

## Review

### 8-Hydroxyguanine: From its discovery in 1983 to the present status

By Susumu NISHIMURA<sup>†)</sup>

University of Tsukuba, Ibaraki, Japan  
(Communicated by Takao SEKIYA, M. J. A.)

**Abstract:** 8-Hydroxyguanine (8-OH-G) was discovered in 1983 in our laboratory at the National Cancer Center Research Institute, Tokyo. Since it could be formed in DNA not only *in vitro* but also *in vivo* by oxygen radical forming agents, we immediately hypothesized the importance of this discovery in connection with its biological consequence. Further intensive efforts by us from 1983 to 1990 confirmed that 8-OH-G is a highly significant oxidated DNA lesion involved in mutation and/or carcinogenesis in mammals, including humans. With the subsequent entry of many investigators to this research field the number of publications on 8-OH-G increased exponentially, reaching more than several thousands by the end of 2005. In this article, a summary is given of the important works carried out in the early days, and further notable contributions by many investigators are reviewed, focusing on 8-OH-G in the mammalian system. A special emphasis is given to research on knockout mice that are deficient in genes involved in the repair systems of the 8-OH-G lesion. Lastly, our own recent work is summarized involving a one-year carcinogenesis study of *Ogg1* (the gene for 8-OH-G specific glycosylase/AP lyase<sup>†1)</sup> knockout mice that have been exposed to oxidative stress.

**Key words:** 8-Hydroxyguanine; oxygen radical; DNA repair; mutagenesis, carcinogenesis; *Mut M*.

**Discovery of 8-hydroxyguanine.** In the early 1980s, Takashi Sugimura (Director of the National Cancer Center Research Institute of Tokyo and Chief of the Biochemistry Division at the time) discovered that when fish and meat are broiled by cooking, large amounts of mutagens are produced. Sugimura and his colleagues subsequently defined the mutagenic properties as Glu-P-1, Glu-P-2, Trp-P-1 and Trp-P-2 which were isolated from pyrolysis products of pure glutamic acid and tryptophan, respectively. This finding heralded the beginning of a new research field, namely the exploration of carcinogenic heterocyclic amines produced by cooking.<sup>1-3)</sup> In order to characterize mutagens present in broiled fish and meat, a collaborative study was initiated between the Biology Division, which I directed at the time, and the

Biochemistry Division.

The scientists of the Biology Division had considerable experiences in chemistry from previous studies that involved the structural characterization of modified nucleosides present in tRNA. Minako Nagao of the Biochemistry Division prepared quite a large amount of extract from broiled fish as well as broiled beef (minced beef used for hamburgers). Hiroshi Kasai in our Division subsequently attempted to purify and identify active mutagenic components from these materials through various purification procedures. This work resulted in the successful isolation of IQ and MeIQ from fish and MeIQx from beef.<sup>4-6)</sup> When assayed by the Ames' *Salmonella* test using TA 98 strain, these isolates proved to be the most active mutagens discovered so far and were later demonstrated to be carcinogenic in rodents and monkey.<sup>1-3)</sup>

During the course of the characterization of mutagenic isolates, Kasai became aware of large amounts of mutagenic activity not requiring activation by microso-

<sup>†)</sup> University of Tsukuba, Tennodai, 1-1-1, Tsukuba, Ibaraki 305-8577, Japan (nishimura@tara.tsukuba.ac.jp).

Abbreviations: 8-OH-G, 8-hydroxyguanine; 8-OH-dG, 8-hydroxydeoxyguanosine; HPLC, high performance liquid chromatography.

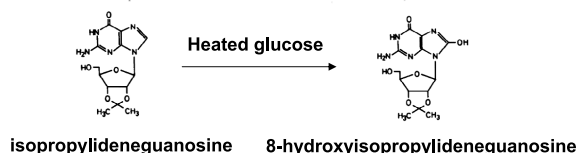
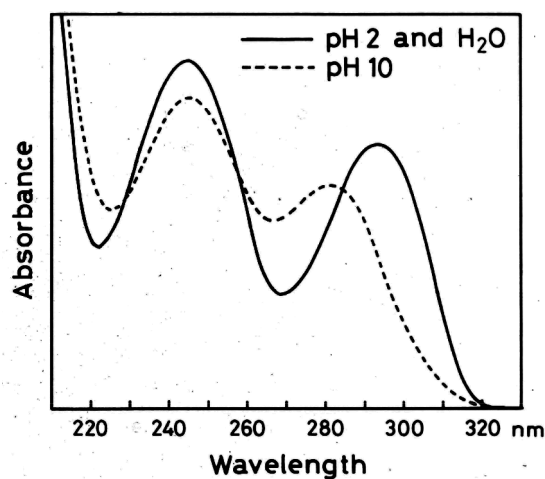


Fig. 1. Structure and unique UV spectra of 8-hydroxyisopropylidenguanosine.

mal enzymes that were lost during their enrichment, presumably due to their instability. Thus, Kasai adopted an alternative approach to identify such unstable mutagens, that is, isolation of guanine adducts by HPLC. Among the four DNA constituents, deoxyguanosine is the most important for adduct formation with mutagens/carcinogens. Isopropylidenguanosine instead of deoxyguanosine was used for identification of guanine adducts, because it is easily separated by organic solvent extraction. Serving as a model compound from broiled food, heated glucose was first tested. When isopropylidenguanosine was mixed with heated glucose and then fractionated by HPLC, two additional peaks could be distinguished. Surprisingly, one of them turned out to be 8-hydroxyisopropylidenguanosine, a simple guanine adduct having just one oxygen atom (Fig. 1).<sup>7</sup> The chemical structure was established by NMR and massspectral analysis and the comparison with authentic 8-hydroxyisopropylidenguanosine which was prepared from 8-bromoguanosine.<sup>7</sup> This finding suggested that oxygen radicals are involved in the reaction.<sup>82</sup> Reactive oxygen species are generated endogenously by cellular oxygen metabolism and exogenously by ionizing radiation, environmental mutagens and carcinogens. Thus, we quickly realized the importance of this discovery, and Kasai set about showing, by *in vitro* reaction, that various kinds of oxygen radical forming agents,

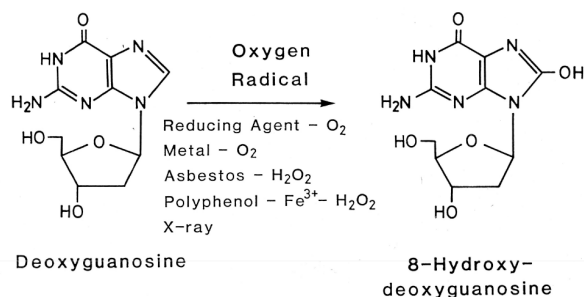


Fig. 2. Formation of 8-OH-G in DNA by oxygen radical-forming agents.

including x-ray irradiation, had the ability to catalyze 8-hydroxylation of deoxyguanosine as well as the deoxyguanosine residue present in DNA (Fig. 2).<sup>8-12</sup> Interestingly, 8-hydroxydeoxyguanosine was a new compound not previously reported, although 8-hydroxyguanosine was previously synthesized by Morio Ikehara and his colleagues. It was rather surprising that 8-hydroxyguanine had not already been found in DNA, considering the numerous studies carried out in the radiation biology field. Apparently, such studies had been focusing on pyrimidine modifications following from the discovery of the biological significance of thymine dimers by Richard B. Setlow.<sup>13</sup>

#### Early accomplishments in our laboratory.

Whether 8-OH-G could be formed by oxidative stress *in vivo* was a key question at the time of discovery of 8-OH-G, because many modified bases that can be produced in a test tube, may not necessarily be found in nature. The challenge was quickly contended with by the breakthrough discovery of Robert A. Floyd of University of Oklahoma, who showed that the use of an electrochemical detection system instead of ultraviolet absorption gave an enhanced sensitivity of more than one thousand fold.<sup>14</sup> We immediately adopted this technique and found that 8-OH-G was naturally present in DNA and could be increased in liver DNA when the whole body of mouse was exposed to  $\gamma$ -radiation (Fig. 3).<sup>15</sup> It was also shown that 8-OH-G in liver DNA decreased during recovery phase from  $\gamma$ -radiation, indicating that a repair system(s) for the removal of 8-OH-G from DNA exists in mice.<sup>15</sup> This was a fascinating finding suggesting that 8-OH-G has an important role in biological processes. Myong C. Chung, who came from Seoul National University as a visiting scientist supported by the First 10-Year Strategy of Cancer Control Program of the Japanese government, became involved in isolating a specific enzyme for the repair of 8-OH-G in DNA. To sim-

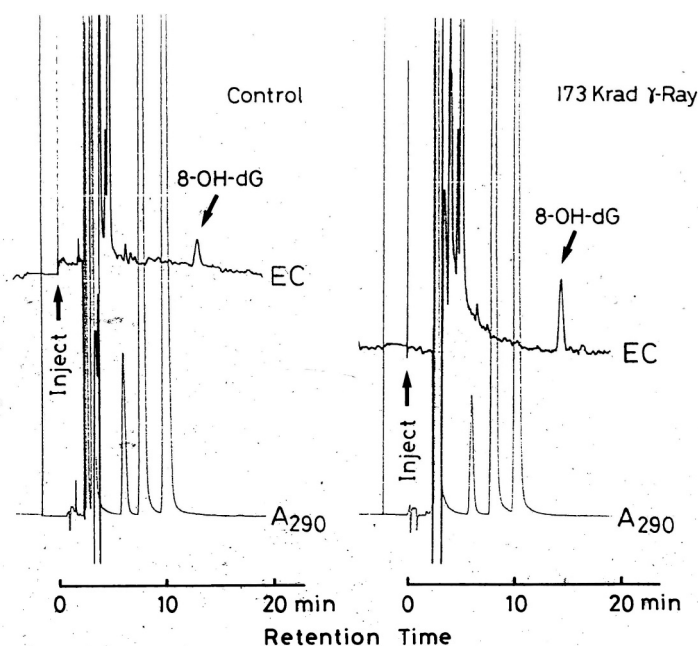


Fig. 3. Detection of 8-OH-G in mouse liver DNA by HPLC-coupled electrochemical detection system.

ply the experiment we used *Escherichia coli* instead of mammalian cells. Detection of an 8-OH-G specific repair enzyme was achieved using a chemically synthesized deoxyribopolynucleotide with 8-OH-G located in a specific position (Fig. 4).<sup>16)</sup> This substrate was prepared by Eiko Ohtsuka and her colleagues at Hokkaido University. Such a deoxyribopolynucleotide, having a modified base in a specific position, was the first to be chemically synthesized. Ultimately, an 8-OH-G specific enzyme was successfully isolated from *E. coli*.<sup>16)</sup> This 8-OH-G specific glycosylase/AP lyase was later found to be identical to formamidopyrimidine DNA glycosylase (FPG protein) which was previously discovered as an enzyme with the ability to remove formamidopyrimidine.<sup>17)</sup>

Further important discoveries on 8-OH-G from these early days, mostly accomplished within our laboratory, will be briefly summarized.

1) *Misreading of 8-OH-G during DNA replication:*

We firstly showed that 8-OH-G is misread by *E. coli* Klenow fragment when 8-OH-G containing deoxyribopolynucleotide is used as a template. In this reaction, 8-OH-G was read not only by cytosine (C), but also by adenine (A) and other bases. Significantly, neighboring bases in the vicinity of the 8-OH-G residue were also

found to be misread.<sup>18)</sup> Later Arthur P. Grollman and his colleague extended this study using a highly elegant approach<sup>19)</sup> and showed that an 8-OH-G residue is principally misread as adenine, when mammalian polymerase  $\alpha$  is used.

2) *Conformation of 8-OH-G and its electrostatic potential:*

By using an Ab initio molecular orbital method, it was shown that the favored conformation of 8-OH-G is the 6,8-diketo form (8-oxo form) rather than the 8-hydroxy form (Fig. 5 and Fig. 6).<sup>20)</sup> It also showed that the addition of an oxygen atom to the 8 position of guanine base changed the electrostatic potential of the molecule entirely and gave it a negative character (Fig. 5).<sup>20)</sup> X-ray crystallographic analysis of 9-ethyl-8-hydroxyguanine also showed its 8-oxo form.<sup>21)</sup>

3) *NMR study of double-stranded deoxyribopolynucleotides having 8-OH-G:*

Our NMR study indicated that an 8-OH-G residue located in a deoxyribopolynucleotide can be paired with a cytosine in the opposite strand.<sup>22)</sup> Later Michael Kouchakdian *et al.* showed by NMR that if the opposite strand had adenine instead of cytosine, 8-OH-G can also make a base-pair with adenine, in a syn conformation with the 8-oxo form (Fig. 7).<sup>23)</sup> This was also confirmed by X-ray crystallography.<sup>24)</sup> These observations indicated

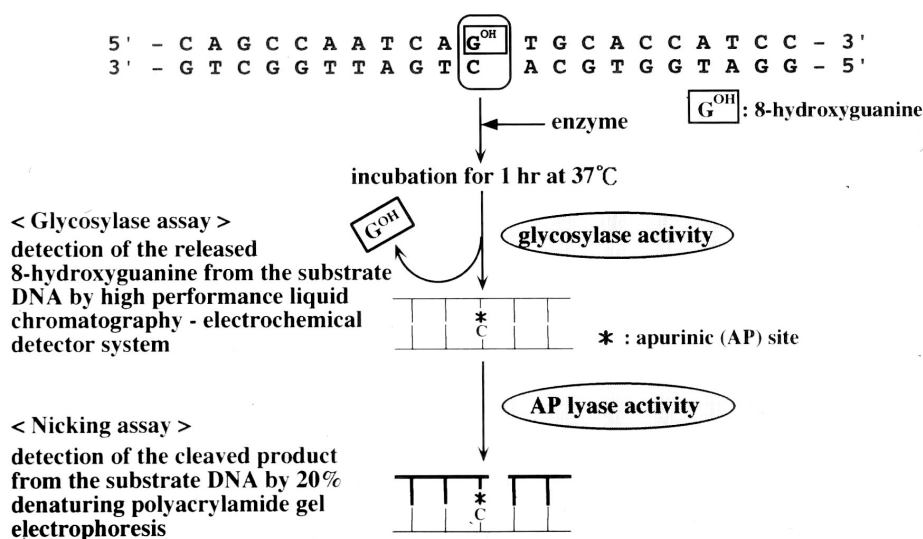


Fig. 4. Glycosylase and nicking assay for MutM protein by using synthetic deoxyribopolynucleotide having 8-OH-G.

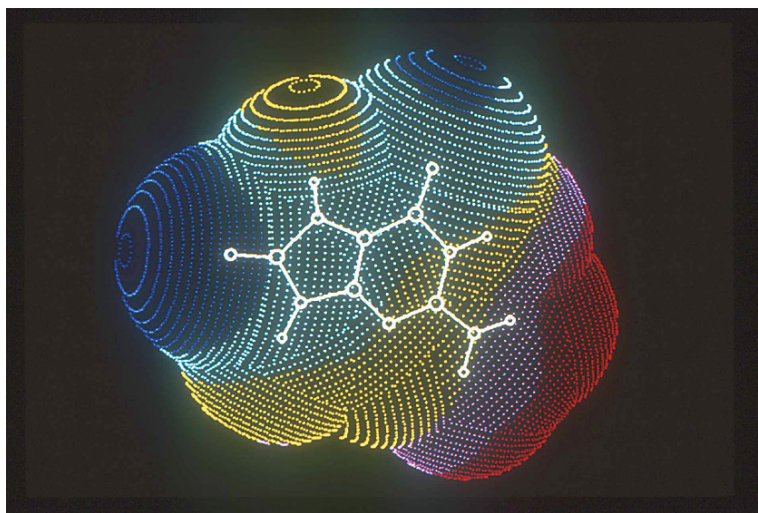


Fig. 5. Conformation of 8-hydroxyguanine obtained by ab initio molecular orbital method. Map of electrostatic potential (ESP) at the molecular surface, 1.5 Å distant from van der Waals spheres. Dot: red,  $9 \leq \text{ESP}$ ; pink,  $3 \leq \text{ESP} < 9$ ; yellow,  $-3 < \text{ESP} < 3$ ; light blue,  $-9 < \text{ESP} \leq -3$ ; blue,  $-15 < \text{ESP} \leq -9$ ; green,  $\text{ESP} \leq -15$  (Kcal/mole).

that the conformation of 8-OH-G in DNA can be altered depending upon the identity of the base paired with 8-OH-G in the opposite strand.

4) *Formation of 8-OH-G in kidney DNA by oral administration of potassium bromate (KBrO<sub>3</sub>) to rats:*

In collaboration with Yuji Kurokawa and his coworkers, we showed that oral administration of the renal carcinogen KBrO<sub>3</sub> to rats induced the formation of

8-OH-G in kidney DNA, but with far less being generated in liver DNA.<sup>25)</sup> These results suggested that formation of 8-OH-G in tissue DNA is target organ specific for carcinogenesis. Later, many investigators adopted this approach in the assessment of target organ specificity during carcinogenesis.<sup>26)</sup>

5) *Formation of 8-OH-G by asbestos:*

We showed that the carcinogenic potential of asbestos correlates with the extent of 8-OH-G formation

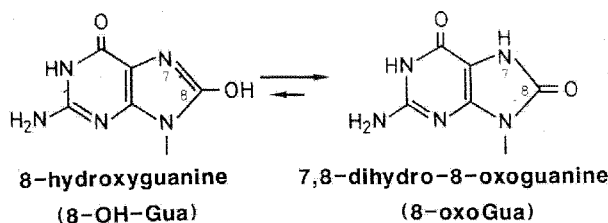


Fig. 6. Tautomeric form of 8-OH-G.

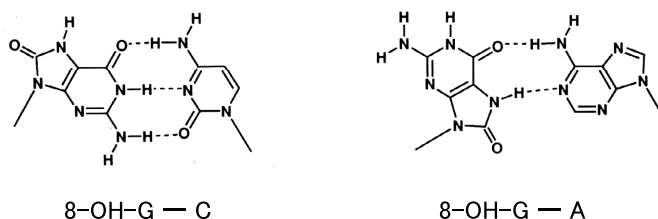
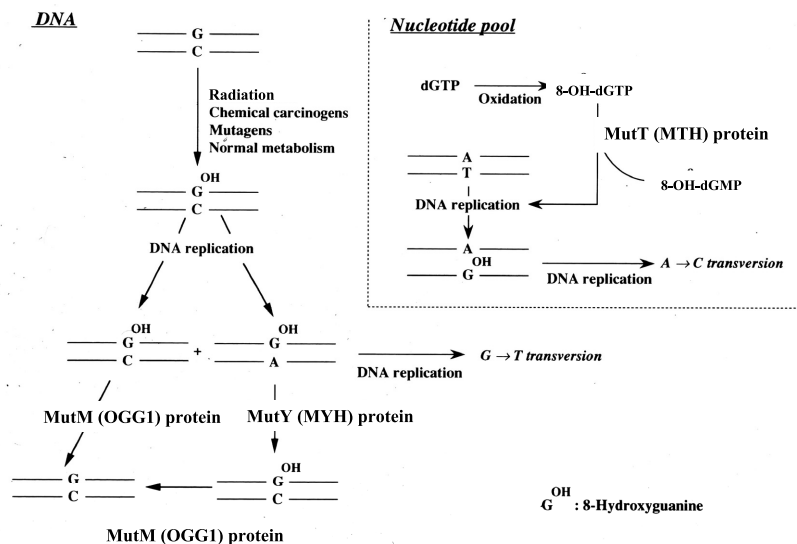


Fig. 7. Formation of base pairs of 8-OH-G with C or A in the opposite strands.

Fig. 8. DNA repair systems for 8-OH-G in *E. coli* and mammalian cells. The names of the enzymes in parenthesis are that from mammalian cells.

by *in vitro* reaction, when it was incubated with DNA and hydrogen peroxide.<sup>27)</sup> The results strongly suggested that the carcinogenic activity of asbestos could be explained by its potential to produce 8-OH-G in DNA.

6) *Detection of an 8-OH-G specific glycosylase/AP lyase in various rat and mouse tissues:*

An 8-OH-G specific glycosylase/AP lyase, similar to MutM/FPG protein, was identified in various tissues from rats and confirmed the ubiquitous presence of the

8-OH-G specific repair enzyme(s) not only in bacteria, but also in mammals.<sup>28),29)</sup>

In March 1986, the US-Japan Cooperative Cancer Research Program on "Oxygen Radicals in Cancer" was held in Honolulu, Hawaii. This meeting permitted the first presentation of our work on 8-OH-G to an international audience.<sup>30)</sup> Bruce N. Ames from the USA side, among other participants from the USA, took considerable interest in our work and in fact, Ames also directed

some of his efforts towards investigating this important subject.

**The presence of the three genes involved in 8-OH-G repair.** From around 1990 there was a large influx of investigators to the 8-OH-G research field. An outcome of particular notice from this period was the discovery of three genes that are engaged in 8-OH-G repair both in microorganisms and mammals (Fig. 8). Initially, much work had been done in *E. coli* to clarify the mechanism of 8-OH-G repair in DNA. As mentioned previously, FPG (the *MutM* gene product) is a specific glycosylase/AP lyase for 8-OH-G, and following its inactivation, an enhancement of G to T transversions is observed.<sup>31),32)</sup> *MutY*, identified by Jeffrey F. Miller and his colleagues, is a gene encoding a specific mismatch repair glycosylase enzyme which removes an adenine base when paired to 8-OH-G.<sup>33)</sup> *MutT*, discovered by Mutsuo Sekiguchi and his coworkers, codes for a specific pyrophosphatase with the ability to cleave 8-OH-dGTP to 8-OH-dGMP.<sup>34)</sup> Oxygen radicals produce not only 8-OH-G in DNA, but also 8-OH-dGTP from dGTP in the nucleotide pool. 8-OH-dGTP is incorporated into DNA opposite adenine (A) instead of cytosine (C), and thus, if not repaired prior to a subsequent round of replication, induces a mutation. Therefore, inactivation of *MutT* can result in A to C transversion. Lawrence A. Loeb and his colleagues in collaboration with us also showed that an A to C transversion takes place when using a plasmid vector system in *E. coli*.<sup>35)</sup> The existence of three gene products in *E. coli* responsible for the repair of 8-OH-G in DNA, sometimes called the GO system, demonstrates a functional cooperation to minimize mutagenesis<sup>36)</sup> and is a reflection of the importance of 8-OH-G repair to living organisms.

An important question is whether a similar mechanism for the repair of 8-OH-G exists in mammalian cells. It has been shown that homologs of both *MutY* and *MutT* are present in mammalian cells.<sup>37),38)</sup> As described in the previous section,<sup>28),29)</sup> we found a similar enzymatic activity to the *MutM* product (FPG protein) in mouse and rat tissues. An activity with a similar function was also found in human leukocytes.<sup>39)</sup> Subsequent attempts to purify the enzyme from human cells showed the presence of at least two isoforms.<sup>40)</sup> The gene coding for the *MutM* homolog was not identified in mammalian cells until 1997. Meanwhile, a gene encoding a functional *MutM* homolog called *OGG1* was isolated from the yeast *Saccharomyces cerevisiae* in 1996 by Serge Boiteux and his coworkers,<sup>41)</sup> and independently by Gregory L. Verdine and his colleagues.<sup>42)</sup> Suddenly in

1997, seven groups independently obtained a human or mouse homolog of *OGG1* through similarity searches of the respective EST database using the yeast *OGG1* sequence.<sup>43)-49)</sup> Among them, Hiroyuki Aburatani and his group of the University of Tokyo in collaboration with us, isolated four isoforms of cDNA produced by alternative splicing, as well as the related genomic DNA from human and mouse cells.<sup>43)</sup> This gene is referred to as *hMMH/ hOGG1* or *mMMH/ mOGG1* in human and mouse, respectively.<sup>43)</sup> One of the human isoforms, type 1a, was expressed in *E. coli* and its gene product was isolated. It was shown to function as a glycosylase/AP lyase specific for the 8-OH-G residue. Transfection of each of the four cDNA isoforms into *MutM*<sup>-</sup> and *MutY*<sup>-</sup> *E. coli* caused a reduced mutation frequency from G to T transversions. The type 1a isoform encodes a nuclear localization signal at its 3' end whereas the other does not have such signal, suggesting that the type 1a variant is involved in the repair of 8-OH-G in nuclear DNA, and the other is directed towards the repair of mitochondrial DNA. Later, this was confirmed by Akira Yasui and his colleagues.<sup>50)</sup> Although expression of human and mouse *Ogg1* was confirmed at mRNA level, it had not yet been shown at the protein level. In addition, it had not been clarified as to whether hOGG1 type 1a, type 1b, type 1c, type 2, or some other enzyme ranks as the most important member in the repair of 8-OH-G lesions. To answer this question, we prepared an OGG1 type 1a specific antibody. Using this antibody, we confirmed that the hOGG1 type 1a protein is indeed expressed in many human cell lines and that endogenous hOGG1 type 1a protein has repair activity for 8-OH-G lesions. In addition, we showed that upon depletion of the type 1a protein in a whole cell extract by its antibody, most of Ap lyase activity was lost, indicating that the type 1a protein is a major contributor to the repair of 8-OH-G lesions.<sup>51)</sup>

**Studies using *OGG1* knockout mice.** An important outstanding question was whether OGG1 is in fact involved in repair of 8-OH-G lesions *in vivo*. To answer this question, we generated *Ogg1* knockout mice (C57BL/6J) in collaboration with Tetsuo Noda of the Cancer Institute (Tokyo) and Aburatani.<sup>52)</sup> Genotype analysis of F<sub>2</sub> offspring revealed typical Mendelian ratios of wild-type, heterozygous, and homozygous pups amounting to 25, 48, and 25% respectively, indicating that the *Ogg1* gene product is not essential for embryonic development. Both *Ogg1*<sup>-/-</sup> and *Ogg1*<sup>+/-</sup> mice appeared healthy with respect to their survival and gross phenotypic appearance and male and female homozygous mice were found to be fertile. A sig-



nificant increase of 8-OH-G was observed in the liver DNA of *Ogg1*<sup>-/-</sup> mice. At 9 weeks of age, the level of 8-OH-G in liver DNA of *Ogg1* homozygous mice was 4.2-fold higher than that of wild-type mice. A further increase in 8-OH-G levels was observed at 14 weeks representing a 7-fold increase over wild-type mice of the same age. The mutation frequency of *gpt* liver tissue of *Ogg1*<sup>-/-</sup> mice was 2.3-fold higher than that of the wild type mice, indicating that 8-OH-G in DNA stimulates the induction of mutations. It is worth highlighting that Tomas Lindahl and his colleagues also reported similar results from an independently derived gene-targeted mouse line.<sup>53)</sup>

**High accumulation of 8-OH-G in kidney DNA of *Ogg1* knockout mice by chronic oxidative stress.** Since the mutation frequency of *Ogg1* knockout mice was marginal, it was difficult to clarify the type of mutation induced by 8-OH-G *in vivo*. To obtain more information regarding the involvement of 8-OH-G in mutation and/or carcinogenesis, it was necessary to promote oxidative stress in mice. We concluded that potassium bromate (KBrO<sub>3</sub>) was best suited for this purpose because of its specific oxygen radical forming property without formation of complexed DNA adduct and the fact it is a known renal carcinogen for rodents.<sup>54),55)</sup> *Ogg1*<sup>-/-</sup>, *Ogg1*<sup>-/+</sup>, and *Ogg1*<sup>+/+</sup> mice carrying a *gpt* marker were treated with KBrO<sub>3</sub> in their drinking water over a long period (12 weeks). The level of 8-OH-G in kidney DNA of *Ogg1*<sup>-/-</sup> mice was tremendously increased in a time-dependent manner (~70 times above that of *Ogg1*<sup>+/+</sup> mice treated with KBrO<sub>3</sub>). KBrO<sub>3</sub> treatment also gave rise to an increased mutation frequency. Another interesting observation is that the accumulated 8-OH-G did not decrease following discontinuation of KBrO<sub>3</sub> treatment, indicating the absence of significant repair in the total genome.<sup>56)</sup>

With the extensive exposure to KBrO<sub>3</sub> as described, the amounts of 8-OH-G in the liver DNA of *Ogg1*<sup>-/-</sup> mice also showed significant increases; one fifth that of kidney DNA. Such accumulated DNA lesions did not decrease even 4 weeks after termination of KBrO<sub>3</sub> treatment. Therefore, in order to assess whether cell proliferation influences the extent of mutation when 8-OH-G is present in DNA, we performed partial hepatectomy on *Ogg1*<sup>-/-</sup> and *Ogg1*<sup>+/+</sup> mice after being treated with KBrO<sub>3</sub>. The remnant liver from *Ogg1*<sup>-/-</sup> mice treated with KBrO<sub>3</sub> regenerated to the same extent as nontreated *Ogg1*<sup>+/+</sup> mice. Interestingly, 8-OH-G was not repaired during cell proliferation following partial hepatectomy, indicating that there is no appreciable replication cou-

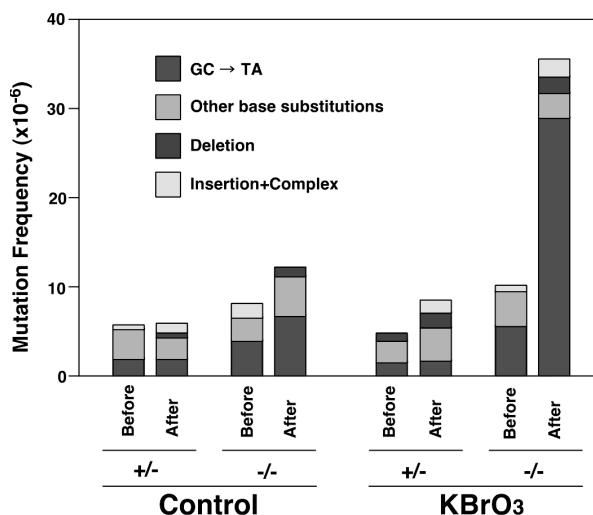


Fig. 9. Mutation spectra of *gpt* gene from liver of *gpt/Ogg1* mutant mice which had been exposed to KBrO<sub>3</sub>, followed by partial hepatectomy.

pled repair of preexisting 8-OH-G, induced by DNA replication or cell proliferation. The mutation frequency after the regeneration of liver from treated *Ogg1*<sup>-/-</sup> mice showed a 3.5-fold increase as compared with that before regeneration. The proliferation of cells with accumulated amounts of 8-OH-G caused mainly GC→TA transversions, as would be predicted from previous *in vitro* experiments (Fig. 9).<sup>57)</sup>

Next, in order to assess the effect of KBrO<sub>3</sub> on tumor induction, a one year carcinogenesis study was performed. *Ogg1*<sup>-/-</sup> and *Ogg1*<sup>+/+</sup> mice were chronically exposed to KBrO<sub>3</sub> for 29 weeks. After termination of the treatment, mice were kept for an additional 23 weeks. The amount of 8-OH-G in kidney DNA after 29 weeks of KBrO<sub>3</sub> exposure reached 500 residues of 8-OH-dG/10<sup>6</sup>dG, almost 250 fold that of untreated wild type mice. During the course of the study the mice appeared normal, although a decrease of body weight gain and some kidney malfunction was observed. When *Ogg1*<sup>-/-</sup> and *Ogg1*<sup>+/+</sup> mice were sacrificed at 52 weeks, tumor formation could not be found in the kidney or other organs, such as the lung, liver, spleen, thymus, stomach and intestine. Microscopic examination also failed to discern precancerous foci in any tissues from *Ogg1*<sup>-/-</sup> and *Ogg1*<sup>+/+</sup> mice.<sup>58)</sup>

**Evidence showing the involvement of 8-OH-G in carcinogenesis in rodents and human.** Kunihiro Sakumi *et al.*<sup>59)</sup> reported that *Ogg1* deficient mice derived from C57BL/6J kept under normal conditions developed a greater number of lung adenoma and carci-

noma compared to wild type mice at an age of one and half years. A plausible explanation for the discrepancy between the results of our groups may arise from the fact that Sakumi *et al.*<sup>59)</sup> showed that even wild type mice produced tumors in various organs, although the number of the tumors was less than that found in *Ogg1*<sup>-/-</sup> mice. On the contrary, in our experiment, no tumors were found in control wild type mice whether or not they had been exposed to KBrO<sub>3</sub>. One could argue that in the study of Sakumi *et al.*, the mice were kept for a longer period of time (78 weeks compared to 60 weeks in our experiment). KBrO<sub>3</sub> is known to be a specific oxygen radical forming agent. Thus, one can speculate that mutation of the genome alone is insufficient for tumor formation, and a promoting process or inflammation is necessary for tumor induction. In order to explore this possibility, we are currently performing a carcinogenesis study in which partially hepatectomized *Ogg1*<sup>-/-</sup> mice are kept for a long period of time after treatment with KBrO<sub>3</sub>, since partial hepatectomy is known to have tumor promoting activity.

Other knockout mice strains related to 8-OH-G repair, such as *MYH* and *MTH* also display a higher tumor incidence compared to wild type mice, even when not exposed to oxidative stress.<sup>60)-63)</sup> *MYH* or *MTH* deficiency may be of greater importance to tumor induction in comparison to *Ogg1* deficiency. However, the actual increase of 8-OH-G and the mutation frequency in these mice without applied oxidative stress is comparable or less than that found in *Ogg1*<sup>-/-</sup> mice exposed to KBrO<sub>3</sub>. Incidentally, Yoshimichi Nakatsu *et al.* recently reported that *Mutyh* deficient mice exposed to KBrO<sub>3</sub> showed increased G→T mutation frequency and subsequently produce a large number of tumors in the intestine.<sup>64)</sup>

Other 8-OH-G repair systems are known to exist in addition to *Ogg1*. Tapas K. Hazra *et al.* found an OGG-2 enzyme which cleaves 8-OH-G/G and 8-OH-G/A lesions more efficiently than 8-OH-G/C.<sup>65)</sup> The same group also identified another repair enzyme, NEH 1 which has a wide substrate specificity.<sup>66)</sup> The nucleotide excision pathway may also play a role in the repair of 8-OH-G, because an 8-OH-G base was shown to be removed from synthetic oligonucleotides by a reconstituted system consisting of XPA, RPA, TFIIH, XPC-HHR23B, XPG and XPF-ERCCI.<sup>67)</sup> Transcription-coupled repair system can remove 8-OH-G in the transcription strand of *Ogg1*<sup>-/-</sup> cell lines, though at a slower rate than *Ogg1*<sup>+/+</sup> cell lines.<sup>68),69)</sup> In addition, reports have shown that other enzymes, such as N-methylpurine-DNA glycosylase<sup>70)</sup>

and ribosomal protein S3<sup>69)</sup> have the capacity to cleave 8-OH-G containing DNA. Therefore, the possibility exists that although the contribution of other enzymes to the removal of 8-OH-G within the overall genome is marginal, their activity could be of greater relevance to preventing carcinogenesis.

In the case of humans, it is now clearly established that the presence of 8-OH-G in DNA correlates with carcinogenesis. Allelic polymorphisms in the *Ogg1* exonic region, which is translated to an altered enzymatic activity of the OGG1 protein, were found to correlate with the incidence of lung cancer in humans.<sup>71)-73)</sup> A strong association was also found between a reduced activity in OGG1 protein and lung cancer incidence.<sup>74)</sup> Some hereditary colorectal cancer patients were found to have a deficiency in the *MYH* gene.<sup>75)-77)</sup>

The induction of cancers in human such as that occurs in lung and colon, is the results of multiple processes over a long period of time. Under these circumstances the significance of 8-OH-G in carcinogenesis might be clearly seen. Therefore, a more sophisticated animal model to elucidate the molecular mechanism of 8-OH-G in human cancer may be needed.

**Transcriptional misreading of a human *c-Ha-ras* fragment within an 8-OH-G hot spot by DNA polymerase  $\eta$  and  $\kappa$ .** Recent studies have provided interesting data on the participation of non-replicative DNA polymerases in mutagenesis. A group of minor polymerases were found to have the unique ability of translesion DNA synthesis. Enzymes of particular interest are the Y-family DNA polymerases  $\iota$ ,  $\eta$  and  $\kappa$ , which were shown to bypass various forms of DNA damage.<sup>78)</sup> This prompted us to examine the mechanism of how 8-OH-G residues in DNA are recognized by such Y-family polymerases.

We previously reported that a chemically synthesized form of the human *c-Ha-ras* gene having 8-OH-G in codon 12 or 61 showed remarkable transforming activity when transfected into NIH3T3 cells.<sup>79)-81)</sup> An interesting observation was that the mutation found in transfected human *c-Ha-ras* consisted not only of G→T transversions on the position of 8-OH-G, but also G→C transversions. In addition, neighboring bases to the 8-OH-G residue were also found to be mutated (Table I). To examine this phenomenon further, fragments of the human *c-Ha-ras* gene that contained 8-OH-G in codon 12 were used as a template for DNA polymerase  $\eta$  using an *in vitro* reaction.<sup>82)</sup> It was shown that DNA polymerase  $\eta$  not only misincorporated dAMP, but also dGMP, while DNA polymerases  $\alpha$  and  $\beta$  exclusively led to



Table I. Mutations induced by 8-OH-G containing *c-Ha-ras* genes transfected into NIH3T3 cells

|                   | Position of 8-OH-G |               |    |               |                |               |    |  |
|-------------------|--------------------|---------------|----|---------------|----------------|---------------|----|--|
|                   | 34                 |               | 35 |               | 181            |               | 35 |  |
| G*→T <sup>a</sup> | 14                 | G*→T          | 22 | G*→T          | 35             | G*→T          | 8  |  |
| G*→A              | 1                  | G*→A          | 8  | G*→A          | 0 <sup>b</sup> | G*→A          | 3  |  |
| G*→C              | 0                  | G*→C          | 1  | G*→C          | 0 <sup>b</sup> | G*→C          | 1  |  |
| 3'-G→T            | 0                  | 5'-G→T        | 4  | 5'-T→A        | 1              | 5'-G→T        | 1  |  |
| 3'-G→A            | 0                  | 5'-G→A        | 5  | 5'-T→C        | 4              | 5'-G→A        | 1  |  |
| 3'-G→C            | 0                  | 5'-G→C        | 2  | 5'-T→G        | 0 <sup>b</sup> | 5'-G→C        | 0  |  |
| total mutants     | 15                 | total mutants | 42 | total mutants | 40             | total mutants | 14 |  |

<sup>a</sup>G\* represents 8-OH-G.

<sup>b</sup>Mutations at this position do not activate the *c-Ha-ras* gene.

misincorporation of dAMP. In addition, we observed that when using polymerase  $\eta$ , an 8-OH-G residue was capable of propagating a mutation as a result of an “action-at-a-distance” effect. The replication catalyzed by this enzyme resulted in misincorporation of dAMP, dTMP and dGMP opposite a normal guanine that was flanked on the 3'-position by an 8-OH-G residue. Intriguingly, with two adjacent 8-OH-G residues the specificity of polymerase  $\eta$  was even more compromised, leading to the incorporation all four nucleotides. By contrast, two adjacent 8-OH-G residues almost completely blocked synthesis by polymerases  $\alpha$  and  $\beta$ . These results suggest an important role for polymerase  $\eta$  in conferring hypermutability to codon 12. Similar results were also obtained in the case of polymerase  $\kappa$ .<sup>83)</sup> Louise Prakash and her colleagues previously reported that polymerase  $\eta$  is able to carry out an efficient and accurate translesion replication at the site of the 8-OH-G residue.<sup>84)</sup> Apparently, misreading of 8-OH-G by polymerase  $\eta$  depends on the sequence context of DNA.

Motoya Katsuki and his colleagues in collaboration with us previously reported that transgenic mice having a normal human *c-Ha-ras* gene are quite sensitive to spontaneous tumor induction or by various chemical carcinogens and the resulting tumors always contained activated human *c-Ha-ras* that had been altered at either at codon 12 or 61.<sup>85),86)</sup> Thus, experiments are now underway to test whether *Ogg1* knockout mice harboring a normal human *c-Ha-ras* gene develop tumors upon exposure to compounds that induce oxidative stress. Should this be the case, it would provide direct proof that mutagenesis by 8-OH-G in DNA is linked to carcinogenesis.

**Future aspects of 8-OH-G involvement in carcinogenesis.** From what has been described in the pre-

ceding sections, it is reasonable to conclude that 8-OH-G is involved in the induction of cancer in humans, although some discrepancy has been found with the rodent model. Obviously, a more suitable rodent experimental model is required to elucidate how 8-OH-G is involved in cancer induction at a molecular level. However, the negative results with respect to carcinogenesis in *OGG1* knockout mice exposed to high oxidative stress might offer some clues; for example, the possibility that another process in addition to a point mutation in the genome is necessary for the induction of cancer.

Giving consideration to future approaches, several other factors need to be addressed. It is possible that 8-OH-G formation in DNA by oxygen radicals is not evenly distributed throughout the genome, and indeed, the distribution of 8-OH-G in DNA also may differ depending upon which reagents are used to generate oxygen radicals. It is also true that the pattern of 8-OH-G repair in DNA by OGG1 is not uniform through the genome, as it has been shown that 8-OH-G residues in the transcribed DNA strand are resistant to repair by OGG1.<sup>68),69)</sup> Therefore, studies based on other 8-OH-G repair enzymes and further genetically engineered mouse models are needed. It is also important to assess the effect of post-translational modification(s) on OGG1 activity and other 8-OH-G related repair enzymes.<sup>87)</sup>

Oxygen radicals are generated not only by exogenous reagents such as radiation and environmental chemical carcinogens, but also by internal cellular metabolism. Oxygen radicals have the ability to react with various cellular components such as proteins and lipids; being in some cases beneficial to the cell. Therefore, the administration of antioxidants may not be

a wholly appropriate solution for the prevention of carcinogenesis. The inhibition of 8-OH-G formation in DNA by more specific means may be a better alternative. In other words, it would be interesting to find specific reagents that could inhibit formation of 8-OH-G in DNA by oxygen radicals, or inhibit misreplication in translesion DNA synthesis at the site of the 8-OH-G residue. Another possible alternative would be to use an agonist(s) to enhance the enzymatic activity of *OGG1*. Although admittedly the successful attainment of such goals would be a challenge, it is certainly worthwhile to consider the pursuit of these long-term objectives.

<sup>\*1</sup> The enzyme *OGG1*, as well as *MutM*, has two enzymatic activities, glycosylase and AP lyase in a single molecule; see Fig. 4.

<sup>\*2</sup> The actual mutagens present in heated glucose were later defined as methylreductic acid and hydroxymethylreductic acid.<sup>88)</sup>

<sup>\*3</sup> Other groups refer to this as *OGG1*, because it was isolated by a similarity search using yeast *OGG1*. We named it *MMH* based on its similarity to the *MutM* activity of *E. coli*. Hereafter, to avoid confusion, we will use the term *OGG1*.

## Appendix

**Analysis of 8-hydroxydeoxyguanosine:** It is worth emphasizing the need to properly analyze the amount of 8-hydroxydeoxyguanosine (8-OH-dG) present in DNA, isolated from various sources including mammalian tissues, with the minimal amount of artifacts. The lowest amount of background 8-OH-dG (for example, DNA from wild type mice not exposed to exogenous oxidative stress) is approximately 1~2 8-OH-dG residue per  $10^6$  dG of DNA. With such a low background of 8-OH-dG, an increase of 8-OH-dG in tissue DNA can be observed in *OGG1* knockout mice with or without exposure to oxidative stress.<sup>52)</sup> The method utilized in our studies is presently the best procedure to avoid the artificial generation of 8-OH-dG during isolation of deoxynucleosides from tissue DNA. Dai Nakae *et al.* were the first to show the usefulness of NaI in extracting DNA from tissue.<sup>89)</sup> Later, Ames and his colleagues endorsed this procedure in a subsequent detailed study.<sup>90)</sup> It does not appear to matter whether the final quantitative analysis is made by means of HPLC-coupled electrochemical detection or HPLC-MS since the crucial steps in avoiding artifacts are the isolation of DNA and

the subsequent conversion of DNA to deoxynucleosides. Consequently, many previous reports have inadvertently expressed the background 8-OH-dG levels as 10 to 100 times above that which it should be. In these cases, the conclusion of the report must be cautiously considered.

**Nomenclature:** 8-Hydroxyguanine was also later referred to as 7,8-dihydro-8-oxoguanine, since its favored tautomeric confirmation is the 8-oxo-form. However, personally I prefer to use 8-hydroxyguanine rather than 7,8-dihydro-8-oxoguanine. As a comparison, malonaldehyde which is a known DNA modifier has been widely adopted as the name, even though its favoured tautomeric conformation is a hydroxyl form. Additionally, 7,8-dihydro-8-oxoguanine does not actually exist in nature, whereas 8-hydroxyguanine is derived from guanine. Perhaps, 7,8-dihydro-8-oxoguanine is a more biologically biased name. Finally, 8-oxoguanine is an abbreviated term, and therefore not formally correct.

**Analysis of urinary 8-OH-dG:** The amount of 8-OH-dG present in the urine of animals is regarded as a reliable marker to assess the extent of exposure to oxidative stress. For example, it was shown that the urinary level of 8-OH-dG from smokers is statistically higher than that of non-smokers.<sup>91)</sup> However, there is no direct relationship between the amounts of 8-OH-dG in urine and the extent of 8-OH-dG formation in the DNA of tissues, since 8-OH-G is removed from DNA in the form of the base, 8-hydroxyguanine. It is very likely that the removal of 8-OH-G residues in DNA is principally carried out by base-excision repair enzymes such as *OGG1*. Nucleotide excision repair enzymes, involved in transcription-coupled repair processes may not be a major contributor to 8-OH-G repair in DNA. 8-Hydroxydeoxyguanosine found in urine is perhaps derived from the free nucleotide pool.

**Acknowledgements.** I thank my colleagues who were involved in the studies on 8-OH-G described in this article, while I was in the National Cancer Center Research Institute, Tokyo from 1965 to 1992 and Tsukuba Research Institute, Banyu Pharmaceutical Co. Ltd., Tsukuba from 1992 to 2004. Particularly I thank Dr. H. Kasai who did his pioneering work of discovery of 8-OH-G. I also thank Drs. E. Ohtsuka, H. Aburatani, T. Noda, F. Hanaoka, H. Ohmori, S. Mitra, A. P. Grollman and L. A. Loeb for their important and crucial collaborations with us. I am also grateful to Drs. T. Lindahl, P. C. Hanawalt, R. A. Floyd, and M. Dizdaroglu for their interest and discussion on our work.

### References

- 1) Sugimura, T. (2004) Establishment of the concept that cancer is a disease of DNA: Serendipitous discoveries in my research career concerning the science of carcinogenesis. Selected Topics in the History of Biochemistry: Personal Recollections VIII. *In* Comprehensive Biochemistry, Vol 43 (eds. by Semenza, G., and Turner, A. J.). Elsevier B. V., pp. 355-392.
- 2) Sugimura, T., Wakabayashi, K., Nakagama, H., and Nagao, M. (2004) Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci.* **95**, 290-299.
- 3) Sugimura, T. (1997) Overview of carcinogenic heterocyclic amines. *Mutation Res.* **376**, 211-219.
- 4) Kasai, H., Yamaizumi, Z., Wakabayashi, K., Nagao, M., Sugimura, T., Yokoyama, S., Miyazawa, T., Springan, N. E., Weisburger, J. H., and Nishimura, S. (1980) Potent novel mutagens produced by broiling fish under normal conditions. *Proc. Jpn. Acad., Ser. B* **56**, 278-283.
- 5) Kasai, H., Yamaizumi, Z., Wakabayashi, K., Nagao, M., Sugimura, T., Yokoyama, S., Miyazawa, T., and Nishimura, S. (1980) Structure and chemical synthesis of Me-IQ, a potent mutagen isolated from broiled fish. *Chem. Lett.*, 1931-1934.
- 6) Kasai, H., Yamaizumi, Z., Shiomi, T., Yokoyama, S., Miyazawa, T., Wakabayashi, K., Nagao, M., Sugimura, T., and Nishimura, S. (1981) Structure of a potent mutagen isolated from fried beef. *Chem. Lett.*, 485-488.
- 7) Kasai, H., Hayami, H., Yamaizumi, Z., Saito, H., and Nishimura, S. (1984) Detection and identification of mutagens and carcinogens as their adducts with guanosine derivatives. *Nucl. Acids Res.* **12**, 2127-2136.
- 8) Kasai, H., and Nishimura, S. (1983) Hydroxylation of the C-8 position of deoxyguanosine by reducing agents in the presence of oxygen. *Nucl. Acids Res., Symp. Ser., No 12*, s165-s167.
- 9) Kasai, H., and Nishimura, S. (1984) Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucl. Acids Res.* **12**, 2137-2145.
- 10) Kasai, H., and Nishimura, S. (1984) Hydroxylation of deoxyguanosine at the C-8 position by polyphenols and aminophenols in the presence of hydrogen peroxide and ferric ion. *Gann* **75**, 565-566.
- 11) Kohda, K., Tada, M., Kasai, H., Nishimura, S., and Kawazoe, Y. (1986) Formation of 8-hydroxyguanine residues in cellular DNA exposed to the carcinogen 4-nitroquinoline 1-oxide. *Biochem. Biophys. Res. Commun.* **139**, 626-632.
- 12) Kasai, H., Tanooka, H., and Nishimura, S. (1984) Formation of 8-hydroxyguanine residues in DNA by X-irradiation. *Gann* **75**, 1037-1039.
- 13) Setlow, R. B., and Setlow, J. K. (1962) Evidence that ultraviolet-induced thymine dimers in DNA cause biological damage. *Proc. Natl. Acad. Sci. USA*, **48**, 1250-1257.
- 14) Floyd, R. A., Watson, J. J., Wong, P. K., Altmiller, D. H., and Rickard, R. C. (1986) Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanism of formation. *Free Radical Res. Commun.* **1**, 163-172.
- 15) Kasai, H., Crain, P. F., Kuchino, Y., Nishimura, S., Ootsuyama, A., and Tanooka, H. (1986) Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* **7**, 1849-1851.
- 16) Chung, M. H., Kasai, H., Jones, D. S., Inoue, H., Ishikawa, H., Ohtsuka, E., and Nishimura, S. (1991) An endonuclease activity of *Escherichia coli* that specifically removes 8-hydroxyguanine residues from DNA. *Mutation Res.* **254**, 1-12.
- 17) Tchou, J., Kasai, H., Shibutani, S., Chung, M.-H., Laval, J., Grollman, A. P., and Nishimura, S. (1991) 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proc. Natl. Acad. Sci. USA* **88**, 4690-4694.
- 18) Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E., and Nishimura, S. (1987) Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. *Nature* **327**, 77-79.
- 19) Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* **349**, 431-434.
- 20) Aida, M., and Nishimura, S. (1987) An ab initio molecular orbital study on the characteristics of 8-hydroxyguanine. *Mutation Res.* **192**, 83-89.
- 21) Kasai, H., Nishimura, S., Toriumi, Y., Itai, A., and Iitaka, Y. (1987) The crystal structure of 9-ethyl-8-hydroxyguanine. *Bull. Chem. Soc. Jpn.* **60**, 3799-3800.
- 22) Oda, Y., Uesugi, S., Ikehara, M., Nishimura, S., Kawase, Y., Ishikawa, H., Inoue, H., and Ohtsuka, E. (1991) NMR studies of a DNA containing 8-hydroxydeoxyguanosine. *Nucl. Acids Res.* **19**, 1407-1412.
- 23) Kouchakdjian, M., Bodepudi, V., Shibutani, S., Eisenberg, M., Johnson, F., Grollman, A. P., and Patel, D. J. (1991) NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7H-dG) opposite deoxyadenosine in a DNA duplex. 8-oxo-7H-dG (syn), dA (anti) alignment at lesion site. *Biochemistry* **30**, 1403-1412.
- 24) Lipscomb, L. A., Peek, M. E., Morningstar, M. L., Verghis, S. M., Miller, E. M., Rich, A., Essigmann, J. M., and Williams, L. D. (1995) X-ray structure of a DNA decamer containing 7,8-dihydro-8-oxoguanine. *Proc. Natl. Acad. Sci. USA* **92**, 719-723.
- 25) Kasai, H., Nishimura, S., Kurokawa, Y., and Hayashi, Y. (1987) Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA. *Carcinogenesis* **8**, 1959-1961.
- 26) Kasai, H., Okada, Y., Nishimura, S., Rao, M. S., and Reddy, J. K. (1989) Formation of 8-hydroxydeoxyguanosine in liver DNA of rats following long-term exposure to a peroxisome proliferator. *Cancer Res.* **49**, 2603-2605.
- 27) Kasai, H., and Nishimura, S. (1984) DNA damage induced by asbestos in the presence of hydrogen peroxide. *Gann* **75**,

- 841-844.
- 28) Yamamoto, F., Kasai, H., Bessho, T., Chung, M. H., Inoue, H., Ohtsuka, E., Hori, T., and Nishimura, S. (1992) Ubiquitous presence in mammalian cells of enzymatic activity specifically cleaving 8-hydroxyguanine-containing DNA. *Jpn. J. Cancer Res.* **83**, 351-357.
  - 29) Yamamoto, F., Kasai, H., Togashi, Y., Takeichi, N., Hori, T., and Nishimura, S. (1993) Elevated level of 8-hydroxydeoxyguanosine in DNA of liver, kidney and brain of Long-Evans cinnamon rats. *Jpn. J. Cancer Res.* **84**, 508-511.
  - 30) Nishimura, S., and Ames, B. N. (1986) US-Japan meeting on "Oxygen radicals in cancer". *Jpn. J. Cancer Res. (Gann)* **77**, 843-848.
  - 31) Michaels, M. L., Pham, L., Cruz, C., and Miller, J. H. (1991) MutM, a protein that prevents GC  $\rightarrow$  TA transversions, is formamidopyrimidine-DNA glycosylase. *Nucl. Acids Res.* **19**, 3629-3632.
  - 32) Bessho, T., Tano, K., Kasai, H., and Nishimura, S. (1992) Deficiency of 8-hydroxyguanine DNA endonuclease activity and accumulation of the 8-hydroxyguanine in mutator mutant (*MUTM*) of *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **188**, 372-378.
  - 33) Michaels, M. L., Cruz, C., Grollman, A. P., and Miller, J. H. (1992) Evidence that *MutY* and *MutM* combine to prevent mutation by oxidatively damaged form of guanine in DNA. *Proc. Natl. Acad. Sci. USA* **89**, 7022-7025.
  - 34) Maki, H., and Sekiguchi, M. (1992) Mut T protein specifically hydrolyzes a potent mutagenic substrate for DNA synthesis. *Nature* **355**, 273-275.
  - 35) Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. (1992) 8-Hydroxyguanine, an abundant product of oxidative DNA damage, causes G  $\rightarrow$  T and A  $\rightarrow$  C substitutions. *J. Biol. Chem.* **267**, 166-172.
  - 36) Michaels, M. L., and Miller, J. H. (1992) The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). *J. Bacteriol.* **174**, 6321-6325.
  - 37) Slupska, M. M., Baikalov, C., Luther, W. M., Chiang, J.-H., Wei, Y. F., and Miller, J. H. (1996) Cloning and sequencing a human homolog (*hMYH*) of the *Escherichia coli mutY* gene whose function is required for the repair of oxidative DNA damage. *J. Bacteriol.* **178**, 3885-3892.
  - 38) Sakumi, K., Furuichi, M., Tsuzuki, T., Kakuma, T., Kawabata, S., Maki, H., and Sekiguchi, M. (1993) Cloning and expression of cDNA for a human enzyme that hydrolyzes 8-oxodGTP, mutagenic substrate for DNA synthesis. *J. Biol. Chem.* **268**, 23524-23530.
  - 39) Chung, M.-H., Kim, H.-S., Ohtsuka, E., Kasai, H., Yamamoto, F., and Nishimura, S. (1991) An endonuclease activity in human polymorphonuclear neutrophils that removes 8-hydroxyguanine residues from DNA. *Biochem. Biophys. Res. Commun.* **178**, 1472-1478.
  - 40) Bessho, T., Tano, K., Kasai, H., Ohtsuka, E., and Nishimura, S. (1993) Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in human cells. *J. Biol. Chem.* **268**, 19416-19421.
  - 41) van der Kemp, P. A., Thomas, D., Barbey, R., de Oliveira, R., and Boiteux, S. (1996) Cloning and expression in *Escherichia coli* of the *OGG1* gene of *Saccharomyces cerevisiae* which codes for a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine. *Proc. Natl. Acad. Sci. USA* **93**, 5197-5202.
  - 42) Nash, H. M., Bruner, S. D., Schärer, O. D., Kawate, T., Addona, T. A., Spooner, E., Lane, W. S., and Verdine, G. L. (1996) Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA repair protein superfamily. *Current Biology* **6**, 968-980.
  - 43) Aburatani, H., Hippo, Y., Ishida, T., Takashima, R., Matsuba, C., Kodama, T., Takao, M., Yasui, A., Yamamoto, K., Asano, M. *et al.* (1997) Cloning and characterization of mammalian 8-hydroxyguanine-specific glycosylase/apurinic, apyrimidinic lyase, a functional mutM homologue. *Cancer Res.* **57**, 2151-2156.
  - 44) Rosenquist, T. A., Zharkov, D. I., and Grollman, A. P. (1997) Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc. Natl. Acad. Sci. USA* **94**, 7429-7434.
  - 45) Rodlan-Arjona, T., Wei, Y.-F., Carter, K. C., Klungland, A., Anselmino, C., Wang, R.-P., Augstutand, M., and Lindahl, T. (1997) Molecular cloning and functional expression of a human cDNA encoding the antitumor enzyme 8-hydroxyguanine-DNA glycosylase. *Proc. Natl. Acad. Sci. USA* **94**, 7429-7434.
  - 46) Radicella, J. P., Dherin, C., Desmaze, C., Fox, M. S., and Boiteux, S. (1997) Cloning and characterization of *hOGG1*, a human homolog of the *OGG1* gene of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **94**, 8010-8015.
  - 47) Arai, K., Morishita, K., Shinmura, K., Kohno, T., Kim, S.-R., Nohmi, T., Taniwaki, M., Ohwada, S., and Yokota, J. (1997) Cloning of a human homolog of the yeast *OGG1* gene that is involved in the repair of oxidative DNA damage. *Oncogene* **14**, 2857-2861.
  - 48) Lu, R., Nash, H. M., and Verdine, G. L. (1997) A mammalian DNA repair enzyme that excises oxidatively damaged guanines maps to a locus frequently lost in lung cancer. *Current Biology* **7**, 397-407.
  - 49) Bjørås, M., Luna, L., Johnsen, B., Hoff, E., Haug, T., Rognes, T., and Seeberg, E. (1997) Opposite base-dependent reactions of a human base excision repair enzyme on DNA containing 7,8-dihydro-8-oxoguanine and abasic site. *EMBO J.* **16**, 6314-6322.
  - 50) Takao, M., Aburatani, H., Kobayashi, K., and Yasui, A. (1998) Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage. *Nucl. Acids Res.* **26**, 2917-2922.
  - 51) Monden, Y., Arai, T., Asano, M., Ohtsuka, E., Aburatani, H., and Nishimura, S. (1999) Human MMH (OGG1) type 1 a protein is a major enzyme for repair of 8-hydroxyguanine lesions in human cells. *Biochem. Biophys. Res. Commun.* **258**,

- 605-610.
- 52) Minowa, O., Arai, T., Hirano, M., Monden, Y., Nakai, S., Fukuda, M., Itoh, M., Takano, H., Hippou, Y., Aburatani, H. *et al.* (2000) *Mmh/Ogg1* gene inactivation results in accumulation of 8-hydroxyguanine in mice. *Proc. Natl. Acad. Sci. USA* **97**, 4156-4161.
  - 53) Klungland, A., Rosewell, I., Hollenbach, S., Larsen, E., Daly, G., Epe, B., Seeberg, E., Lindahl, T., and Barnes, D. E. (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc. Natl. Acad. Sci. USA* **96**, 13300-13305.
  - 54) Kurokawa, Y., Hayashi, Y., Maekawa, A., Takahashi, M., Kokubo, T., and Odashima, S. (1983) Carcinogenicity of potassium bromate administered orally to F344 rats. *J. Natl. Cancer Inst.* **71**, 965-972.
  - 55) Kurokawa, Y., Aoki, S., Matsushima, Y., Takamura, N., Imazawa, T., and Hayashi, Y. (1986) Dose-response studies on the carcinogenicity of potassium bromate in F344 rats after long-term oral administration. *J. Natl. Cancer Inst.* **77**, 977-982.
  - 56) Arai, T., Kelly, V. P., Minowa, O., Noda, T., and Nishimura, S. (2002) High accumulation of oxidative DNA damage, 8-hydroxyguanine, in *Mmh/Ogg1* deficient mice by chronic oxidative stress. *Carcinogenesis* **23**, 2005-2010.
  - 57) Arai, T., Kelly, V. P., Komoro, K., Minowa, O., Noda, T., and Nishimura, S. (2003) Cell proliferation in liver of *Mmh/Ogg1*-deficient mice enhances mutation frequency because of the presence of 8-hydroxyguanine in DNA. *Cancer Res.* **63**, 4287-4292.
  - 58) Arai, T., Kelly, V. P., Minowa, O., Noda, T., and Nishimura, S. (2006) The study using wild-type and *Ogg1* knockout mice exposed to potassium bromate shows no tumor induction despite an extensive accumulation of 8-hydroxyguanine in kidney DNA. *Toxicology* **221**, 179-186.
  - 59) Sakumi, K., Tominaga, Y., Furuichi, M., Xu, P., Tsuzuki, T., Sekiguchi, M., and Nakabeppu, Y. (2003) *Ogg1* knockout-associated lung tumorigenesis and its suppression by *Mth1* gene disruption. *Cancer Res.* **63**, 902-905.
  - 60) Russo, M. T., De Luca, G., Degan, P., Parlanti, E., Dogliotti, E., Barnes, D. E., Lindahl, T., Yang, H., Miller, J. H., and Bignami, M. (2004) Accumulation of the oxidative base lesion of 8-hydroxyguanine in DNA of tumor-prone mice defective in both *Myh* and *Ogg1* DNA glycosylases. *Cancer Res.* **64**, 4411-4414.
  - 61) Xie, Y., Yang, H., Cunanan, C., Okamoto, K., Shibata, D., Pan, J., Bernes, D. E., Lindahl, T., McIlhatton, M., Fishel, R., and Miller, J. H. (2004) Deficiencies in mouse *Myh* and *Ogg1* result in tumor predisposition and G to T mutations in codon 12 of the *K-Ras* oncogene in lung tumors. *Cancer Res.* **64**, 3096-3102.
  - 62) Cheadle, J. P., Dolwani, S., and Sampson, J. R. (2003) Inherited defects in the DNA glycosylase MYH cause multiple colorectal adenoma and carcinoma. *Carcinogenesis* **24**, 1281-1282.
  - 63) Tsuzuki, T., Egashira, A., Igarashi, H., Iwakuma, T., Nakatsuru, Y., Tominaga, Y., Kawate, H., Nakao, K., Nakamura, K., Ide, F. *et al.* (2001) Spontaneous tumorigenesis in mice defective in the *MTH1* gene encoding 8-oxodGTPase. *Proc. Natl. Acad. Sci. USA* **98**, 11456-11461.
  - 64) Nakatsu, Y., Yamauchi, K., Isoda, T., Nakabeppu, Y., and Tsuzuki, T. (2005) Oxidative stress-induced intestinal tumors in *Mutyh*-deficient mice. The Proceedings of Sixty-Fourth Annual Meeting of the Japanese Cancer Association, pp. 1-337.
  - 65) Hazra, T. K., Izumi, T., Maitt, L., Floyd, R. A., and Mitra, S. (1998) The presence of two distinct 8-oxoguanine repair enzymes in human cells: their potential complementary roles in preventing mutation. *Nucl. Acids Res.* **26**, 5116-5122.
  - 66) Hazra, T. K., Izumi, T., Boldogh, I., Imhoff, B., Kow, Y. W., Jaruga, P., Dizdaroglu, M., and Mitra, S. (2002) Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. *Proc. Natl. Acad. Sci. USA* **99**, 3523-3528.
  - 67) Reardon, J. T., Bessho, T., Kung, H. C., Bolton, P. H., and Sancar, A. (1977) *In vitro* repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in *Xeroderma pigmentosum* patients. *Proc. Natl. Acad. Sci. USA* **94**, 9463-9468.
  - 68) Le Page, F., Randrianarison, V., Marot, D., Cabannes, J., Perricaudet, M., Feunteun, J., and Sarasin, A. (2000) BRCA1 and BRCA2 are necessary for the transcription-coupled repair of the oxidative 8-oxoguanine lesion in human cells. *Cancer Res.* **60**, 5548-5552.
  - 69) Le Page, F., Klungland, A., Barnes, D. E., Sarasin, A., and Boiteux, S. (2000) Transcription coupled repair of 8-oxoguanine in murine cells: the *Ogg1* protein is required for repair in nontranscribed sequences but not in transcribed sequences. *Proc. Natl. Acad. Sci. USA* **97**, 8397-8402.
  - 70) Bessho, T., Roy, R., Yamamoto, K., Kasai, H., Nishimura, S., Tano, K., and Mitra, S. (1993) Repair of 8-hydroxyguanine in DNA by mammalian *N*-methyl-DNA glycosylase. *Proc. Natl. Acad. Sci. USA* **90**, 8901-8904.
  - 71) Yacoub, A., Augeri, L., Kelley, M. R., Doetsch, P. W., and Deutsch, W. A. (1997) A *Drosophila* ribosomal protein contains 8-oxoguanine and abasic site DNA repair activities. *EMBO J.* **15**, 2306-2312.
  - 72) Ishida, T., Takashima, R., Fukayama, M., Hamada, C., Hippo, Y., Fujii, T., Moriyama, S., Matsuba, C., Nakahori, Y., Morita, H. *et al.* (1999) New DNA polymorphism of human *MMH/OGG1* gene: prevalence of one polymorphism among lung-adenocarcinoma patients in Japanese. *Int. J. Cancer* **8**, 18-21.
  - 73) Sugimura, H., Kohno, T., Wakai, K., Nagura, K., Genka, K., Igarashi, H., Morris, B. J., Baba, S., Ohno, Y., Gao, C. M. *et al.* (1999) *hOGG1* Ser326Cys polymorphism and lung cancer susceptibility. *Cancer Epidemiol. Biomarkers Prev.* **8**, 669-674.
  - 74) Paz-Elizur, T., Krupsky, M., Blumenstein, S., Elinger, D.,



- Schechtman, E., and Livneh, Z. (2003) DNA repair activity for oxidative damage and risk of lung cancer. *J. Natl. Cancer Inst.* **95**, 1312-1319.
- 75) Sieber, O. M., Lipton, L., Crebtree, M., Heiniman, K., Fidalgo, P., Phillips, R. K. S., Bisgaard, M.-L., Orntoft, T. F., Aaltonen, L. A., Hodgson, S. V. *et al.* (2003) Multiple colorectal adenoma, classic adenomatous polyposis, and germ-line mutations in *MYH*. *New Engl. J. Med.* **348**, 791-799.
- 76) Al-Tassan, N., Chemiel, N. H., Maynard, J., Fleming, N., Livingston, A. L., Williams, G. T., Hodges, A. K., Davies, D. R., David, S. S., Sampson, J. R., and Cheadle, J. P. (2002) Inherited variants of *MYH* associated with somatic G:C→T:A mutations in colorectal tumors. *Nature Genet.* **30**, 227-232.
- 77) Cheadle, J. P., Dolwani, S., and Sampson, J. R. (2003) Inherited defects in the DNA glycosylase MYH cause multiple colorectal adenoma and carcinoma. *Carcinogenesis* **24**, 1281-1282.
- 78) Prakash, S., and Prakash, L. (2002) Translesion DNA synthesis in eukaryotes: a one- or two-polymerase affair. *Genes Dev.* **16**, 1872-1883.
- 79) Kamiya, H., Miura, K., Ohtomo, N., Koda, T., Kakinuma, M., Nishimura, S., and Ohtsuka, E. (1989) Transformation of NIH3T3 cells with synthetic *c-Ha-ras* genes (1989) *Jpn. J. Cancer Res.* **80**, 200-203.
- 80) Kamiya, H., Murata-Kamiya, N., Koizumi, S., Inoue, H., and Nishimura, S. (1995) 8-Hydroxyguanine (7,8-dihydro-8-oxoguanine) in hot spots of the *c-Ha-ras* gene: effect of sequence contexts on mutation spectra. *Carcinogenesis* **16**, 883-889.
- 81) Kamiya, H., Miura, K., Ishikawa, H., Inoue, H., Nishimura, S., and Ohtsuka, E. (1992) *c-Ha-ras* containing 8-hydroxyguanine at codon 12 induces point mutations at the modified and adjacent positions. *Cancer Res.* **52**, 3483-3485.
- 82) Jałozzyński, P., Masutani, C., Hanaoka, F., Perez, A. B., and Nishimura, S. (2003) 8-Hydroxyguanine in a mutational hotspot of the *c-Ha-ras* gene causes misreplication, 'action-at-a-distance' mutagenesis and inhibition of replication. *Nucl. Acids Res.* **31**, 6085-6095.
- 83) Jałozzyński, P., Ohashi, E., Ohmori, H., and Nishimura, S. (2005) Error-prone and inefficient replication across 8-hydroxyguanine (8-oxoguanine) in human and mouse *ras* gene fragments by DNA polymerase  $\kappa$ . *Genes to Cells* **10**, 543-550.
- 84) Haracska, L., Yu, S.-L., Johnson, R. E., Prakash, L., and Prakash, S. (2000) Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase  $\eta$ . *Nature Genet.* **25**, 458-461.
- 85) Saitoh, A., Kimura, M., Takahashi, R., Yokoyama, M., Nomura, T., Izawa, M., Sekiya, T., Nishimura, S., and Katsuki, M. (1990) Most tumors in transgenic mice with human *c-Ha-ras* gene contained somatically activated transgenes. *Oncogene* **5**, 1195-1200.
- 86) Ando, K., Saitoh, A., Hino, O., Takahashi, R., Kimura, M., and Katsuki, M. (1992) Chemically induced forestomach papillomas in transgenic mice carry mutant human *c-Ha-ras* transgenes. *Cancer Res.* **52**, 978-982.
- 87) Bhakat, K. K., Mokkaapati, S. K., Boldogh, I., Hazra, T. K., and Mitra, S. (2006) Acetylation of human 8-oxoguanine-DNA glycosylase by p300 and its role in 8-oxoguanine repair *in vivo*. *Mol. Cell. Biol.* **26**, 1654-1665.
- 88) Kasai, H., Nakayama, M., Toda, N., Yamaizumi, Z., Oikawa, J., and Nishimura, S. (1989) Methylreductic acid and hydroxymethylreductic acid: oxygen radical-forming agents in heated starch. *Mutation Res.* **214**, 159-164.
- 89) Nakae, D., Mizumoto, Y., Kobayashi, E., Noguchi, O., and Konishi, Y. (1995) Improved genomic/nuclear DNA extraction for 8-hydroxydeoxyguanosine analysis of small amounts of rat liver tissue. *Cancer Lett.* **97**, 233-239.
- 90) Helbock, H. J., Beckman, K. B., Shigenaga, M. K., Walter, P. B., Woodall, A. A., Yeo, H. C., and Ames, B. N. (1998) DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-guanosine and 8-oxo-guanine. *Proc. Natl. Acad. Sci. USA* **95**, 288-293.
- 91) Loft, S., Vistisen, K., Ewertz, M., Tjønneland, A., Overvad, K., and Poulsen, H. E. (1992) Oxidative DNA-damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis* **13**, 2241-2247.

(Received Jan. 30, 2006; accepted Feb. 13, 2006)

## Profile

Susumu Nishimura was born in 1931 in Tokyo. He graduated from Department of Chemistry, Faculty of Science, University of Tokyo in 1955 and received PhD in Department of Biochemistry and Biophysics, Faculty of Science, University of Tokyo in 1960. After briefly joining in Cancer Institute, Tokyo as a research associate, he did his postdoctoral training in Biology Division, Oak Ridge National Laboratory under the late G. David Novelli in 1961-1963, and then in Institute for Enzyme Research, University of Wisconsin in 1963-1965 under H. Gobind Khorana, where he was involved in elucidation of genetic code.

After his postdoctoral training in US, he joined National Cancer Center Research Institute, Tokyo in 1965. He worked there for 26 years. In the earlier phase of his study in National Cancer Center Research Institute, he discovered and characterized many hyper-modified nucleosides present in tRNA and elucidated their biological roles in tRNA functions. The example is discovery of queosine with J. A. McClosky, and elucidation of its unique biosynthesis catalyzed by tRNA-guanine transglycosylase.

Later his studies were shifted to cancer. By collaboration with T. Sugimura, Nishimura's group was involved in characterization of IQ, MeIQ and MeIQx which are potent mutagens/carcinogens produced by broiling fish and meat. In the field of oncogene research, Y. Taya and Nishimura first showed combination of *K-ras* and *myc* amplification accompanied by point mutational activation of *K-ras* in human lung cancer cells. Nishimura was also involved in elucidation of crystal structure of human *c-Ha-ras* protein, P21, carried out with S.-H. Kim of University of California, Berkeley, and E. Ohtsuka of Hokkaido University. Another notable finding by Nishimura was the discovery of 8-hydroxyguanine with H. Kasai in his laboratory as reviewed in this article.

S. Nishimura moved to Tsukuba Research Institute of Banyu Pharmaceutical Co., Ltd. in 1992 as Director, after mandatory retirement from National Cancer Center Research Institute at the age of 60. There he continued to work on the basic problem on cancer, but also participated in discovery of a new anticancer agent with indolocarbazole structure, targeting topoisomerase 1. From 2004, he moved to University of Tsukuba, and he is now a Visiting Professor at Center for TARA of University of Tsukuba.

S. Nishimura has published more than 450 papers and his work has been cited more than 15,000 times. He is one of the most cited 100 researchers in the field of Biochemistry. He is a foreign honorary member of the American Society for Biochemistry and Molecular Biology, and American Academy of Arts and Sciences. He is also a honorary member of the Japanese Cancer Association, the Japanese Biochemical Society and the Chemical Society of Japan. He was awarded Naito Science Award in 1980, Princess Takamatsu Cancer Research Fund Prize in 1988, and Fujihara Prize in 1990. He is also a recipient of the Imperial Prize and Japan Academy Prize in 1988.

