MINI-REVIEW

Reactive Oxygen and Nitrogen Oxide Species-induced Stress, a Major Intrinsic Factor Involved in Carcinogenic Processes and a Possible Target for Cancer Prevention

Dai Nakae[1*], Takashi Umemura[2], Yuji Kurokawa[3]

Abstract

Reactive oxygen and nitrogen oxide species and their inducing stress are involved in a variety of physiological and pathological phenomena in aerobes, including humans. For multistage carcinogenic processes, reactive oxygen and nitrogen oxide species-induced stress (RONOSS) serves as a major intrinsic factor and is involved in every step. This means that free radicals, RONOSS and their inducing downstream events may be targets for cancer prevention. It is therefore of importance to elucidate the mechanisms underlying the participation of RONOSS in carcinogenesis and to apply the obtained results for establishment of strategies to control cancer development. Despite the large body of accumulated knowledge due to worldwide efforts dealing with this research field, there still remain numerous uncertainties. In this mini-review, we introduce two examples of such efforts, one concerning a renal carcinogen $KBrO_3$ and the other dealing with hepatocarcinogenesis caused by a choline-deficient, L-amino acid-defined diet, in order to give some idea about the current understanding of the roles of RONOSS in carcinogenesis.

Key Words: Reactive oxygen species - 8-hydroxydeoxyguanosine - potassium bromate - signal transduction abnormalities - choline-deficient-L-amino acid-defined diet - carcinogenesis

Introduction

Carcinogenesis involves a series of qualitatively different stages that occur as a result of the sequential accumulation of genetic and epigenetic lesions. While these lesions are induced mainly by exposure to extrinsic factors such as various substances present in the environment, intrinsic factors have also attracted attention for their participation in carcinogenic processes (Ames et al., 1995; Loeb, 1989; Pitot et al., 1991). Among such intrinsic factors, reactive oxygen and nitrogen oxide species-induced stress (RONOSS) have been shown to play significant roles through a wide variety of alterations including injury to subcellular components, mutations, abnormal gene regulation and signal transduction abnormalities (Deshpande and Irani, 2002; Epe, 2002; Jackson and Loeb, 2001; Kovacic and Jacintho, 2001; Lala and Chakraborty, 2001). On the other hand, it has become well recognized that the presence of continuous stimulation, such as chronic inflammation, is crucially involved in many cases of development of cancers. Typical examples of factors causing such continuous stimulation include chronic viral hepatitis for hepatocellular carcinomas (Montalto et al., 2002), cholelithiasis and chronic inflammation due to varied infectious and non-infectious reasons for biliary tract cancers (Lazcano-Ponce et al., 2001; Tazuma and Kajiyama, 2001), chronic inflammation due to the infection of *Helicobacter pylori* for gastric cancers (Ebert et al., 2002; Peek and Blaser, 2002; Terry et al., 2002), and hormonal imbalance for mammary and genital cancers (Cavalieri and Rogan, 2002, Imagawa et al., 2002; Liao and Dickson, 2002). Such continuous stimulation may cause tumor-induction *per se*, but in most cases it serves to provide a "hypercarcinogenic state" which enhances carcinogenic processes (Shimizu et al., 1999 and 2000; Umeda and Hino,

[1]*Department of Pathology, Sasaki Institute, Sasaki Foundation, Tokyo, Japan;* [2]*Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan;* [3]*Sasaki Foundation, Tokyo, Japan*
To whom all correspondence should be addressed at: Department of Pathology, Sasaki Institute, Sasaki Foundation, 2-2 Kanda-Surugadai, Chiyoda, Tokyo 101-0062, Japan; phone, +81-3-3294-3286; facsimile, +81-3-5259-9301; e-mail, dai.nakae@sasaki.or.jp

2001). RONOSS play significant roles in most of these continuous stimulation cases, and this is one of the most typical pathways for stress to be involved in neoplasia (Ambs et al., 1999; Deshpande and Irani, 2002; Lala and Chakraborty, 2001; Shacter and Weitzman, 2002). In this sense, free radicals, RONOSS and their downstream events are essential intrinsic factors and may thus be good targets for cancer prevention. It is then of importance to investigate concrete examples of their participation in carcinogenesis to draw conclusions for practical strategies to control cancers. In this mini-review, we introducing two models, one concerning the renal carcinogen $KBrO_3$ and the other dealing with hepatocarcinogenesis caused by a choline-deficient, L-amino acid-defined (CDAA) diet in the absence of any carcinogen exposure, in order to give some idea about the current understanding of the roles of RONOSS in carcinogenesis.

Possible Roles for an 8-Hydroxydeoxyguanosine (8-OHdG) Adduct in $KBrO_3$ Carcinogenesis

$KBrO_3$ has been classified as a "genotoxic" carcinogen based on positive mutagenicity in the Ames (Ishidate et al., 1984), chromosome aberration (Ishidate and Yoshioka, 1980) and micronuclear (Hayashi et al., 1988) tests, and positive carcinogenicity in rats after a 2-year oral administration with the standard long-term bioassay protocol (Kurokawa et al., 1982). We have shown that antioxidants, especially sulfhydryl compounds, are protective against the clastogenicity of $KBrO_3$, suggesting the participation of reactive oxygen species in its underlying mechanisms of action (Sai et al., 1992a). In fact, ribo- and deoxyribonucleosides of 8-hydroxyguanine induce sister chromatid exchange in human lymphocytes (Arashidani et al., 1998). We have, therefore, hypothesized that the oxidizing property of $KBrO_3$ is responsible for its "genotoxicity" and carcinogenicity.

Generation of Reactive Free Radicals by $KBrO_3$

We have analyzed reactive oxygen species caused by the interaction of $KBrO_3$ with cells from rat kidney by means of electron spin resonance spectrometry and revealed generation of 5,5-dimethyl-1-pyrroline-*N*-oxide-hydroxide, this being inhibited by singlet oxygen scavengers such as dimethylsulfoxide and ethanol. Superoxide dismutase, catalase and various hydroxyl radical scavengers, were not found to exert any significant inhibition. Furthermore, using 2,2,6,6-tetramethylpiperidine, a singlet oxygen-trapping agent, its oxide could be detected in mixtures of $KBrO_3$ with homogenates of kidney tissue. Together with our data from the chemiluminescence analysis, the results suggested that singlet oxygen is the most probable candidate for the reactive oxygen species generated by $KBrO_3$ (Sai et al., 1992b). On the other hand, it was recently reported that the reduction of $KBrO_3$ by sulfhydryl compounds such as glutathione (GSH) and cysteine (Cys) results in yields of bromine oxides and bromine radicals, which can effectively cause the guanine oxidation (Murata et al., 2001).

8-OHdG Formation by $KBrO_3$

It is well known that reactive free radicals attack cellular DNA and nucleotide pools to form various types of oxidized bases; e.g., 8-OHdG (Kasai et al., 1998), 5-hydroxydeoxycytidine (Feig et al., 1994) and 2-hydroxydeoxyadenosine (Kamiya and Kasai, 1995). Among these oxidized bases, 8-OHdG has especially received much attention because of the accumulated data indicating that it may play crucial roles in a chain of events from RONOSS to carcinogenesis (Kasai, 1997). 8-OHdG pairs with adenine as well as cytosine, subsequently yielding GC-to-TA transversions upon replication by DNA polymerases (Cheng et al., 1992; Shibutani et al., 1991). This mutation is considered to contribute to the activation of oncogenes and/or the inactivation of tumor suppressor genes, leading to carcinogenesis (LePage et al., 1995). In fact, it has been demonstrated that $KBrO_3$ has the potential to induce 8-OHdG formation in both *in vitro* and *in vivo* situations.

In vitro: The 8-OHdG levels were increased in renal proximal tubular cells isolated from rats using collagenase and incubated with $KBrO_3$ and in isolated rat renal nuclei incubated with a lipid-peroxidation system (Sai et al., 1994). In the presence of GSH, $KBrO_3$ also induces 8-OHdG modification in DNA of bacteriophage PM2 (Ballmaier and Epe, 1995), calf thymus DNA (Chipman et al., 1998; Persons and Chipman, 2000) and the human leukemia cell line HL-60 and its H_2O_2-resistant clone, HP100 (Murata et al., 2001). This 8-OHdG formation by $KBrO_3$ then indeed causes gene mutations, as shown at the *HPRT* locus of V79 Chinese hamster cells (Speit et al., 1999) and in *p53* exon 7 of LLC-PK1 cells (Richter and Vamvakas, 1998). Additionally, depletion of GSH by buthionine sulfoximine exerts only a limited inhibitory effect on $KBrO_3$-induced 8-OHdG formation in DNA of human leukemia cell lines, suggesting an important role for Cys as well as GSH (Murata et al., 2001). It is thus indicated that $KBrO_3$ requires reduction systems, especially those featuring sulfhydryl groups provided by GSH and Cys, to exert its oxidizing properties leading to oxidative DNA damage. In the kidney, GSH is filtered through glomeruli into the lumen of renal tubules and then hydrolyzed by the brush-border enzymes to the constituent amino acids including Cys, which are reabsorbed in the proximal tubules. The location of the 8-OHdG formation by $KBrO_3$ may thus be in accordance with the target site of the carcinogen.

In vivo: A single administration of $KBrO_3$ to rats by gavage causes elevation of 8-OHdG levels in the kidney DNA, but not in liver DNA (Kasai et al., 1987). A single intraperitoneal injection of $KBrO_3$ also increases the 8-OHdG level in rat kidney DNA (Sai et al., 1991). In addition, such increase in the 8-OHdG level following a single administration of $KBrO_3$ is inhibited by co-treatment with various antioxidants; such as vitamin C (Sai et al., 1992c), resveratol, melatonin and vitamin E (Cadenas and Barja, 1999). While the doses of $KBrO_3$ used in these studies were

considerably higher than its carcinogenic dose, we have shown that the 8-OHdG levels in the kidney DNA, but not in liver DNA, significantly increase from the ends of weeks 1 and 4, respectively, in male and female rats continuously administered $KBrO_3$ at an concentration of 500 ppm in the drinking water, and that high 8-OHdG levels are maintained until the end of week 13 (Umemura et al., 1995 and 1998). A 13-week administration of $KBrO_3$ at 500 ppm in the drinking water is sufficient to induce renal cell tumors in male rats after a subsequent 91-week maintenance of animals on drinking water without any carcinogen (Kurokawa et al., 1990). However, another group claimed that a single intragastric administration of $KBrO_3$ to rats at a dose enough to elevate the 8-OHdG level fails to exert initiating activity in a renal carcinogenesis model (Kurata et al., 1992). It is, therefore, plausible that persistent increase of the 8-OHdG level is required for generation of gene mutations sufficient for carcinogenicity of $KBrO_3$.

Repair Enzymes for 8-OHdG

In *Escherichia coli*, three kinds of DNA glycosylases have been found to prevent mutagenesis caused by 8-OHdG. Fpg protein acts in the excision of 8-OHdG (Michaels et al., 1991). MutY protein, in contrast, excises adenine residues incorporated by DNA polymerases opposite 8-OHdG (Tajiri et al., 1995) and Mut T protein hydrolyses 8-OHdGTP from the pool of DNA precursors (Maki and Sekiguchi, 1992). Recently, a gene encoding a human repair glycosylase for 8-OHdG, *hOGG1*, was cloned (Rosenquist et al., 1997). It has been shown that the repair rate of 8-OHdG induced by $KBrO_3$ in a Chinese hamster ovary cell line is more rapid in *hOGG1* transfectants than in the parental cells. The overexpression of *OGG1*, however, does not affect mutation frequencies of the *gpt* locus in AS52 cells exposed to $KBrO_3$ (Hollenbach et al., 1999). At present, therefore, the relationship between the repair systems for 8-OHdG and $KBrO_3$-initiated carcinogenesis is still a matter of controversy, although the *OGG1* deficient mouse might provide some information (Klungland et al., 1999).

Hepatocarcinogenesis in Rats Fed a CDAA Diet and the Participation of RONOSS in Its Underlying Mechanisms

Dietary choline deficiency with limited methionine availability in rats induces hepatocellular carcinomas in male Fischer 344 rats in the absence of any exposure to exogenous carcinogenic substances (Ghoshal and Farber, 1984; Locker et al., 1986; Newberne et al., 1982). The cancer incidence, however, is low at around 30-40% when using conventional choline-deficient diets, which makes it difficult to explore underlying carcinogenic mechanisms (Ghoshal and Farber, 1984; Locker et al., 1986; Newberne et al., 1982). To overcome this problem, we have produced a new diet deficient in choline and low in methionine and demonstrated strong hepatocarcinogenicity of this CDAA diet in male Fischer 344 rats (Nakae et al., 1990 and 1992). Using this diet, we have been investigating the mechanisms underlying

"endogenous" carcinogenesis and exploring strategies for cancer chemoprevention (Nakae, 1999 and 2000).

Characteristics of Hepatocarcinogenesis in Rats fed the CDAA diet

When male Fischer 344 or Wistar rats are fed the CDAA diet continuously, hepatocellular carcinomas begin to develop at the end of week 40 and reach an incidence of almost 100% at the end of week 104 (Nakae, 1999; Nakae et al., 1992). The hepatocarcinogenesis proceeds with background induction of fatty liver (by day 2), apoptotic death and proliferation of hepatocytes (from day 3 and thereafter repeatedly continuing) and fibrosis (from week 4 and thereafter progressing) (Nakae, 1999). Fibrosis becomes frank cirrhosis by the end of week 30 (Nakae, 1999). In the presence of these non-tumor changes, foci of cellular alteration with positive glutathione *S*-transferase placental form (GST-P) phenotype are induced and grow (Nakae, 1999). The foci then progress to hepatocellular adenomas and are finally converted into hepatocellular carcinomas (Nakae, 1999). The histological sequence for the development of hepatocellular carcinoma in rats fed the CDAA diet thus closely resembles that seen in human cases especially those preceded by hepatitis C virus infection (Yao and Terrault, 2001). The liver damage induced in rats by the CDAA diet feeding and chronically present during the hepatocarcinogenic processes may serve as a continuous stimulation to cause a "hypercarcinogenic state", as does chronic hepatitis induced in humans by hepatitis C virus infection.

Involvement of RONOSS in Hepatocarcinogenesis in Rats fed the CDAA diet

When male Fischer 344 or Wistar rats are fed the CDAA diet, hydrogen peroxide is over-produced in hepatocyte mitochondria 3 days after the commencement (Hensley et al., 2000), suggesting the induction of RONOSS in the livers from a very early time-point. The hepatocyte nuclear DNA is oxidatively injured, and the levels of 8-OHdG are significantly elevated by the end of the first day of the feeding and accumulate thereafter (Yoshiji et al., 1992). Other sub-hepatocellular components are then oxidatively injured, detected as the significantly increased levels of 2-thiobarbituric acid-reacting substances (TBARS) at the end of day 3, the levels increasing continuously thereafter (Yoshiji et al., 1992). Whereas the exact nature of the TBARS is not as yet identified, byproducts of peroxidation of lipids and proteins are suggested to contribute (Nakae, 1999). In fact, ω-6 lipid hydroperoxide-mediated DNA adducts are generated in the hepatocytes of rats fed the CDAA diet for 3 days (Kawai et al., 2002). These findings indicate that RONOSS is indeed induced in hepatocytes of rats from the very beginning of the CDAA diet feeding and that the stress continues as long as the diet is fed. Then the next query must be whether RONOSS is biologically significant for "endogenous" carcinogenesis. The time-course of the accumulation of TBARS significantly

correlates with those of hepatocyte apoptosis and proliferation (Nakae, 1999). Furthermore, it appears that 8-OHdG formation and TBARS generation are critically involved in the induction and growth of putatively preneoplastic, GST-P-positive, hepatocyte focal lesions (Kobayashi et al., 1998; Nakae et al., 1994). In addition, various chemicals modify the extent of apoptosis and proliferation of hepatocytes and hepatocarcinogenesis itself in rats fed the CDAA diet in association with the concurrent modification of RONOSS-related changes (Denda et al., 2002; Kobayashi et al., 1998; Nakae , 1999 and 2000; Nakae et al., 1994 and 1998). It is thus strongly suggested that RONOSS induced by the continuous feeding of the CDAA diet is causally involved in the induction of a "hypercarcinogenic state" due to continuous liver damage and, in turn, progress of "endogenous" hepatocarcinogenesis in rats. While the underlying mechanisms remain largely obscure, work has revealed the generation of reactive nitrogen oxide species via the induction of inducible nitric oxide synthase, activation of several transcription factors including nuclear factor-κB and activator protein-1, induction and activation of inducible cyclo-oxygenase (Denda et al., 2002; Nakae et al., 1998), and the sequential and accumulating overexpression of cell cycle regulating protooncogenes (Tsujiuchi et al., 1995) and various cytokines (Nakae, 1999). It is, therefore, indicated that RONOSS causes a variety of signal transduction alterations, and these may provide a main clue as to the causative involvement of stress in "endogenous" hepatocarcinogenesis in rats fed the CDAA diet.

Conclusions

Multi-stage carcinogenic mechanisms are mediated by both extrinsic and intrinsic factors. It is thus necessary to elucidate how these act alone and in combination to participate in neoplastic processes. Assuming the importance of RONOSS as a major intrinsic factor, in this context, the precise mechanisms underlying the influence of RONOSS itself and/or its induction of a "hypercarcinogenic state" on the carcinogenic behaviors of various other factors either intrinsic or extrinsic, including environmental chemicals, are expected to be studied using appropriate *in vivo* animal models as well as the modifying effects due to various natural and synthetic agents. $KBrO_3$ can serve as a good model compound, because it is a "genotoxic" carcinogen causing RONOSS during its carcinogenicity, it therefore no longer being used as a food additive. The hepatocarcinogenesis model in rats fed the CDAA diet also provides a good tool, because it causes a "hypercarcinogenic state" closely resembling the human "counterpart" of chronic hepatitis. It is strongly expected that studies on the roles of RONOSS in carcinogenesis using $KBrO_3$, the CDAA diet and various other tools will provide information useful for the understanding of the carcinogenic mechanisms and in turn the establishment of the practical strategy to control cancers.

References

Ambs S, Hussain SP, Marrogi AJ, Harris CC (1999). Cancer-prone oxyradical overload disease. *IARC Sci Publ*, **150,** 295-302.

Ames BN, Gold LS, Willen WC (1995). The causes and prevention of cancer. *Proc Natl Acad Sci USA*, **92,** 5258-65.

Arashidani K, Iwamoto TN, Muraoka M, Kasai H (1998). Genotoxicity of ribo- and deoxyribonucleosides of 8-hydroxyguanine, 5-hydroxycytosine, and 2-hydroxyadenine: induction of SCE in human lymphocytes and mutagenicity in Salmonella typhimurium TA 100. *Mutat Res*, **403,** 223-7.

Ballmaier D, Epe B (1995). Oxidative DNA damage induced by potassium bromate under cell-free conditions and in mammalian cells. *Carcinogenesis*, **16,** 335-42.

Cadenas S, Barja G (1999). Resveratol, melatonin, vitamin E and PBN protect against renal oxidative DNA damage induced by the kidney carcinogen $KBrO_3$. *Free Radic Biol Med*, **26,** 1531-7.

Cavalieri EL, Rogan EG (2002). A unified mechanism in the initiation of cancer. *Ann N Y Acad Sci*, **959,** 341-54.

Cheng KC, Cahill DS, Kasai H, et al (1992). 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G-T and A-C substitutions. *J Biol Chem*, **267,** 166-72.

Chipman JK, Davies JE, Persons JL, et al (1998). DNA oxidation by potassium bromate; a direct mechanism or linked to lipid peroxidation. *Toxicology*, **126,** 93-102.

Denda A, Kitayama W, Murata A, et al (2002). Increased expression of cyclooxygenase-2 protein during rat hepatocarcinogenesis caused by a choline-deficient, L-amino acid-defined diet and chemopreventive efficacy of a specific inhibitor, nimesuride. *Carcinogenesis*, **23,** 245-56.

Deshpande SS, Irani K (2002). Oxidant signalling in carcinogenesis: a commentary. *Hum Exp Toxicol*, **21,** 63-4.

Ebert MP, Schandl L, Malfertheiner P (2002). *Helicobacter pylori* infection and molecular changes in gastric carcinogenesis. *J Gastroenterol*, **37 Suppl 13,** 45-9.

Epe B (2002). Role of endogenous oxidative DNA damage in carcinogenesis: What can we learn from repair-deficient mice?. *Biol Chem*, **383,** 467-75.

Feig DI, Sowers LC, Loeb LA (1994). Reverse chemical mutagenesis: identification of the mutagenic lesions resulting from reactive oxygen species-mediated damage to DNA. *Proc Natl Acad Sci USA*, **91,** 6609-13.

Ghoshal AK, Farber E (1984). The induction of liver cancer by dietary deficiency of choline and methionine without added carcinogens. *Carcinogenesis*, **5,** 1367-70.

Hayashi M, Kishi M, Sofuni T, Ishidate M Jr (1988). Micronucleus tests with mice on 39 food additives and 8 miscellaneous chemical substances. *Chem Toxicol*, **26,** 487-500.

Hensley K, Kotake Y, Sang H, et al (2000). Dietary choline restriction causes complex I dysfunction and increased H_2O_2 generation in liver mitochondria. *Carcinogenesis*, **21,** 983-9.

Hollenbach S, Dhenaut A, Eckert I, et al (1999). Overexpression of *Ogg1* in mammalian cells: effects on induced and spontaneous oxidative DNA damage and mutagenesis. *Carcinogenesis*, **20,** 1863-8.

Imagawa W, Pedchenko VK, Helber J, Zhang H (2002). Hormone/growth factor interactions mediating epithelial/stromal communication in mammary gland development and carcinogenesis. *J Steroid Biochem Mol Biol*, **80,** 213-30.

Ishidate M Jr, Sofuni T, Yoshikawa K, et al (1984). Primary mutagenicity screening of food additivies currently used in Japan. *Food Chem Toxicol*, **22,** 623-36.

Ishidate M Jr, Yoshioka K (1980). Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation- a comparative study on mutagens and carcinogens. *Arch Toxicol*, **Suppl 4**, 41-4.

Jackson AL, Loeb LA (2001). The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. *Mutat Res*, **477,** 7-21.

Kamiya K, Kasai H (1995). Formation of 2-hydroxydeoxyadenosine triphosphate, an oxidatively damaged nucleotide, and its incorporation by DNA polymerases: Steady-state kinetics of the incorporation. *J Biol Chem*, **270,** 19446-50.

Kasai H, Crain PF, Kuchino Y, et al (1986). Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis*, **7,** 1849-51.

Kasai H, Nishimura S, Kurokawa Y, Hayashi Y (1987). Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA. *Carcinogenesis*, **8,** 1959-61.

Kasai H (1997). Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat Res*, **387,** 147-63.

Kawai Y, Kato Y, Nakae D, et al (2002). Immunohistochemical detection of a substituted 1, N^2-ethenodeoxyguanosine adduct by w-6 polyunsaturated fatty acid hydroperoxide in the liver of rats fed a choline-deficient, L-amino acid-defined diet. *Carcinogenesis*, **23,** 485-9.

Klungland A, Rosewell I, Hollenbach S, et al (1999). Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc Natl Acad Sci USA*, **96,** 13300-5.

Kobayashi Y, Nakae D, Akai H, et al (1998). Prevention by 1'-acetoxychavicol acetate of the induction but not growth of putative preneoplastic, glutathione *S*-transferase placental form-positive, focal lesions in the livers of rats fed a choline-deficient, L-amino acid-defined diet. *Carcinogenesis*, **19,** 1809-14.

Kovacic P, Jacintho JD (2001). Mechanisms of carcinogenesis: Focus on oxidative stress and electron transfer. *Curr Med Chem*, **8,** 773-96.

Kurata Y, Diwan BA, Ward JM (1992). Lack of renal tumor-initiating activity of a single dose of potassium bromate, a genotoxic renal carcinogen in male F344/NCr rats. *Food Chem Toxicol*, **30,** 251-9.

Kurokawa Y, Hayashi Y, Maekawa A, et al (1982). Induction of renal cell tumors in F344 rats by oral administration of potassium bromate, a food additive. *Jpn J Cancer Res*, **73,** 335-8.

Kurokawa Y, Maekawa A, Takahashi M, Hayashi Y (1990). Toxicity and carcinogenicity of potassium bromate: A new renal carcinogen. *Environ Health Perspect*, **87,** 309-35.

Lala PK, Chakraborty C (2001). Role of nitric oxide in carcino-genesis and tumour progression. *Lancet Oncol*, **2,** 149-56.

Lazcano-Ponce EC, Miquel JF, Munoz N, et al (2001). Epidemiology and molecular pathology of gallbladder cancer. *Can Cancer J Clin*, **51,** 349-64.

LePage F, Margot A, Grollman AP, et al (1995). Mutagenicity of a unique 8-oxoguanine in a human *Ha-ras* sequence in mammalian cells. *Carcinogenesis*, **16,** 2779-84.

Liao DJ, Dickson RB (2002). Roles of androgens in the development, growth, and carcinogenesis of the mammary gland. *J Steroid Biochem Mol Biol*, **80,** 175-89.

Locker J, Reddy TV, Lombardi B (1986). DNA methylation and hepatocarcinogenesis in rats fed a choline-devoid diet. *Carcinogenesis*, **7,** 1309-12.

Loeb LA (1989). Endogenous carcinogenesis: Molecular oncology into the twenty-first century. *Cancer Res*, **49,** 5489-96.

Maki H, Sekiguchi M (1992). MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature*, **355,** 273-5.

Michaels ML, Pham L, Cruz C, Miller JH (1991). MutM, a protein that prevents G:C-T:A transversion, is formamidopyrimidine-DNA glycosylase. *Nucleic Acids Res*, **19,** 3629-32.

Montalto G, Cervello M, Giannitrapani L, et al (2002). Epidemiology, risk factors, and natural history of hepatocellular carcinoma. *Ann N Y Acad Sci*, **963,** 13-20.

Murata M, Bansho Y, Inoue S, et al (2001). Requirement of glutathione and cystein in guanine-specific oxidation of DNA by carcinogenic potassium bromate. *Chem Res Toxicol*, **14,** 678-85.

Nakae D (1999). Endogenous liver carcinogenesis in the rat. *Pathol Int*, **49,** 1028-42.

Nakae D (2000). Modulation by environmental chemicals of liver carcinogenesis in rats. *Recent Res Devel Cancer*, **2,** 143-5.

Nakae D, Kotake Y, Kishida H, et al (1998). Inhibition by phenyl *N-tert*-butyl nitrone of early phase carcinogenesis in the livers of rats fed a choline-deficient, L-amino acid-defined diet. *Cancer Res*, **58,** 4548-51.

Nakae D, Mizumoto Y, Yoshiji H, et al (1994). Different roles of 8-hydroxyguanine formation and 2-thiobarbiruric acid-reacting substances generation in the early phase of liver carcinogenesis induced by a choline-deficient, L-amino acid-defined diet in rats. *Jpn J cancer Res*, **85,** 499-505.

Nakae D, Yoshiji H, Maruyama H, et al (1990). Production of both 8-hydroxydeoxyguanosine in liver DNA and gamma-glutamyltransferase-positive hepatocellular lesions in rats given a choline-deficient, L-amino acid-defined diet. *Jpn J Cancer Res*, **81,** 1081-4.

Nakae D, Yoshiji H, Mizumoto Y, et al (1992). High incidence of hepatocellular carcinomas induced by a choline deficient L-amino acid defined diet in rats. *Cancer Res*, **52,** 5042-5.

Newberne PM, de Camargo JLV, Clark AJ (1982). Choline deficiency, partial hepatectomy and liver tumors in rats and mice. *Toxicol Pathol*, **19,** 2342-52.

Peek RM Jr, Blaser MJ (2002). *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nature Rev Cancer*, **2,** 28-37.

Persons JL, Chipman JK (2000), The role of glutathione in DNA by potassium bromate in vitro. *Mutagenesis*, **15,** 311-6.

Pitot HC, Dragan Y, Xu YH, et al (1991). Pathways of carcinogenesis – genetic and epigenetic. *Princess Takamatsu Symp*, **22,** 21-33.

Richter H, Vamvakas S (1998). *S*-(1,2-Dichlorovinyl)-L-cysteine-induced dedifferentiation and *p53* gene mutations in LLC-PK1 cells: A comparative investigation with *S*-(2-chloroethyl) cysteine, potassium bromate, *cis*-platinum and styrene oxide. *Toxicol Lett*, **94,** 145-57.

Rosenquist TA, Zharkov DO, Grollman AP (1997). Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc Natl Acad Sci USA*, **94,** 7429-34.

Sai K, Hayashi M, Takagi A, et al (1992a). The micronuleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. *Mutat Res*, **269,** 113-8.

Sai K, Takagi A, Umemura T, et al (1991). Relation of 8-hydroxydeoxyguanosine formation in rat kidney to lipid peroxidation, glutathione level and relative organ weight after

a single administration of potassium bromate. *Jpn J Cancer Res*, **82,** 165-9.

Sai K, Takagi A, Umemura T, et al (1992c). The protective role of glutathione, cysteine and vitamin C against oxidative DNA damage induced in rat kidney by potassium bromate. *Jpn J Cancer Res*, **83,** 45-51.

Sai K, Tyson C A, Thomas D W, et al (1994). Oxidative DNA damage induced by potassium bromate in isolated rat renal proximal tubules and renal nuclei. *Cancer Lett*, **87,** 1-7.

Sai K, Uchiyama S, Ohno Y, et al (1992b). Generation of active oxygen species in vitro by the interaction of potassium bromate with rat kidney cell. *Carcinogenesis*, **13,** 333-9.

Shacter E, Weitzman SA (2002). Chronic inflammation and cancer. *Oncology*, **16,** 217-32.

Shibutani S, Takeshita M, Grollman AP (1991). Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*, **349,** 431-4.

Shimizu N, Ikehara Y, Inada K, et al (2000). Eradication diminishes enhancing effects of *Helicobacter pylori* infection on glandular stomach carcinogenesis in Mongolian gerbils. *Cancer Res*, **60,** 1512-4.

Shimizu N, Inada KI, Tsukamoto T, et al (1999). New animal model of glandular stomach carcinogenesis in Mongolian gerbils infected with *Helicobacter pylori* and treated with a chemical carcinogen. *J Gastroenterol*, **34 Suppl 11,** 61-6.

Speit G, Haupter S, Schutz P, Kreis P (1999). Comparative evaluation of the genotoxic properties of potassium bromate and potassium superoxide in V79 Chinese hamster cells. *Mutat Res*, **439,** 213-21.

Tajiri T, Maki H, Sekiguchi M (1995). Functional cooperation of MutT, MutM and MutY proteins in preventing mutations caused by spontaneous oxidation of guanine nucleotide in *Escherichia coli*. *Mutat Res*, **336,** 257-67.

Tazuma S, Kajiyama G (2001). Carcinogenesis of malignant lesions of the gall bladder. The impact of chronic inflammation and gallstones. *Langenbecks Arch Surg*, **386,** 224-9.

Terry MB, Gaudet MM, Gammon MD (2002). The epidemiology of gastric cancer. *Semin Radiat Oncol*, **12,** 111-27.

Tsujiuchi T, Kobayashi E, Nakae D, et al (1995). Prevention by methionine of enhancement of hepatocarcinogenesis by coadministration of a choline-deficient, L-amino acid-defined diet and ethionine in rats. *Jpn J Cancer Res*, **86,** 1136-42.

Umeda T, Hino O (2001). Hypercarcinogenic state in chronic liver disease. *Intern Med*, **40,** 555-6.

Umeda T, Hino O (2002). Molecular aspects of human hepatocarcinogenesis mediated by inflammation: from hypercarcinogenic state to normo- or hypocarcinogenic state. *Oncology*, **62 Suppl 1,** 38-42.

Umemura T, Sai K, Takagi A, et al (1995). A possible role for oxidative stress in potassium bromate ($KBrO_3$). *Carcinogenesis*, **16,** 593-7.

Umemura T, Takagi A, Sai K, et al (1998). Oxidative DNA damage and cell proliferation in kidneys of male and female rats during 13-weeks exposure to potassium bromate ($KBrO_3$). *Arch Toxicol*, **72,** 264-9.

Yao F, Terrault N (2001). Hepatitis C and hepatocellular carcinoma. *Curr Treat Options Oncol*, **2,** 473-83.

Yoshiji H, Nakae D, Mizumoto Y, et al (1992). Inhibitory effect of dietary iron deficiency on inductions of putative preneoplastic lesions as well as 8-hydroxydeoxyguanosine in DNA and lipid peroxidation in the livers of rats caused by exposure to a choline-deficient, L-amino acid-defined diet. *Carcinogenesis*, **13,** 1227-33.