Oxidative Stress-Induced Carcinogenesis and Its Prevention
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Molecular Mechanisms of Inflammation-Induced Carcinogenesis

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Summary  Chronic inflammation has been thought as the major risk factor for various types of human cancer. The International Agency for Research on Cancer (IARC) has estimated that approximately one-fifth of cancer cases worldwide is attributable to infectious/inflammatory diseases. While we fundamentally understand that persistent inflammation plays a role in carcinogenesis, recent advances in the molecular study of the mechanisms have revealed that reactive oxygen and nitrogen species, harmful endogenous genotoxic substances, produced by inflammatory cells are largely involved in the carcinogenic process. In this article, we review the instances demonstrating the definite link between inflammation and cancer, and shed light on the molecular mechanisms proved to be responsible for the inflammation-based carcinogenesis.

Key Words: inflammation, carcinogenesis

Introduction

Cancer epidemiologists have pointed out three undoubtedly cancer-causing factors: daily diet, smoking and infection/inflammation. In the 1980’s, they estimated around 75% of cancers was due to those factors; most recently the percentage is thought to be 43% [1]. Proportion of the involvement of each factor to the total cancer death differs among countries because individual’s life style differs from country to country, which will make up the characteristic of his or her country as a whole. For example, in 2005, the ratio of infection/inflammation to all the causes for cancer death was around 25% in sub-Saharan Africa while that in Europe was around 6% [1]. Collectively, underlying infections and inflammation are linked to around 15–20% of all cancer deaths [2].

Among the three factors, infection/inflammation is an unquestionable factor of carcinogenesis. For instance, chronic hepatitis viruses and fluke infections of the liver increase liver cancers; infection with papilloma viruses associate with anogenital cancers, especially cervical cancers; Helicobacter pylori (H. pylori) infection of the stomach tends to increase the risk of stomach cancer; inhalational irritants such as asbestos increase lung cancers, even in non-smokers, and autoimmune diseases such as ulcerative colitis and Crohn’s disease are typical examples of tissue-inflammation-associated carcinogenesis. What is important is that the pathogens to cause those inflammatory diseases are unrelated to each other, whereas the underlying pathogenesis in common is inflammation.

In this article, we review the currently accumulating
evidence of inflammation-associated carcinogenesis, and present up-to-date molecular findings of the carcinogenesis.

**Inflammation-Based Carcinogenesis**

*Registry of inflammation-based carcinogenesis*

In 1863 Rodolf Virchow suggested that inflammation might give rise to tumors from his observation that lymphoreticular infiltrates, namely chronic inflammation, preceded cancer [3]. His description is presumably the first report of the link between inflammation and cancer. In the 1970s, Prehn proposed that immune effector cells contributed to carcinogenesis and named the phenomenon as “immunostimulation theory of tumor development” [4]. In the 1980s, cancer epidemiological studies identified chronic infections and inflammation as the major risk factors of various types of cancer. Chronic infection and tissue inflammation are now undoubtedly recognized as the risk factors of various types of human cancers and more evidence of the close link between inflammation and cancer is accumulated [2, 5, 6] (Table 1).

**Typical examples of inflammation-based carcinogenesis in human**

*Bacteria infection—*Helicobacter pylori* is the most common bacterial pathogen of the gastrointestinal tract in human and transmissible in oral to oral or fecal to oral mode primarily within families. Due to more than 20% of genomic diversity in each *H. pylori* strain, varieties of pathogenicity have been confirmed in each strain [7]. *H. pylori* can be divided into two subpopulations based on their ability or disability to produce a 120–145 kDa protein named cytotoxin-associated gene A (CagA) antigen [8]. The CagA protein is secreted directly from *H. pylori* into gastric epithelial cells via the type-IV secretion system. Chronic infection with cagA-positive *H. pylori* causes severe gastroduodenal mucosal inflammation, atrophic gastritis, and gastric carcinoma [9].

There is positive association between *H. pylori* infection and gastric carcinogenesis since gastric carcinoma arises in around 0.4% of *H. pylori*-infected patients, and approximately 80% of gastric cancer patients have been infected with *H. pylori*. Gastric carcinomas can be divided into intestinal and diffuse type. Genetic alterations associated with gastric cancer have been identified as *E-cadherin* gene mutations in the diffuse type [10]; however, entire genetic alterations induced by *H. pylori* infection are not fully understood yet.

CagA interacts with cellular proteins that regulate cell growth, motility and polarity in both CagA phosphorylation-dependent and -independent manners [11]. Tyrosine phosphorylation of CagA is driven by *c-Src*, *Fyn*, *Lyn* and *Yes*. One of the target proteins for tyrosine-phosphorylated CagA is SHP-2, a cytoplasmic tyrosine phosphatase [12]. Physical complex of CagA and SHP-2 induces morphological transformation of epithelium by promoting cell proliferation and motility [12]. A typical molecular signaling of CagA without tyrosine phosphorylation is to directly activate Ras-MAP kinase signaling through binding of CagA with Grb2 and to promote proliferation of gastric epithelial cells [13]. Moreover, CagA transduces signals through interaction with *c-Met*, a hepatocyte growth factor receptor, and elicits morphogenesis [14]. This signaling pathway requires tyrosine phosphorylation of *c-Met* via *H. pylori* infection; however, the phenomenon is independent of CagA tyrosine phosphorylation.

The degree of *H. pylori* infection and the severity of mucosal injury directly correlate with the extent of neutrophil infiltration [15]. Soluble proteins released by *H. pylori* such as urease are capable of activating monocytes to produce neutrophil chemotactic cytokines such as IL-1β, IL-6, IL-8 and tumor necrosis factor-α (TNF-α) [9]. Moreover, extracts or sonicates of *H. pylori* promote neutrophils to adhere to endothelial cells and to stimulate chemotactic activity, which results in emigration and accumulation of activated neutrophils into the interstitium of the stomach [16]. Whereas CagA protein of *H. pylori* induces proliferation of gastric epithelial cells in the manner of tumor promoter [12], emigrated neutrophil-derived reactive oxygen species (ROS) and reactive

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nitrogen species (RNS) become the major cause for genetic alterations, and they act as initiator for proliferative epithelial cells [17].

Parasite infection—Infestation of the liver flukes Opisthorchis viverrini or Clonorchis sinensis has been known to be associated with the high incidence of cholangiocarcinoma in South-East Asia [18]. Ohshima et al. have detected that increased nitrosation potential is mediated by nitric oxide synthase (NOS) induced by Opisthorchis viverrini infestation in the liver fluke model they established using a Syrian golden hamster [19]. Moreover, they noticed that the patients infested with the liver fluke had increased urinary excretion of nitrate and N-nitrosoprolinone (NPRO) [19], and that, by administration of ascorbic acid, the levels of NPRO were lowered significantly in the parasite-positive patients due to inhibition of endogenous nitrosation [19]. It has recently been found that NOS persistently associated with Opisthorchis viverrini play a role in DNA damage and participate in the development of cholangiocarcinoma [20].

From those clinical and experimental results, it is suggested that NOS is induced by Opisthorchis viverrini infestation and that the nitric oxide (NO) can develop cholangiocarcinoma in its infested host [6]. Parasite-related carcinogenesis is also found in Northern Africa and the Middle East, in patients with schistosomiasis (Schistosoma haematobium) accompanying chronic bacterial infection of the urinary tract causing bladder cancer [21].

Virus infection—Hepatitis viruses A-G (hepatitis F is still controversial) have been identified; of those only B (HBV) and C (HCV) are the major risk factors for the development of hepatocellular carcinoma (HCC) [22]. There are differences in the patients’ clinical course between the two viruses [22]. The hepatitis viruses do not involve any known oncogene, and only rarely an integrated virus sequence activates cellular proto-oncogenes [23]. However, persistent hepatocellular inflammation associated with chronic and active hepatitis can induce mutations in tumor suppressor genes and extensive chromosomal abnormalities [24].

In the development of HCC, virus envelope protein is largely involved, which is assumed from the finding that transgenic mice, expressing HBV large envelope protein, cause oxidative DNA damage in the liver during chronic and active hepatitis [25]. ROS are involved in the HCC related with chronic HCV infection [26]. In fact, potential source of ROS in patients with chronic hepatitis is activated phagocytes and cytotoxic T-lymphocytes [24]. These cell-derived ROS injure hepatocytes, and then compensatory cell division occurs and DNA alterations will be accumulated [26]. Niitsu and his group treated chronic HCV patients with therapeutic iron reduction (phlebotomy and low iron diet) and measured hepatic 8-hydroxyl-2'-deoxyguanosine (8-OHdG) formation, as a marker of ROS-induced DNA damage; they found that the elevated hepatic 8-OHdG levels were markedly lowered and none of the patients developed HCC [26].

Human papilloma viruses (HPVs) are another major cause for infection-related carcinogenesis. To date, over 100 different HPVs have been identified, and one-third of people are infected with the virus in the genital tract epithelia. Since around 99% of analgenital cancers including cervical cancers are positive for sexually transmittable HPVs DNA [27], persistent HPV infection is thought to be a causative factor for development of genital tumors such as squamous cell carcinoma or adenocarcinoma of the cervix [28]. Cancer-prone types of the viruses have been identified, which are HPV-16, -18, -31, -33, and -45 [28]. Infection with those high-risk HPVs is not limited to the genital tract; approximately 20% of oropharynx cancers contains DNA from these HPVs [29].

Once HPV infects its target cells, viral gene expression is activated and approximately 20 to 100 extra-chromosomal copies of the viral DNA are produced per cell [28]. The major cellular targets for HPVs-associated carcinogenesis have been identified as E6 and E7 proteins, which are well known viral oncoproteins. Enforced expression of E6 or E7 protein or both can immortalize and transform normal cells [30, 31]. E6 is known to bind the p53 tumor suppressor protein to the cellular ubiquitin ligase E6AP, which leads to a rapid turnover of p53 [32]. p53 is one of the best known tumor suppressor genes and its molecular interactions are well characterized. It regulates cell cycle, mainly through the activity of p21, a cyclin kinase inhibitor [33]. The typical example of its cell cycle regulation is recognized when any of DNA damaging agents has been administered; p53 is activated, and induces p21 protein to arrest cell cycle, leading to apoptosis or DNA repair [33]. The ternary complex of E6, p53 and E6AP brings about ubiquitination of p53 and its degradation with 26S proteasome. These proteolytic cascades make the p53 half-life less than 20 minutes in vivo [34]. Moreover, E6 indirectly downregulates p53 activity through association with various intracellular signaling molecules, i.e., p300/CBP (a coactivator of p53) and PDZ family proteins (cell adhesion and junction molecules). Intriguingly, E6 protein functions as immortalization inducer, which we know from the fact that E6 activates transcription of a telomerase, hTERT, in cooperation with Myc and Sp-1 genes [35]. Namely, E6 plays roles in inhibition of pro-apoptotic activities of p53, in prevention of senescence and in acceleration of cell growth.

Oncogenic E7 protein is known to bind to RB tumor suppressor protein and relieve the control of RB-mediated cell cycle [36]. RB protein is another major cell cycle regulator, functioning through its phosphorylation. E7 acts as degradator of RB protein through the ubiquitin proteasome
pathway [37]. Cell-cycling-associated proteins, cyclins and cyclin-dependent kinase (cdk) inhibitors are regulated by phosphorylated Rb protein. E7 proteins interact with cyclins A and E, as well as with the cdk inhibitors p21 and p27. The precise mechanism responsible for the disturbance of cell cycle associated with HPV infections is not fully determined yet; however, it is probably due to the blockage of strict control of cell cycle regulation either by p53 or RB genes, or both.

Autoimmune reaction—Chronic inflammatory bowel diseases, such as ulcerative colitis and Crohn’s disease, are the examples of autoimmunity-based carcinogenesis [38]. It is estimated that around 1% of inflammatory bowel disease patients would develop colorectal tumors [39]. Choi and Zelig found that the carcinomas complicated with the two bowel diseases presented strikingly similar clinicopathological features, and suggested that the common underlying feature, namely chronic inflammation, was significant for the pathogenesis of colorectal carcinoma [40].

Recent studies have shown that autoimmune diseases in human are fundamentally associated with the defective function or increased numbers of CD4+ CD25+ regulatory T cells [41]. Autoimmunity can be characterized as disregulated immune function which will cause reaction to self antigen(s). In H. pylori infection, regulatory T cells contribute to suppress responsiveness of memory T cells to the bacteria, since H. pylori-infected patients have an increased population of pre-regulatory T cells in the stomach and duodenal mucosa [42]. Chronic hepatitis virus infections also increase regulatory T cells in peripheral circulation [43]. Reversely, depletion of regulatory T cells from the patients results in increased T-cell responses to hepatitis viruses, which implies that regulatory T cells derange effective anti-viral immunity in the host [43], and may decrease the immunofunction of T cells, causing immunosuppression, which is evidenced in patients with cancer in the lung, pancreas, breast, liver or skin, where large numbers of regulatory T cells are detected [44].

Irritant-induced inflammation—Chronic airway irritation and inflammation caused by airborne particles such as asbestos [45] or tobacco smoke [46] are closely associated with lung cancer development. Based on the epidemiological evidence, asbestos fibers (at a fibre length of 20 µm or more, and thickness below 1 µm) have been classified as causative agents of human lung and pleural tumors [45].

The putative mechanism for asbestos-related carcinogenicity is basically chronic inflammation caused from inhalation of the fibers, genotoxicity of the ROS and RNS generated in the inflammation or from the redox reactions on the fiber surface [47].

Phagocytes as a driving force for inflammation-based carcinogenesis

Bacteria-based mutation assay (Ames assay) revealed that inflammatory cells could be mutagenic. Phagocytes were found to induce mutagenicity through generation of ROS/RNS initiated by respiratory burst metabolism [48]. This finding was supported by the fact that the phagocytes from patients with chronic granulomatous disease, which is characterized by a genetic defect in the nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (ROS-generating) system, are unable to increase the mutation frequency, suggesting their role as intermediates of ROS in phagocyte-induced mutagenesis. Interestingly, tumor-infiltrating phagocytes also show mutagenic activity through production of ROS [49], which will be one of the answers to the question why malignant tumor cells arise from the primarily growing tumors. Tumor-infiltrating lymphocytes are, as it were, ‘double-edged sword’ since they act, on one hand, as immune effector cells for tumor cell lysis, and on the other hand, they promote tumor malignancy with their own mutagenic potential. Their orientations will be determined either by length of inflammation or the degree of secretion of mutagenic substances by phagocytes, or both.

Direct evidence of phagocytes-mediated transformation of mammalian cells was presented by Weitzman et al. in 1985 [50]. They utilized in vitro co-culture system, and discovered that human activated neutrophils converted a mouse immortalized fibroblast cell line, 10T1/2 cells, to tumorigenic cells [50]. And the conversion was inhibited by ROS scavengers added into the coculture system; moreover, the same conversion occurred in the cells when they were exposed to an enzyme-based ROS-generating system. Thus they demonstrated neutrophil-derived ROS as a factor for carcinogenic conversion of normal cells.

The same is true in the involvement of inflammation and its-derived ROS/RNS in malignant progression of benign mouse tumors. By using experimentally induced regressive tumors, weakly tumorigenic and non-metastatic, we succeeded in establishing a model in which we can consistently observe that the tumor cells are converted into tumorigenic and metastatic tumors once they have grown in vivo in contact with cells inflamed by a foreign-body implantation [51], particularly neutrophils [52]. The malignant conversion is not restricted to mouse cells; we also observed the same phenomenon in human colonic adenoma cells [53] or in rat regressive mammary tumor cells [54]. The concept of the foreign body-induced carcinogenesis was established by Boone & Takeichi [55]. They attached immortalized but non-tumorigenic mouse fibroblast cell lines to a piece of plastic plate or glass beads and implanted them into a subcutaneous space in mice; then the fibroblast cells were converted into tumorigenic ones. First, they speculated that some carcinogenic chemical substances were diffused by the
foreign body [55]. Heppner, a contemporary researcher, speculated that foreign-body-induced inflammation was one of the possible causative factors for the conversion [56]. Since then, we expanded the experimental systems to demonstrate that inflammation is the definite cause for the foreign body-induced carcinogenesis and tumor progression (Figure 1).

Genetic Mechanisms in Inflammation-Induced Carcinogenesis

Cancer genetics revealed the evidence that tumor development is not only due to gene mutational events but also epigenetic events. We herein focus on solid genetic mechanisms for chronic inflammation-based carcinogenesis and summarize evidence for the definite intrinsic factor, i.e., ROS/RNS on inflammation-induced carcinogenesis.

p53—Somatic mutations in the p53 tumor suppressor gene have widely been recognized in the development of various types of human cancer. Abnormalities associated with p53 deficiency are known to induce growth arrest or trigger apoptosis. Thus p53 gene is generally called gatekeeper gene for genotoxic injury. Several studies support the idea that p53 gene mutation is associated with elevated levels of inflammation-associated ROS. Moreover, p53 is a transcriptional transrepressor of iNOS and thus p53 deficiency leads to an excessive NO production and an accumulation of genetic abnormality [57]. Endogenous NO production also causes oxidative DNA damage by generating peroxynitrite through the stoichiometric fluxes of NO and superoxide [58].

More than 80% of HCCs is known to arise in the persistent inflammation with hepatitis virus infections or dietary aflatoxin B1 intake [59]. Aflatoxin B1 is mycotoxin that may contaminate corn, rice, and peanuts. Point mutation is found in the exposure to aflatoxin B1 or in chronic viral hepatitis, at the third base of p53 codon 249; transversion from G:C to T:A is common in HCC [60].

The same transversion occurs in human fibroblasts after they are treated with H2O2 and FeCl3 [61]. Such an effect of ROS on the amino acid transversions is also seen in other diseases. Hemochromatosis (an iron overload disease) and Wilson disease (a copper overload disease) are the genetic disorders characterized by an excessive absorption and an accumulation of iron and copper, respectively, in hepatocytes, and are associated with a risk of HCC [62, 63]. In these diseases, the transversion of G:C to T:A is frequent at codon 249 in the liver, accompanied with concomitant generation of NO and ROS, compared to normal liver counterparts [64]. The hepatocytes are thus vulnerable, and prone to transversions at the p53 genes in the presence of inflammatory-cell-derived or transition-metal-mediated ROS. The same p53 mutations is observed in patients with colon [65] or lung cancer [66].

Dysfunction of p53 gene accelerates inflammation-associated carcinogenesis. Tazawa et al. observed a high incidence of spontaneous autologous tumors in p53 gene-disrupted mice after subcutaneous implantation of a small piece of plastic plate [67]. The plastic plate was recognized as foreign body in the mice, and the foreign body-induced inflammation caused the tumor at the implantation site. They evidenced that p53 gene was one of the important molecules which regulated inflammation-associated carcinogenesis.

NF-κB—The transcription factor NF-κB has a role as master gene in inflammation-induced carcinogenesis. NF-κB regulates various pathophysiological functions mainly through activating Toll-like receptor and receptors for pro-inflammatory cytokines. The cellular functions regulated by NF-κB are cell growth signals, apoptosis, tissue invasion/metastasis and angiogenesis which are essential for tumor development and progression [68]. Moreover, NF-κB has a central role in inflammation and innate/adaptive immune responses. In other words, NF-κB acts as a bridge between inflammation and carcinogenesis. Regulation of NF-κB expression would be a shortcut strategy for prevention of inflammation-based carcinogenesis and its therapy; however, prolonged and substantial inhibition of NF-κB might not be practical because the inhibition will cause immunodeficiency [68]. Undoubtedly NF-κB is a candidate to regulate inflammation-induced carcinogenesis. However, when we administer NF-κB inhibitor(s) to host, we must know the timing, administration dose, and delivery system for a targeted organ, as in the application for most candidate genes/proteins in cancer prevention and therapy.

DNA methylation—One of the recent topics of cancer genetics is the involvement of epigenetic alterations in
carcinogenesis. Aberrant DNA methylation is a feature of cancers that causes gene silencing [69]. Cancer cells often contain promoter methylation of the genes related to cell cycle control, DNA repair, angiogenesis, and that of tumor suppressor genes [69]. Hypermethylation of CpG-rich sequences (CpG islands), located in the promoter of the target gene, recruits to form a complex with methylated-DNA binding proteins and histone deacetylases. Deacetylation of the histone backbones makes a closed chromatin structure from the DNA structure of the promoter so that it becomes inaccessible to transcription factors, ready for gene inactivation [70]. The stability of such gene silencing is thought to be irreversible in somatic cells and such transcriptional silencing is also a fundamental feature in the normal physiological development process such as aging and chromosome X inactivation [71, 72]. Recent studies have demonstrated that apart from age and gender, chronic inflammation is one of the internal factors associated with methylation of promoter CpG islands [73, 74].

Involvement of inflammation-related ROS has been suggested in the etiology of human lung cancer [75]. In fact, lung tumors induced by using genotoxic carcinogens show that the p16INK4a tumor suppressor gene is frequently methylated [76]. Moreover, tobacco smoke, which notably causes inflammation, accompanies methylation of p16INK4a in the induced cancers [77]. Jang et al. revealed that inactivation of the promoter region of p16INK4a would be a new prognostic biomarker of the risk of gastritis-associated gastric cancers [78]. Moreover, Epstein-Barr (EB) virus-positive stomach cancer with inflammation has more methylated CpG islands than stomach cancer without EB virus infection [79]. It is apparent that inflammation in the liver stimulates DNA methylation, since aberrant methylation is detected not only in hepatocellular carcinomas but also non-cancerous liver tissues such as chronic hepatitis or liver cirrhosis [80]; and the p16INK4a promoter is inactivated in hepatitis virus infection [81]. Chronic inflammation of the gallbladder is also a high risk for tumor development and progression accompanying hypermethylation at multiple tumor-suppressor gene-promoter sites such as p16, p73, APC, and hMLH1 [82]. Colorectal cancers associated with ulcerative colitis are distinguished from the sporadic ones by their genetic pathways, i.e., infrequent K-Ras mutations and presence of p53 mutations. Colon mucosa of a patient with ulcerative colitis exhibits increased methylation of p16, E-cadherin and MYOD [83]. On the other hand, a DNA repair gene, O6-methylguanine-DNA methyltransferase, is methylated in sporadic cancer, while in case of ulcerative colitis it is not inactivated [84].

In an inflammatory condition, CpG island hypermethylation is one of the important mechanisms to inactivate carcinogenesis-responsible genes. The p16INK4a inactivation is supposed to be a possible target for inflammation-induced carcinogenesis and causes adverse effect of inflammatory cell-derived ROS. p16INK4a inactivation is observed in the inflamed organs themselves or tumor cells that arose in the inflammatory environment, as described above. Inactivation and/or loss of p16INK4a is also seen in rodent renal carcinogenesis induced by ferric nitrilotriacetate administration, as a model of Fenton-type free-radical-induced cancer [85, 86]. Methylation of p16INK4a gene is thus one of the fragile/vulnerable genes for inflammation- or ROS/RNS-associated carcinogenesis.

Reactive nitrogen species—Ames estimated that ROS derived from inflammatory cells might be the primary factor in the development of up to one-third of all cancers [87]. Accumulated studies in this field revealed that ROS produced by inflammatory cells not only cause direct damage to DNA but also exert indirect effects such as deregulation of cell proliferation and apoptosis, stimulation of angiogenesis, and modification of gene/protein expressions and protein activities; all these are a critical step toward carcinogenesis.

Evidence indicates that NO, an important bioregulatory and signaling molecule, may play a significant role in carcinogenesis, especially inflammation-based ones. NO is catalyzed by a family of three enzymes known as NOS. One of the inducible NOS (iNOS) gene expressions can be upregulated by either bacterial endotoxins or pro-inflammatory cytokines in many cell types including phagocytes as well as in a variety of human tumors. An inflammatory environment, e.g., hepatitis, is a typical example to present how NO is involved in the inflammation-based carcinogenesis. During chronic hepatitis, TNF-α and IFN-γ are upregulated and then those cytokines induce iNOS gene expression to enhance NO concentrations in hepatocytes [88]. iNOS expression is also induced directly by the hepatitis virus infection. Such changes are thought to be due to transcriptional transactivation of iNOS gene via HBx protein. Clinically in patients with hepatitis virus infection, hepatic iNOS is consistently upregulated.

NO-mediated RNS are known to act not only as oxidants but also potent nitrating molecules. Thus RNS damage nucleic acids, proteins, lipids, and carbohydrate through nitrosation, nitration, and oxidation reactions [89].

Advances have been made by Akaike and his colleagues in this field recently. They discovered that 8-nitroguanosine was an adverse mediator of NO since NO itself is not a highly genotoxic molecule [90]. According to them, the marked feature of 8-nitroguanosine is that its entire structure, or its components 5-monophosphates and 5-triphosphates are active in redox reaction, and stimulate superoxide generation in the presence of various NADPH-dependent reductases (including NADPH-cytochrome P450 reductase) and all isoforms of NOS [90, 91]. Therefore endogenously formed
8-nitroguanosine is not only the hallmark of nucleic acid damage, but it stimulates de novo production of ROS at inflammation sites [90].

Kawanishi et al. extensively investigated the in vivo 8-nitroguanosine formation in inflammation-based carcinogenesis, in clinical cases and experimental models. They observed extensive 8-nitroguanosine formation in the typical cases of inflammation-associated carcinogenesis: *Opisthorchis viverrini* infection [92], *H. pylori* infection [93], hepatitis C virus infection [94], inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease [95], oral lichen planus, and inflammation-associated precancerous lesions [96]. More importantly, 8-nitroguanine in DNA was potentially mutagenic, which preferentially yields G:C to T:A transversions, possibly through its rapid depurination to form an apurinic site and/or miscoding in DNA was potentially mutagenic, which preferentially yields G:C to T:A transversions, possibly through its rapid depurination to form an apurinic site and/or miscoding. Indeed, G-T transversions have been observed in vivo in the ras gene [97] and the p53 tumor suppressor gene in many human cancers [98]. These findings imply that DNA damage mediated by RNS may participate in carcinogenesis via activation of proto-oncogenes and inactivation of tumor suppressor genes [99].

On the other hand, 8-nitroguanine in DNA may interfere with RNA functions and metabolism. Nitrated guanine nucleosides and nucleotides in the nucleotide pool may contribute to oxidative stress, via production of superoxide which is mediated by various reductases, and may disturb important enzymes such as GTP-binding proteins and cGMP-dependent enzymes [89]. 8-Nitroguanine formed in the nucleic acid will exert pernicious effects on oncogenes, tumor suppressor genes and cellular signaling.

Conclusion

The pathogens that cause various types of inflammation-based carcinogenesis are obviously unrelated, whereas the essential pathological feature in common is continuous inflammation and infiltration of activated phagocytes and lymphocytes. It is assumed, therefore, that continuous generation of ROS/RNS by phagocytes may injure cells. This could, in turn, cause compensatory cell proliferation, which will effectively accumulate DNA damage and gene mutations; all these steps are essential to carcinogenesis.

In this regard, a shortcut to cancer prevention would be to elucidate the background factors distinct in frequent cancers. We believe that the elucidation of precise molecular mechanisms between inflammation and carcinogenesis is most important for this purpose.

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References


