

Review Article

Role of Nitrative and Oxidative DNA Damage in Inflammation-Related Carcinogenesis

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Chronic inflammation induced by biological, chemical, and physical factors has been found to be associated with the increased risk of cancer in various organs. We revealed that infectious agents including liver fluke, *Helicobacter pylori*, and human papilloma virus and noninfectious agents such as asbestos fiber induced iNOS-dependent formation of 8-nitroguanine and 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG) in cancer tissues and precancerous regions. Our results with the colocalization of phosphorylated ATM and γ -H2AX with 8-oxodG and 8-nitroguanine in inflammation-related cancer tissues suggest that DNA base damage leads to double-stranded breaks. It is interesting from the aspect of genetic instability. We also demonstrated IL-6-modulated iNOS expression via STAT3 and EGFR in Epstein-Barr-virus-associated nasopharyngeal carcinoma and found promoter hypermethylation in several tumor suppressor genes. Such epigenetic alteration may occur by controlling the DNA methylation through IL-6-mediated JAK/STAT3 pathways. Collectively, 8-nitroguanine would be a useful biomarker for predicting the risk of inflammation-related cancers.

1. Introduction

Chronic inflammation induced by biological, chemical, and physical factors has been found to be associated with the increased risk of cancer in various organs [1–3] (Table 1). Inflammation activates a variety of inflammatory cells, which trigger oxidant-generating enzymes such as inducible nitric oxide synthase (iNOS), NADPH oxidase, and myeloperoxidase to produce high concentrations of free radicals including reactive nitrogen species (RNS) and reactive oxygen species (ROS) [1]. Overproduction of RNS and ROS can change the balance of oxidants and antioxidants and cause nitrative and oxidative stress which contributes to the damage of biomolecules such as DNA, RNA, lipid and proteins, leading to an increase in mutations, genomic instability, epigenetic changes, and protein dysfunction and play roles in the multistage carcinogenic process.

ROS generate 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG, also known as 8-hydroxydG (8-OHdG)), a marker of oxidative DNA damage [4, 5]. 8-OxodG, a potentially muta-

genic DNA lesion, leading to the transversion of G : C to T : A (G → T transversion) [6], has been implicated in cancers triggered by infections [7]. The generation of ROS is not confined to inflammatory processes. Carcinogenic chemicals and their metabolites as well as electron transport chains in mitochondria are able to generate ROS. On the other hand, nitric oxide (NO), a primary initiator of RNS, is generated specifically during inflammation via iNOS in inflammatory and epithelial cells [5, 8]. Overproduction of NO participates in the generation of peroxynitrite (ONOO⁻), which can lead to the formation of 8-nitroguanine, an indicator of nitrative DNA damage [9, 10]. 8-Nitroguanine undergoes spontaneous depurination in DNA, resulting in the formation of an apurinic site [11]. Incorporated adenine can form a pair with apurinic sites during DNA replication, leading to the G → T transversion [12] (Figure 1). Moreover, apurinic sites might represent major damage that requires error-prone DNA polymerase ζ for efficient *trans*-lesion DNA synthesis. It was reported that DNA polymerase ζ can efficiently bypass abasic sites by extending from nucleotides

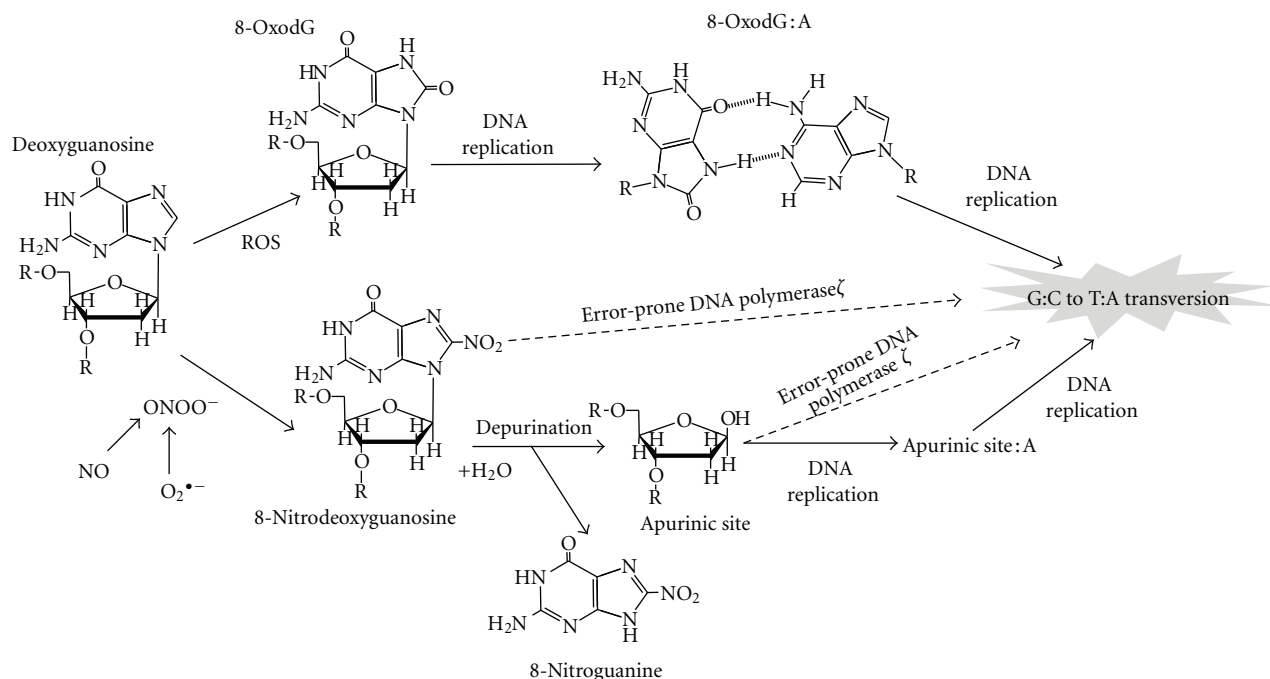


FIGURE 1: Proposed mechanism of point mutation induced by 8-nitroguanine and 8-oxodG through induction of the G:C → T:A transversion.

TABLE 1: Nitrative and oxidative DNA damage in inflammation-induced carcinogenesis.

Etiologic agent/pathologic condition	IARC classification ^a	Cancer site	Associated neoplasm	Detection of DNA lesions ^c [reference no.]
(I) Infection agent				
Viruses				
HPV ^b				
High-risk types	1	Cervix and other site	Cervical carcinoma	IHC [38]
Low-risk types	2A			
HCV, HBV ^b	1	Liver	Hepatocellular carcinoma	IHC [56–59]
EBV ^b	1	Nasopharynx	Nasopharyngeal carcinoma	IHC [38, 49, 50], ELISA [49]
Bacterium				
<i>Helicobacter pylori</i>	1	Stomach	Gastric cancer	IHC [36]
Parasites				
<i>Opisthorchis viverrini</i>	1	Intra- and extrahepatic bile duct	Cholangiocarcinoma	IHC [17, 22–26], HPLC-ECD [23, 27]
<i>Schistosoma haematobium</i>	1	Bladder	Bladder cancer	IHC [60]
(II) Inflammatory disease				
Asbestos fiber	1	Lung	Mesothelioma, lung carcinoma	IHC [61]
Reflux oesophagitis Barrett's oesophagitis		Oesophagus	Oesophageal carcinoma	IHC (In prep.)
Lichen planus		Oral	Oral squamous cell carcinoma	IHC [62]
Inflammatory bowel disease		Colon	Colorectal carcinoma	IHC [63]
Crohn's disease				IHC (this paper)
Chronic ulcerative colitis				
Unknown		Soft tissue	Malignant fibrous histiocytoma	IHC [64, 65]

This table was adapted and modified from the IARC [2] and Coussens and Werb [1].

IARC: International Agency for Research on Cancer. ^aIARC classification: Group 1: carcinogenic to humans; Group 2A: probably carcinogenic to humans.

^bHPV: human papilloma virus; HBV: hepatitis B virus; HCV: hepatitis C virus; EBV: Epstein-Barr virus.

^cDNA lesions: IHC, 8-nitroguanine and 8-oxodG detected by immunohistochemistry; HPLC-ECD: 8-oxodG detected by HPLC-ECD; ELISA: serum 8-oxodG detected by ELISA.

inserted opposite the lesion by other DNA polymerases [13]. Wu et al. suggested that cells deficient in subunits of DNA polymerase ζ were hypersensitive to nitrative stress, and *trans*-lesion DNA synthesis mediated by this polymerase contributes to extensive point mutations [14]. Additionally, DNA polymerases η and κ were also found to be involved in the incorporation of adenine opposite 8-nitroguanine during DNA synthesis in a cell-free system associated with *trans*-lesion DNA synthesis leading to the G \rightarrow T transversion [15]. Therefore, 8-nitroguanine is a potential mutagenic DNA lesion involved in inflammation-mediated carcinogenesis. Relevantly, systematic and comprehensive genome-scale approaches by using the immunoprecipitation-based technique combined with high-density microarrays may be useful to investigate roles of DNA lesions in carcinogenesis [16].

We focus on the roles of nitrative and oxidative DNA damage in infection- and inflammation-related carcinogenesis. We produced a specific anti-8-nitroguanine antibody [17] and examined the localization of DNA lesions by immunohistochemical analysis in animal models and clinical samples (Table 1). Here, we review the effects of RNS-/ROS-mediated DNA damage on genomic instability and epigenetic change in relation to carcinogenesis.

2. DNA Damage in Infection-Related Carcinogenesis

2.1. Liver Fluke Infection and Cholangiocarcinoma. Liver fluke infections of *Opisthorchis viverrini* (*O. viverrini*) are a risk factor for cholangiocarcinoma in Southeast Asia [18]. *O. viverrini* infestations are endemic in Khon Kaen province, northeastern Thailand, and Khon Kaen has the highest incidence of cholangiocarcinoma in the world [19]. *O. viverrini* infections induce inflammation in both animal models [20] and humans [21]. Our previous studies showed that 8-oxodG and 8-nitroguanine levels were increased in *O. viverrini*-infected hamsters compared with uninfected control groups [17, 22–24]. In addition, DNA damage was significantly increased in reinfected hamsters compared with animals infected just once [23]. Notably, repeated infection increased iNOS expression and 8-nitroguanine production in the epithelium of bile ducts even after a decrease in inflammatory cells. To elucidate the mechanism involved, we examined the expression of iNOS, NF- κ B, and Toll-like receptor (TLR) 2 in mouse macrophage cell lines treated with *O. viverrini* crude antigens [25], suggesting that *O. viverrini* infection induced TLR2 activation with NF- κ B-dependent transcription and iNOS expression. Treatment with an antiparasitic drug (praziquantel) significantly improved the DNA lesions [22]. These findings in hamsters were confirmed by the observation that 8-oxodG and 8-nitroguanine accumulated more in cancerous areas than in intrahepatic areas adjacent to tumors in surgical specimens [26]. Furthermore, an epidemiological study of *O. viverrini*-infected subjects and cholangiocarcinoma patients demonstrated that urinary 8-oxodG levels were significantly higher in cholangiocarcinoma patients than in *O. viverrini*-infected patients and healthy subjects and higher in *O. viverrini*-infected

subjects than in healthy subjects [27]. The urinary 8-oxodG levels in *O. viverrini*-infected patients significantly decreased two months after praziquantel treatment and were comparable to levels in healthy subjects one year after treatment [27]. These results indicate that *O. viverrini* causes chronic and recurrent inflammation followed by the accumulation of oxidative and nitrative DNA lesions, which may participate in the development of cholangiocarcinomas.

2.2. *H. pylori* and Gastric Cancer. *Helicobacter pylori* is the main cause of chronic gastritis and a potential risk factor for gastric carcinoma [28]. The molecular mechanisms behind *H. pylori*-induced production of ROS/RNS were wide ranging from activated neutrophils to *H. pylori* itself, as nicely reviewed by Handa et al. [29]. *H. pylori* infections promote the secretion of various inflammatory cytokines, contributing directly to the pronounced inflammatory response. Lipopolysaccharide, a component of Gram-negative bacteria such as *H. pylori*, is a TLR4 ligand that induces inflammatory responses via NF- κ B expression [30]. NF- κ B, which is involved in the regulation of iNOS, had been reported to function as a tumor promoter in inflammation-associated cancer [31, 32]. In patients with *H. pylori*-induced gastritis or gastric ulcers, iNOS is expressed in the infiltrating inflammatory cells [33]. The expression of iNOS mRNA and protein was significantly increased in the epithelial cells of *H. pylori*-positive gastritis patients compared to *H. pylori*-negative patients [34]. Recently, it was also found that *H. pylori* in a Korean isolate induced the expression of iNOS via AP-1 activation [35]. Our previous study [36] demonstrated that levels of 8-nitroguanine and 8-oxodG in gastric gland epithelium were significantly higher in gastritis patients with *H. pylori* infections than in those without infections. A significant accumulation of proliferating cell nuclear antigen (PCNA) was observed in gastric gland epithelial cells in patients infected with *H. pylori* in comparison to those not infected. Interestingly, the accumulation of PCNA was closely correlated with the formation of 8-nitroguanine and 8-oxodG. Collectively, the host response to *H. pylori* mediated NF- κ B expression, resulting in iNOS expression accompanied by 8-nitroguanine and 8-oxodG production in the gastric epithelium. 8-Nitroguanine could be not only a promising biomarker for inflammation but also a useful indicator of the risk of developing gastric cancer in response to chronic *H. pylori* infection.

2.3. HPV and Cervical Carcinoma. Cervical cancer is the second most common cancer among women worldwide and the most common cancer among women in many developing countries [37]. Inflammation is proposed to play an integral role in the development of human papilloma virus (HPV)-induced cervical cancer [1]. Our previous study [38] examined the formation of 8-nitroguanine and 8-oxodG in cells of cervical intraepithelial neoplasia (CIN, grades 1–3) and condyloma acuminatum samples and compared it with the expression of the cyclin-dependent kinase inhibitor p16, considered a biomarker for cervical neoplasia [39–42]. Double immunofluorescence labeling revealed that 8-nitroguanine and 8-oxodG immunoreactivities correlated significantly

with CIN grade. There were no statistically significant differences in p16 expression between CIN and condyloma acuminatum samples. These results suggest that high-risk HPV types promote iNOS-dependent DNA damage, which leads to dysplastic changes and carcinogenesis. Therefore, 8-nitroguanine is a more suitable and promising biomarker for evaluating the risk of inflammation-mediated cervical carcinogenesis than p16.

2.4. EBV and Nasopharyngeal Carcinoma. Nasopharyngeal carcinoma (NPC) is strongly associated with Epstein-Barr virus (EBV) infections [43]. Various transcription factors are known to participate in iNOS expression including signal transducers and activators of transcription (STATs), such as STAT1 α and STAT3 [44, 45]. Epidermal growth factor receptor (EGFR) physically interacts with STAT3 in the nucleus, leading to transcriptional activation of iNOS [44]. STAT3 is repeatedly activated through phosphorylation via the expression of latent membrane protein 1 (LMP1) as well as EGFR [46, 47], and interleukin-6 (IL-6) is required for LMP1-mediated STAT3 activation [46]. In addition, LMP1-mediated iNOS expression was reported in EBV-infected epithelium cell lines, which play a role in colonization independent of anchorage and tumorigenicity in nude mice [48]. Using biopsy and surgical specimens of nasopharyngeal tissues from NPC patients in southern China, we performed double immunofluorescent staining to examine the formation of 8-nitroguanine and 8-oxodG [49, 50]. Intensive immunoreactivity to iNOS was detected in the cytoplasm of 8-nitroguanine-positive cancer cells. DNA lesions and iNOS expression were also observed in epithelial cells of EBV-positive patients with chronic nasopharyngitis but weaker than those in NPC patients. No or few DNA lesions were observed in EBV-negative subjects. EGFR and phosphorylated STAT3 were strongly expressed in cancer cells of NPC patients, suggesting that the STAT3-dependent mechanism is important to the carcinogenesis [50]. IL-6 was expressed mainly in inflammatory cells of nasopharyngeal tissues of EBV-infected patients. We also found that serum levels of 8-oxodG were significantly higher in NPC patients than control subjects [49]. Collectively, these findings indicate that the nuclear accumulation of EGFR and activation of STAT3 by IL-6 play a key role in iNOS expression and resultant DNA damage, leading to EBV-related NPC.

2.5. HCV and Hepatocellular Carcinoma. Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma throughout the world [51]. Hepatocellular carcinoma arises through genetic alterations in hepatocytes during a chronic HCV infection [52–55]. We investigated the extent of nucleic acid damage in HCV-infected individuals and its change after interferon treatment [56]. Immunoreactivities of 8-nitroguanine and 8-oxodG were strongly detected in the liver of patients with chronic hepatitis C, but not control subjects. 8-Nitroguanine was found to be accumulated in hepatocytes particularly in the periportal area. In the sustained virological responder group after interferon therapy, the accumulation of 8-nitroguanine and 8-oxodG was markedly decreased in the

liver. We observed a strong correlation between hepatic 8-oxodG staining and serum ferritin levels, suggesting the iron content to be a strong mediator of oxidative stress and iron reduction to reduce the incidence of hepatocellular carcinoma in patients with chronic hepatitis C [57, 58]. We also demonstrated that oxidative DNA damage widely occurred in the livers of patients with chronic viral hepatitis especially chronic hepatitis C, and the iron load and 8-oxodG-positive hepatocytic count was significantly higher in HCV-infected than in HBV-infected livers [59]. It is plausible that ROS production during chronic HCV infection is the result of high iron levels in hepatic tissues, which lead to progressive liver inflammation and an increased risk of developing liver cancer. These findings indicate that 8-nitroguanine and 8-oxodG are useful as biomarkers for evaluating the severity of HCV-induced chronic inflammation leading to hepatocellular carcinoma and the efficacy of chronic hepatitis C treatment.

3. DNA Damage in Inflammation-Related Carcinogenesis

3.1. Asbestos and Lung Carcinoma. Excessive and persistent production of ROS/RNS by inflammatory cells is considered as a hallmark of the secondary genotoxicity of nonfibrous and fibrous particles including asbestos [66]. Asbestos is a carcinogen (IARC Group1) causing lung cancer and malignant mesothelioma of the pleura and peritoneum [67]. Among the different types of asbestos, crocidolite (blue asbestos) and amosite (brown asbestos) are more potent carcinogens than chrysotile (white asbestos) [67]. Inflammation is a hallmark of the response to exposure to asbestos in both animal and human models [68, 69]. NO and nitrate stress were reported to be involved in the asbestos-derived inflammatory response via myeloperoxidase, a major constituent of neutrophils which generates hypochlorous acid and RNS [70–73]. Myeloperoxidase plays a significant role in asbestos-induced carcinogenesis [74]. However, the precise mechanisms of nitrate DNA damage remain to be clarified. We performed an immunohistochemical analysis to examine the formation of 8-nitroguanine and the expression of iNOS and its transcription factor (NF- κ B) in the lungs of mice intratracheally administered asbestos fibers, including crocidolite and chrysotile [61]. 8-Nitroguanine was significantly detected in bronchial epithelial cells of asbestos-exposed groups compared with the untreated group. Interestingly, the immunoreactivities of 8-nitroguanine, iNOS, and NF- κ B were significantly higher in the crocidolite-exposed group than in the chrysotile-exposed group. Therefore, the formation of nitrate DNA damage could be one of the mechanisms responsible for the difference in carcinogenic potential between crocidolite and chrysotile.

3.2. Inflammatory Bowel Disease and Colon Cancer. Ulcerative colitis and Crohn's disease, which are referred to as inflammatory bowel diseases (IBDs), are well known as chronic inflammatory diseases in the lower bowel. Epidemiological studies have shown that the incidence of colorectal cancer in IBD patients is greater than the expected incidence

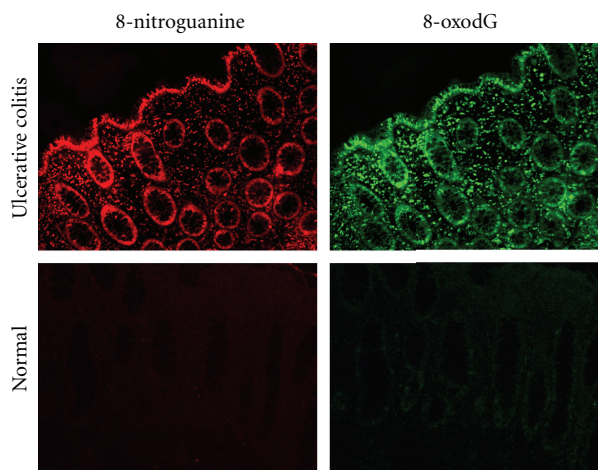


FIGURE 2: 8-Nitroguanine in colon epithelium of a patient with active ulcerative colitis.

in the general population [75]. We hypothesized that an imbalance of helper and regulatory T-cell functions plays a key role in the pathogenesis of IBD. Therefore, we prepared a mouse model of IBD with an imbalance of Th1 and Th2 and, using double immunofluorescence labeling, revealed that both 8-nitroguanine and 8-oxodG were mainly formed in epithelial cells [63]. iNOS, PCNA, and p53 proteins were also expressed in colon epithelium. We observed by using clinical samples that 8-nitroguanine and 8-oxodG were formed in colon epithelium of patients with ulcerative colitis in the active stage (Figure 2). Of relevance, several studies have shown that iNOS is expressed in epithelial cells in colitis patients [76–78]. In noncancerous colon tissues from patients with ulcerative colitis, iNOS protein levels were positively correlated with p53 serine 15 phosphorylation levels [76]. These results suggest that nitrative DNA damage, as well as oxidative DNA damage, participates in colon carcinogenesis in patients with IBD.

3.3. Oral Lichen Planus and Oral Cancer. Oral lichen planus (OLP) is a chronic inflammatory mucosal disease [79] and a risk factor for oral squamous cell carcinoma (OSCC) [80]. Oral leukoplakia is a precancerous lesion characterized by white plaques and hyperkeratosis [81, 82]. We demonstrated that 8-nitroguanine and 8-oxodG accumulated in oral epithelium of biopsy specimens from patients with OLP, leukoplakia, and OSCC, whereas no immunoreactivity was observed in normal oral mucosa [62, 83]. Colocalization of 8-nitroguanine and iNOS was found in oral epithelium of patients with OLP, leukoplakia, and OSCC. Accumulation of p53 was observed in oral epithelium in OLP and leukoplakia patients, and more prominent expression of this protein was observed in OSCC patients. In addition, the immunoreactivity to PCNA was significantly higher in leukoplakia patients than that in normal mucosa, suggesting an increase in cell proliferation [83]. Lee et al. also reported that PCNA and p53 were highly expressed in oral tissues in OLP patients [84]. We conclude that inflammation-mediated DNA damage and additional epithelial cell proliferation promote oral carcinogenesis.

3.4. DNA Damage in Malignant Fibrous Histiocytoma. Malignant fibrous histiocytoma (MFH) is one of the most common soft tissue sarcomas [85, 86] and has a poor prognosis [87, 88]. MFH has been proposed to be accompanied by inflammatory responses [89, 90]. However, the mechanism of its inflammation-induced carcinogenesis is still unclear. We investigated DNA lesions and inflammatory-related molecules including iNOS, NF- κ B, and COX-2 [64]. Immunohistochemical staining revealed that the formation of 8-nitroguanine and 8-oxodG occurred to a much greater extent in MFH tissue specimens from deceased patients than in live patients. iNOS, NF- κ B, and COX-2 were colocalized with 8-nitroguanine in MFH tissues. It is worth noting that a statistical analysis using the Kaplan-Meier method demonstrated strong 8-nitroguanine staining to be associated with a poor prognosis. Furthermore, our study demonstrated significantly higher levels of both 8-nitroguanine and HIF-1 α in the tissue specimens of deceased patients than in those of living subjects. Survival curves analyzed by the Kaplan-Meier method differed significantly between the groups with high and low staining of 8-nitroguanine as well as HIF-1 α [65]. These results suggest a significant role for the iNOS-dependent formation of 8-nitroguanine via HIF-1 α and NF- κ B in the progression of inflammation-related cancer. These results indicate that 8-nitroguanine is involved in not only the initiation of carcinogenesis but also its progression and prognosis in cases of MFH.

4. DNA Damage in relation to Genomic Instability

Genomic instability is a defining characteristic of most carcinogenesis through the accumulation of mutations in several tumor suppressor genes, oncogenes, and genes that are involved in maintaining genomic stability [91]. Events resulting in chromosomal instability, such as amplification and deletions of large segments of DNA, reciprocal and non-reciprocal translocations, aneuploidy, and polyploidy, constitute the large-scale genomic aberrations that characterize the majority of human cancer cells and are thought to accelerate

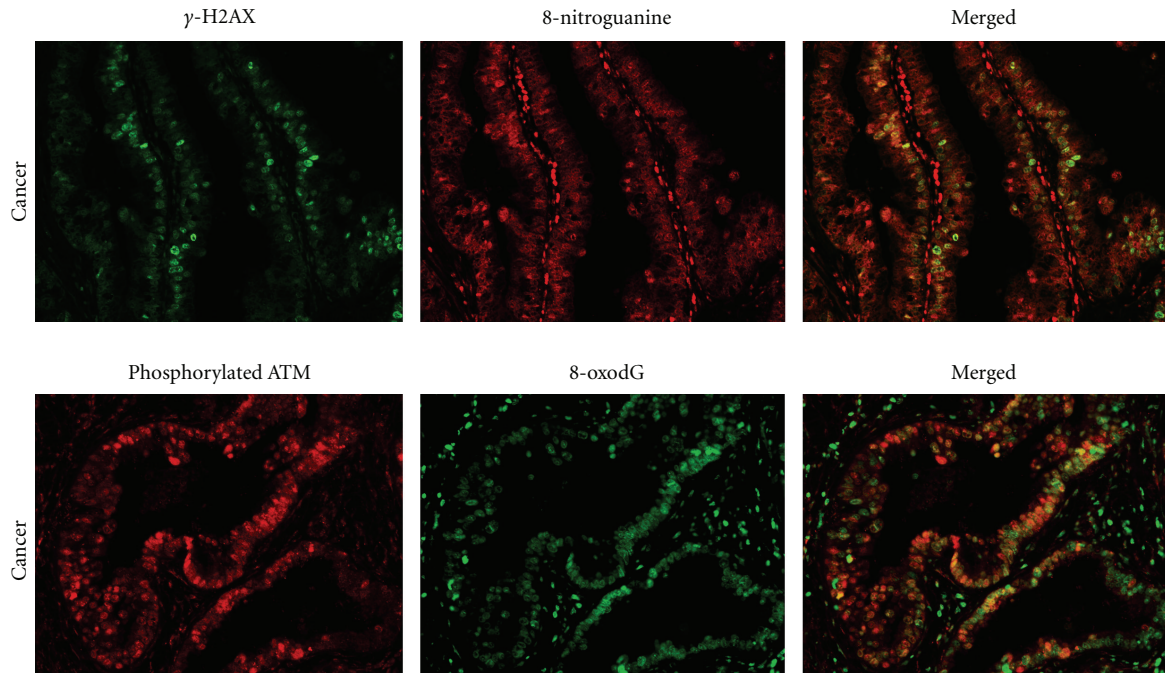


FIGURE 3: Colocalization of DDR proteins and DNA lesions. (a) Colocalization of γ -H2AX (green) and 8-nitroguanine (red). (b) Colocalization of phosphorylated ATM (green) and 8-oxodG (red).

carcinogenesis [91, 92]. Degtyareva et al. demonstrated that chronic oxidative DNA damage due to DNA repair defects induced chromosome instability in a *Saccharomyces cerevisiae* model [92]. Trouiller et al. showed that titanium dioxide, a risk factor for lung cancer, induced oxidative DNA damage, γ -H2AX foci, micronuclei, and DNA deletions, suggesting a link between inflammation-associated DNA damage and genomic instability [93]. The DNA damage response (DDR) is essential for maintaining the integrity of the genome, and a failure of this response results in genomic instability and predisposition to malignancy [94]. Phosphorylated ATM (ataxia telangiectasia mutated) plays a role in DDR to DNA double-stranded breaks. Impaired function of ATM was reported to be involved in DNA damage-induced genomic instability [94, 95]. TNF- α is a proinflammatory cytokine and also acts as an iNOS regulator protein [96, 97]. Natarajan et al. reported that TNF- α induced the formation of 8-oxodG and genomic instability in primary vascular endothelial cells [98]. Yan et al. showed that antioxidants significantly reduced TNF- α -induced genetic damage [99]. Therefore, TNF- α and a dysfunction of ATM could play key roles in the integration between iNOS-mediated DNA damage and genomic instability. Recently, we observed that phosphorylated ATM and γ -H2AX were colocalized with 8-oxodG and 8-nitroguanine in clinical samples of cholangiocarcinoma patients as shown in Figure 3, suggesting that DNA base damage caused double-stranded breaks. DNA lesions were found in infiltrating inflammatory cells, hepatocyte cells of nontumor areas, and cancer cells, whereas γ -H2AX and phosphorylated ATM were expressed in only cancer cells. Moreover, DDR proteins and

DNA lesions were detected only very weakly in normal liver tissues, suggesting that the DNA double-stranded breaks were specific to cancer cells. Our observations also support the idea that highly iNOS-dependent DNA damage causes DNA double-stranded breaks and genomic instability, which play important roles in inflammation-induced carcinogenesis via TNF- α signaling and DDR protein dysfunction.

5. DNA Damage in relation to Epigenetic Change

Diverse cellular functions including the regulation of inflammatory gene expression, DNA repair, and cell proliferation are regulated by epigenetic changes [100]. DNA methylation and histone modifications are the major events involved in epigenetic changes. An important proinflammatory cytokine IL-6 has been reported to control DNA methylation through IL-6-mediated Janus kinase (JAK)/STAT3 pathways [101–105]. We demonstrated that IL-6 modulated iNOS expression via STAT3 and EGFR in EBV-associated nasopharyngeal carcinoma [50]. Accumulating evidence makes it increasingly clear that epigenetic silencing plays an important role in EBV-associated neoplasia [106]. We and our colleagues have found promoter hypermethylation in several candidate genes for tumor suppressor genes [107–110]. Histone modifications play a role in the response to DNA double-stranded breaks through ATM signaling to activate γ -H2AX, resulting in histone ubiquitination and acetylation, and destabilization and conformational changes to nucleosomes lead to DNA repair [111]. RNS cause base lesions, abasic sites, and single-stranded breaks, which may be converted into double-strand

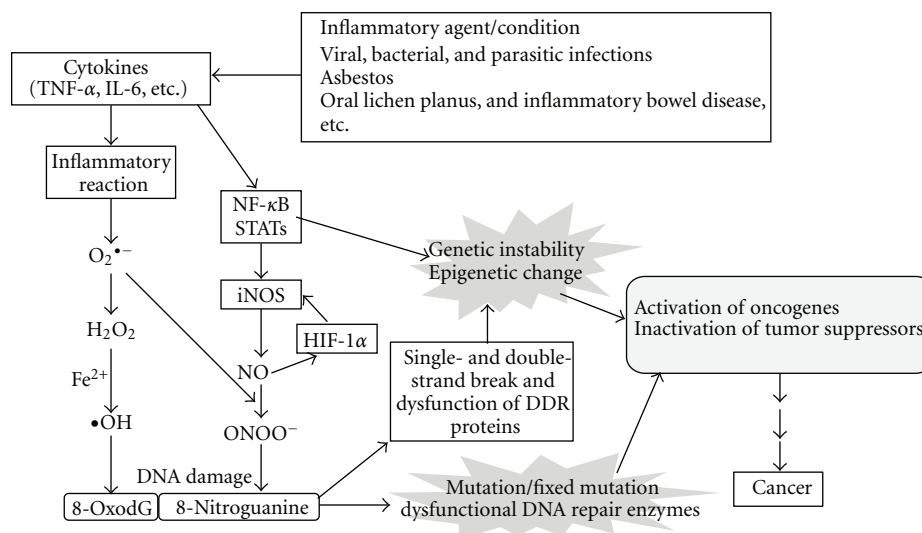


FIGURE 4: Proposed roles of nitritative and oxidative DNA damage in inflammation-related carcinogenesis.

breaks in cells by enzymatic processing, when the damage is in close proximity to or encountered by the replication fork [112]. Collectively, nitritative and oxidative DNA damage may activate epigenetic change via IL-6 signaling and the expression of DDR proteins.

6. Conclusion

We investigated the formation of 8-nitroguanine and 8-oxodG at sites of carcinogenesis in various clinical specimens and animal models in relation to inflammation-related carcinogenesis. We also observed that DNA lesions were formed and significantly increased in *S. haematobium*-induced urinary bladder cancer compared with cancer without such an infection [60]. In addition, Barrett's esophagus, an inflammation-related disease caused by the reflux of gastric acid, also showed greater DNA damage than normal esophageal tissues (unpublished data). Proposed roles of inflammation-related DNA damage in carcinogenesis on the basis of our findings and studies in the literature [94, 113] are summarized in Figure 4. 8-Nitroguanine and 8-oxodG are formed in various inflammation-related cancers and precancerous regions in an iNOS-dependent manner. TNF-α and IL-6 are proinflammatory cytokines which play roles in the control of iNOS expression via the regulation of NF-κB and STAT3 signaling pathways. 8-Nitroguanine and 8-oxodG are mutagenic lesions resulting in the G → T transversion. This type of mutation has been found to occur in vivo in the *ras* gene and the *p53* tumor suppressor gene in various cancers [114]. Nitritative and oxidative DNA damage induce not only mutations but also genomic instability and epigenetic change via TNF-α and IL-6 activities and DNA double-stranded breaks resulting in the activation of oncogenes and inactivation of tumor suppressor genes, which may lead to inflammation-related carcinogenesis.

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