various diseases [12–14], possibly through induction of oxidative, nitrative, and nitrosative damage to nucleic acids, proteins, and lipids. It has been proposed that DNA and tissue damage induced by RNS may contribute to increased mutation rates, genome instability, apoptosis and associated tissue regeneration and cell proliferation, all of which can drive carcinogenesis. Here we discuss the role of DNA damage mediated by RNS in inflammation and its possible implication in carcinogenesis, with special reference to the formation of 8-nitroguanine as an index for nitrative DNA damage. The reader is referred to several useful review

☆ Dedicated to Professor H. Maeda, on the occasion of his retirement from the Kumamoto University Graduate School of Medicine.

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[1] *Abbreviations used:* Fpg, formamidopyrimidine-DNA glycosylase; Gh, guanidinohydantoin; iNOS, inducible nitric oxide synthase; NIm, 5-guanidino-4-nitroimidazole; RNS, reactive nitrogen species; Sp, spiroiminodihydantoin.

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articles for more details of the chemistry and biochemistry of DNA damage induced by RNS [15–18].

iNOS expression in inflammatory conditions associated with cancer susceptibility

Upregulation of iNOS expression has been observed in several inflammatory conditions associated with cancer susceptibility [5] such as Crohn's disease [19,20], ulcerative colitis [19–21], viral hepatitis [22], *H. pylori* gastritis [23–25], and Barrett's esophagus [26]. iNOS is predominantly expressed in inflammatory cells such as macrophages, while epithelial cells from affected tissues also express iNOS. Intense expression of iNOS has been detected in both epithelial and lamina propria mononuclear cells and in neutrophils in patients with ulcerative colitis and Crohn's diseases [19–21]. The level of iNOS expression is well correlated with the degree of inflammation [21]. Similarly, iNOS expression has been found in foveolar cells from *H. pylori*-positive gastric mucosa [24,25]. iNOS expression has also been reported consistently in human cancer cells at variety of sites, including the bladder, prostate, oral cavity, and esophagus and less consistently the stomach, colon, and breast [27].

How iNOS participates in carcinogenesis has not been fully established. Its effect may vary, depending on the type of cancer and experimental factors such as animal model, genetic background, and carcinogenic stimuli (e.g., induced by chemical carcinogens and/or inflammatory reactions) [5,27]. Tatemichi et al. [28] have reported that iNOS may be responsible for the development of thymic lymphomas in tumor suppressor p53-deficient mice which spontaneously develop lymphomas, mainly of thymic origin. In the same experiment, however, they observed an opposite effect of iNOS; lack of iNOS enhanced the development of nonthymic lymphoma, suggesting that iNOS may have protective effects against nonthymic lymphomagenesis. Similar findings have also been reported by Hussain et al. [29]. Ahn and Ohshima [30] observed that iNOS deficiency leads to decreased formation of adenomas in the colon of $Apc^{Min/+}$ mice which have a germline nonsense mutation at codon 850 of the adenomatous polyposis coli (*Apc*) gene and spontaneously develop multiple polyps in the small and large intestines. Pharmacological inhibition of iNOS produced similar results [30,31]. On the contrary, Scott et al. [32] reported that iNOS knockout Min mice developed slightly, but significantly, more intestinal adenomas than iNOS-replete littermates, suggesting that iNOS may have an inhibitory effect on tumor development. Additional studies are needed to clarify the role of iNOS in tumor formation in the context of *Apc* loss [27]. Induction of lung tumors by urethane and of gastric cancer by *N*-methyl-*N*-nitrosourea and *H. pylori* was reduced in iNOS-deficient mice [33,34]. These observations support the hypothesis that iNOS expression, particularly in inflamed tissue, may contribute to carcinogenesis. In this context, it is noteworthy that an inflammatory reaction can produce excess amounts of reactive oxidants such as superoxide and hydrogen peroxide (H_2O_2) simultaneously with iNOS expression, and this can stimulate the formation of more toxic RNS at the site of inflammation [2]. In fact, several of above-mentioned inflammatory diseases are accompanied by elevated formation of 3-nitrotyrosine [19–21,23–25], a marker for exposure to nitrating agents such as peroxynitrite and $^{\bullet}NO_2$ [35,36]. Again, 3-nitrotyrosine staining is not limited to inflammatory cells, but is also clearly visible in affected epithelial cells [19–21,24,25].

We have recently found an association between a specific polymorphism in the promoter region of the iNOS gene (CCTTT pentanucleotide tandem repeat number polymorphism) and the intestinal type of gastric adenocarcinoma in nonsmoking Japanese women [37]. These results imply that subjects with high activity of the iNOS promoter are at elevated risk for *H. pylori*-induced gastric cancer, possibly due to increased tissue damage caused by excess NO generated by iNOS [37].

DNA base modifications induced by RNS and mutagenic spectra

NO itself is not very reactive with DNA, but RNS formed by the reaction of NO and oxygen radicals are potent DNA-damaging agents, which can cause both DNA base modifications and strand breaks. DNA base modifications induced by RNS can occur by four types of chemical reaction: (i) alkylation via nitrosamine formation, (ii) deamination, (iii) oxidation, and (iv) nitration [15–18].

NO is autooxidized under aerobic conditions to form the strong nitrosating agent N_2O_3. Nitrosation of secondary amines by N_2O_3 generates *N*-nitrosamines which can alkylate nucleobases to form mutagenic lesions such as O^6-alkylguanine, inducing a G to A transition [38]. Increased formation of *N*-nitrosamines has been reported to occur in vivo in experimental animals with acute and chronic inflammation as well as in human subjects with infection and inflammation [1,39]. On the other hand, direct attack of N_2O_3 on DNA can lead to DNA deamination by formation of a diazonium ion which is then hydrolyzed to form a deaminated base [38]. Deamination of DNA bases results in the formation of uracil from cytosine, xanthine from guanine, and hypoxanthine from adenine [40]. Deamination of 5-methylcytosine at CpG by N_2O_3 results in the formation of thymine, which can induce C to T transition mutations at CpG, one of the most frequently detected mutations in the *TP53* tumor suppressor gene and the