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Original Contribution

SPONTANEOUS LOSS-OF-FUNCTION MUTATIONS OF THE 8-OXOGUANINE DNA GLYCOSYLASE GENE IN MICE AND EXPLORATION OF THE POSSIBLE IMPLICATION OF THE GENE IN SENESCENCE

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Abstract—8-Oxoguanine is one of the major premutagenic oxidative base legions in vivo and is suspected to play a crucial role in various pathophysiological processes, such as cancer and aging. Mammalian 8-oxoguanine DNA glycosylase (OGG1) is thought to play a major role in the removal of 8-oxoguanine adducts in vivo. We have identified several inbred mouse strains with a spontaneous mutation, OGG1-R336H or double mutations, OGG1-R304W/R336H. R304W mutation caused a complete loss of OGG1 activity, while the R336H mutation led to disruption of nuclear localization of the enzyme although the activity remained normal. Among the double mutants was SAMP1, which exhibits accelerated senescence and short lifespan. We assessed the possible implication of the mutant OGG1 and 8-oxoguanine in aging utilizing SAMP1 mice. SAMP1 retained 1.5- to 1.9-fold increase in 8-oxoguanine level of hepatic nuclear DNA as compared with normal mice, until at least 12 months of age. A genetic association study, however, indicated that the mutant *Ogg1* gene per se is not responsible for the accelerated senescence and short lifespan of SAMP1. Mutant OGG1 may be associated with pathologic conditions in other mouse strains.

Keywords—OGG1, Oxidative DNA damage, 8-Oxoguanine, Senescence, Mutation, Free radicals

INTRODUCTION

Oxidative DNA damage has been implicated in a broad range of pathophysiological processes, such as degenerative diseases, cancer, and aging. Among a variety of oxidatively modified bases, 8-oxoguanine has been the focus of much attention because it is most abundantly produced in vivo [1], and is highly mutagenic, as adenine is frequently misincorporated opposite 8-oxoguanine at DNA replication, leading to G:C to T:A transversion [2–5].

Organisms have pathways dedicated to the removal of 8-oxoguanine adducts from the genome. In mammals, two independent pathways have been demonstrated so far. One is an enzyme 8-oxoguanine DNA glycosylase (OGG1) [6–9] and the other is a transcription-coupled repair pathway, which does not require OGG1 [10,11]. The presence of transcription-coupled repair pathway for 8-oxoguanine was established quite recently and the enzyme(s) involved in it has/have not yet been identified. Molecular cloning of the human *OGG1* gene has already been accomplished [12–15] and paved the way to investigate the possible association of OGG1 with pathophysiological process. Thus far, several studies have suggested likely involvement of OGG1 and 8-oxoguanine in the process of carcinogenesis in humans [15–19]. Also, it was reported recently that cells from patients with Cockayne syndrome B, which is a genetic disorder belonging to the category of segmental progeroid conditions, have

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a deficiency in repair of 8-oxoguanine due to reduced OGG1 activity [20], suggesting the possible association of OGG1 with aging.

Recently, two groups made *Ogg1*-knockout mice to assess the role of OGG1 [21,22]. The biological importance of OGG1, however, is still unclear. Meanwhile, Choi and colleagues identified spontaneous *Ogg1*-mutant mice [23]. In the report, the authors demonstrated that SAMR1 mouse strain carries R336H mutation, and SAMP1 and SAMP8 strains carry R304W and R336H mutations in OGG1. They also reported that SAMP1 and SAMP8 have reduced OGG1 activity and increased 8-oxoguanine level in the genome compared to SAMR1. They deduced that R304W, but not R336H substitution, is directly related to the functional defects of OGG1. It is worthy of note that the mutations were found in Senescence-Accelerated Mouse Prone (SAMP) strains, which show accelerated senescence and short lifespan. The etiology of accelerated senescence of SAMP mice has not been clarified yet [24]. Previous studies, however, indicated possible association of oxidative stress with accelerated senescence of SAMP [25–29]. In addition, accelerated accumulation of somatic mutations in the *Hprt* gene of SAMP1 mice has been demonstrated [30]. These observations suggest the possible implication among oxidative stress, mutant *Ogg1* gene, somatic mutation, and accelerated senescence.

In the present study, we first investigated other mouse strains for mutations in the *Ogg1* gene. We then assessed the effects of mutations on the function of OGG1. Furthermore, we explored whether or not mutant *Ogg1* is the direct cause of accelerated senescence and short lifespan of SAMP mice. We found that other inbred mouse strains also carry R304W and R336H mutations and, hence, they are not specific to SAMP1 and SAMP8 strains. Interestingly, however, some other *Ogg1*-mutant strains are known for their pathologic conditions. We also found that not only R304W, but also R336H substitutions leads to functional defects of OGG1. A genetic association study indicated that the mutant *Ogg1* gene per se is not responsible for the accelerated senescence and short lifespan of SAMP1, although the mice indeed retain increased level of 8-oxoguanine in their genome.

MATERIALS AND METHODS

Mutation analysis of the Ogg1 *gene*

Nine SAMP strains of SAMP1, SAMP2, SAMP3, SAMP6, SAMP7, SAMP8, SAMP9, SAMP10, and SAMP11, and five SAMR strains of SAMR1, SAMR2, SAMR3, SAMR4, and SAMR5 were used. Sixteen other laboratory mouse strains of C57BL/6J, BALB/c, C3H/He, NZB/N, DBA/2, AKR/J, 129/Sv, SJL/J, SWR/J, A/J, SM/J, KK, MRL-*lpr*, NFS, ICR, C57BL/10 were also examined. A genomic DNA fragment, which contains exons 6 and 7, was obtained by PCR amplification with a primer pair of mOgg1-1 (5′-AGGAAAGCACTGGGGTGGAG-3′) and mOgg1-2 (5′-CTTTTGCCCCTGAGCCCTAC-3′), and sequenced using a BigDye Cycle Sequencing FS Ready Reaction Kit and an ABI310 DNA Sequencer (Applied Biosystems; Foster City, CA, USA).

Activity assay for OGG1

The liver, brain, and testis extracts were prepared from male C57BL/6J, C3H/He, SAMR1, SAMR4, NZB/N, SAMP1, and SAMP7 strains of mice.

Recombinant OGG1 fused to glutathione S-transferase (GST) was produced in *E. coli* BL21. The *Ogg1* cDNA fragments were PCR amplified by a primer pair of mOgg1-23 (5′-GCGAATTCCACCATGTTATTCCGTTCCTGGCT-3′) and mOgg1-22 (5′-TGGAATTCCTAGCCCCTCTGGCCTCTT-3′) from first-strand liver cDNA of C57BL/6J, SAMR1, and SAMP1. The cDNAs were inserted into the *Eco*RI site of the pGEX-5X-3 (Amersham Pharmacia Biotech; Little Chalfont, Buckinghamshire, UK). Expression of GST/OGG1 fusion proteins was induced by 0.1 mM IPTG, and the proteins were purified on a glutathione sepharose 4B column (Amersham Pharmacia Biotech).

Twenty-two mer double-strand oligonucleotide, which contained an 8-oxoguanine (^{o}G) in one strand (5′-GGTGGCCTGAC^{o}GCATTCCCCAA-3′) and was labeled with FITC at the 5′ terminus, was used as substrate. The activity assay was carried out at 37°C for 2 h with 40 fmol of substrate DNA and the tissue extract containing 40 μg of protein. The cleaved products were run on 20% denaturing polyacrylamide gel, and visualized using an FMBIO image analyzer (TaKaRa).

Genetic linkage study between mutation and loss of OGG1 activity

Twenty-eight (SAMR1 × SAMP1)F2 mice were used. The mice were genotyped for the *Ogg1* locus by utilizing *Acc*III site polymorphism associated with the C to T point mutation in exon 6. DNA sequence containing exon 6 of the *Ogg1* gene was obtained by PCR amplification by the primer mOgg1-5 (5′-CGCCATTCCTCCACAACAGT-3′) and mOgg1-12 (5′-GAAAGTCCTTTAGGGTCTGG-3′), digested with *Acc*III, and run on a 1.5% agarose gel. The mice with a fragment of 410 bp were judged homozygous for the SAMP1 allele. The mice with a fragment of 335 bp were judged homozygous for the SAMR1 allele. The mice with fragments of

Table 1. List of OGG1-Mutant Mouse Strains

OGG1 type	Mouse strain
OGG1 (normal)	C57BL/6J, A/J, BALB/c, KK, ICR, C57BL/10, DBA/2, 129/Sv, SWR/J, SAMR3
OGG1-R336H	AKR/J, C3H/He, SM/J, MRL-*lpr*, SAMR1, SAMR2, SAMR4, SAMR5
OGG1-R304W/R336H	NZB/N, NFS, SJL/J, SAMP1, SAMP2, SAMP3, SAMP6, SAMP7, SAMP8, SAMP9, SAMP10, SAMP11

410 and 335 bp were judged heterozygous. The OGG1 activity in the liver was examined as described above.

Assay of nuclear localization signal function

cDNA fragments, containing the entire coding sequence of the *Ogg1* gene, were PCR amplified with a primer pair of mOgg1-23 and mOgg1-24 (5′-GCGGATCCCTCTGGCCTCTTAGATCC-3′) from first-strand liver cDNA of C57BL/6J, SAMR1, and SAMP1, and inserted into the *Eco*RI/*Bam*HI site of the vector pEGFP-N3 (Clontech) so that OGG1 protein could be expressed as fusion proteins with green fluorescent protein (GFP). COS cells were transfected by using SuperFect Transfection Reagent (Qiagen; Hilden, Germany). Three days after transfection, the cells were fixed with 4% formalin and observed under fluorescent microscopy.

Analysis of 8-oxoguanine content in the genome

Three, 12, and 24 month old male C57BL/6J mice and 3 and 12 month old SAMR1 and SAMP1 mice were used (all $n = 5$). Nuclear DNA was isolated from 100 mg of the livers using a DNA extractor WB kit (Wako Pure Chemicals Industries; Osaka, Japan). The DNA was digested to deoxyribonucleoside levels by treatment with nuclease P1 and alkaline phosphatase. The content of 8-hydroxy-2′-deoxyguanosine (8-OHdG) was measured as previously described [31]. The difference of mean number of 8-OHdG per 10^6 deoxiguanosine (dG) was evaluated by Student's *t*-test.

Genetic association test between Ogg1 *and accelerated senescence in SAMP1*

(SAMP1 × SAMR1)F_2 mice ($n = 49$) used in this study were described in the previous report [32]. Mice were evaluated for the degree of senescence at 12 or 14 months of age using our grading score system [33]. Mice were genotyped for the *Ogg1* locus by *Acc*III site polymorphism as described above. The difference of mean grading scores was evaluated by the Mann-Whitney U-test with Statview-J 4.5 software.

Genetic association test between Ogg1 *and lifespan in SAMP1*

The (B10.BR × SAMP1) hybrid mouse cohort consisted of 14 (B10.BR × SAMP1)F1 × SAMP1 backcross (BC) mice, 26 (BC × BC)F1, and 30 (BC × BC)F1 × BC. They were raised under conventional conditions, had free access to commercial diet CE-2 (Nihon CLEA; Tokyo, Japan) and tap water, and were observed daily until they died naturally. Mice were genotyped for the *Ogg1* locus as described above. The mean lifespan difference of the mice was evaluated by Student's *t*-test. Survival curves were drawn by the Kaplan and Meier method, and compared by the Logrank test.

RESULTS

Mutations of the Ogg1 *gene in inbred mouse strains*

We confirmed two mutations: a cytosine to thymine change at the twelfth nucleotide in exon 6, and a guanine to adenine change at the fifty-ninth nucleotide in exon 7 of the *Ogg1* gene, as previously reported [23]. The cytosine to thymine change in exon 6 leads to substitution of the 304th amino acid, arginine, by tryptophan (R304W). It also leads to loss of a recognition sequence for a restriction enzyme, *Acc*III. The guanine to adenine change in exon 7 leads to substitution of the 336th amino acid, arginine, by histidine (R336H) in a putative nuclear localization signal. We sequenced the full coding regions of the *Ogg1* gene from SAMP1, SAMP2, SAMR1, and SAMR2, but no other mutations were found. Examination of the remaining SAM strains revealed that all nine SAMP strains, but none of the five SAMR strains, had the R304W substitution (Table 1). All SAM strains, except for SAMR3, had the R336H substitution. The R336H substitution was identified also in C3H/He, AKR/J, SM/J, MRL-*lpr*, NZB/N, NFS, and SJL/J strains. Among them, NZB/N, NFS, and SJL/J strains had the R304W mutation as well.

The R304W substitution leads to loss of OGG1 activity

Both liver and brain cytosol extracts from C57BL/6J with normal OGG1, and C3H/He, SAMR1, and SAMR4 with OGG1-R336H cleaved a substrate oligonucleotide containing 8-oxoguanine (Fig. 1). However, extracts

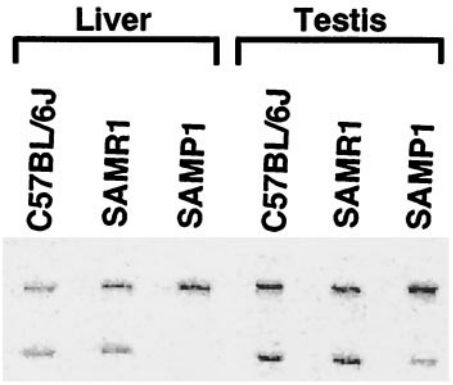

Fig. 1. Gel image of OGG1 activity assay for liver and testis extracts. Only three strains are shown. C3H/He and SAMR4 yielded similar results to that of SAMR1. NZB/N and SAMP7 yielded similar results to that of SAMP1. The upper and lower bands correspond to undigested substrate and cleavage product, respectively. Brain and liver extracts yielded similar results.

from NZB/N, SAMP1, and SAMP7 with OGG1-R304W/R336H yielded no cleavage product. Testis extracts from all strains yielded a cleavage product (Fig. 1). The density of the cleavage products obtained with NZB/N, SAMP1, and SAMP7 testis extracts was lower, as compared to that of the cleavage products obtained with C57BL/6J, C3H/He, SAMR1, and SAMR4 testis extract. Purified recombinant OGG1 protein derived from C57BL/6J and SAMR1 yielded a cleavage product, whereas that derived from SAMP1 yielded no cleavage product (figure not shown).

Of 28 (SAMR1 × SAMP1)F2 mice, four were homozygous for the SAMP1 allele and 17 were heterozygous and seven homozygous for the SAMR1 allele at the *Ogg1* locus. None of the four mice homozygous for the SAMP1 allele showed OGG1 activity, whereas the remaining 24 mice, which were homozygous or heterozygous for the SAMR1 allele had OGG1 activity. Thus, there was a direct correlation between homozygosity of the R304W mutation and loss of OGG1 activity. Taken together, these results indicated that the R304W substitution leads to the loss of OGG1 activity.

The R336H substitution leads to loss of nuclear localization signal function

Accumulation to the cell nucleus was observed for the GFP/OGG1 fusion protein, while the GFP/OGG1-R336H fusion protein was distributed exclusively to the cytosol (Fig. 2). Similar cytosolic localization was observed for the GFP/OGG1-R304W/R336H (figure not shown). These results indicate that the R336H substitution leads to the loss of nuclear localization signal function.

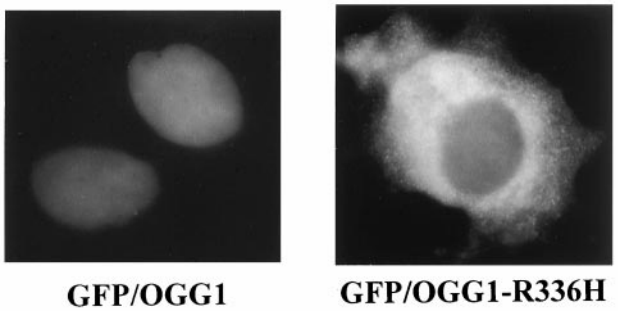

Fig. 2. Subcellular localization of OGG1 proteins in COS cells. COS cells were transfected with plasmid constructs for expression of GFP/OGG1 or GFP/OGG1-R336H. Cell nuclei are labeled N. The Cytosol is practically invisible in the case of GFP/OGG1 (original magnification, × 1000).

8-OHdG content in the liver nuclear DNA of Ogg1*-mutant mice*

At 3 months of age, the mean 8-OHdG content in the liver nuclear DNA of C57BL/6J, SAMR1, and SAMP1 ($n = 5$) was 4.2 ± 1.0 (SD), 7.3 ± 1.5, and 6.3 ± 0.7 in 10^6 deoxyguanosine (dG), respectively. Student's *t*-test of the mean values revealed a significantly higher content in SAMR1 and SAMP1, as compared to C57BL/6J ($p < .01$), but no difference between SAMR1 and SAMP1.

At 12 months of age, the mean 8-OHdG contents in the liver nuclear DNA of C57BL/6J, SAMR1, and SAMP1 mice were 3.4 ± 0.9, 3.9 ± 0.5, and 6.4 ± 1.2 in 10^6 dG, respectively. The mean 8-OHdG content of 24 month old C57BL/6J mice was 3.6 ± 1.4 in 10^6 dG. Thus, no age-dependent change in 8-OHdG content was observed in C57BL/6J and SAMP1 mice. In 12 month old SAMR1 mice, the 8-OHdG content decreased to the same level as in C57BL/6J mice.

Association study between mutant Ogg1 *and accelerated senescence*

In (SAMP1 × SAMR1)F2 mice, the average grading scores of mice homozygous for the SAMP1 allele at the *Ogg1* locus ($n = 10$), heterozygotes ($n = 26$), and homozygous for the SAMR1 allele ($n = 13$) were 6.3, 5.8, and 6.6, respectively. The Mann-Whitney U-test of the mean value revealed no significant difference among the three genotypes.

Association study between mutant Ogg1 *and lifespan*

Seventy hybrid mice between B10.BR and SAMP1 had an average lifespan of 486 days. The mean lifespans of mice homozygous for the SAMP1 allele at the *Ogg1* locus ($n = 47$) and heterozygous for the SAMP1 and

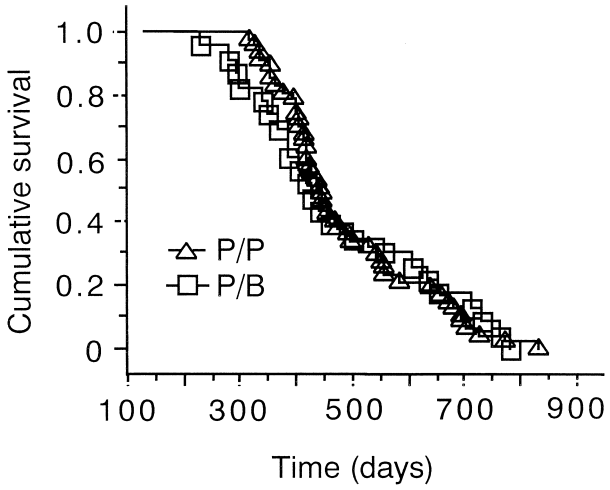

Fig. 3. Survival curves of the mouse cohort with two genotypes at the *Ogg1*. P/P and P/B represent mice homozygous for the SAMP1 allele and mice heterozygous for SAMP1 and B10.BR alleles, respectively.

B10.BR allele at the *Ogg1* locus ($n = 23$) were 490 and 479 days, respectively. Student's *t*-test revealed that mean lifespans of the mice with the two genotypes were not different. Survival curves (Fig. 3) also showed no significant difference between the two genotypes ($p = .93$).

DISCUSSION

Choi and colleagues reported that SAMP1, SAMP8, and SAMR1 mouse strains carry mutations in the *Ogg1* gene, and that the OGG1 activity in SAMP1 and SAMP8 was 10–40% of that in SAMR1 mice in all organs examined [23]. Nine SAMP and five SAMR strains have been established by selective breeding of mice for and against accelerated senescence, respectively, from a progeny of inadvertent crossing between AKR/J and unspecified strain(s) [24]. In the present study, we revealed that all SAM strains, except for SAMR3 have the R336H mutation. All nine SAMP, but none of the SAMR strains, had the R304W mutation. These results indicate that the R304W mutation did not segregate away from accelerated senescence and short lifespan in SAMP strains during the selection and breeding process. However, we found that the mutant *Ogg1* alleles are held not only in SAM strains, but also in various laboratory strains. Accordingly, we explored whether or not mutant *Ogg1* is the direct cause of accelerated senescence and short lifespan of SAMP1 mice.

We demonstrated that the R304W substitution caused a complete loss of OGG1 activity, while nuclear localization of the OGG1 protein was disrupted by the R336H substitution. These results suggested that the strains with the R336H mutation as well as the strains with the R304W mutation have lost the ability to remove 8-oxoguanine from the nuclear DNA. We observed that the strains with the R304W substitution had the OGG1 activity in the testes. It is unlikely that the activity in the testes is ascribed to OGG1 because recombinant OGG1-R304W/R336H did not show activity in vitro. Rather, our observation suggests the presence of other 8-oxoguanine glycosylase in testes. Consistent with this, a slow removal of 8-oxoguanine in vivo from proliferating cells and reduced spontaneous mutation frequency were observed in the testis of the *Ogg1*-knockout mice, although OGG1 activity was not detectable in testes of the mice [21].

We found that both SAMP1 and SAMR1 mice, respectively, had 1.5- and 1.7-fold increase in 8-oxoguanine level in hepatic nuclear DNA at three months of age as compared with age-matched normal mice, consistent with the notion that both mouse strains have lost the functional OGG1. Two strains, however, underwent different patterns of change in 8-oxoguanine level with age. SAMP1 retained the high 8-oxoguanine content at 12 months of age. In SAMR1 mice, on the other hand, it resumed the normal level at 12 months. The reason for this reduction of 8-oxoguanine level in SAMR1 is not clear at present.

Genetic association studies using hybrid progeny from SAMP1 mice demonstrated that the *Ogg1* gene was correlated neither with short lifespan nor with accelerated senescence of the mice. Our data do not preclude the possibility that retention of the high 8-oxoguanine level is associated with accelerated senescence and short lifespan of SAMP1 mice. We could not assess the relationship between 8-oxoguanine content and lifespan in the hybrid progeny, because DNA samples were preserved frozen for a long period, and hence were not suitable for 8-oxoguanine measurement. Further study utilizing SAMP1 mice might elucidate the possible implication of 8-oxoguanine in senescence.

Some spontaneous *Ogg1* mutants are known for their pathologic conditions: AKR/J for lymphatic leukemia [34]; C3H/He for hepatocellular carcinoma [35]; MRL-*lpr* for autoimmunity [36]; NZB/N for autoimmune hemolytic anemia [37]; and SJL/J for reticulum cell sarcomas [38]. The etiology of the hepatocellular carcinoma in C3H/He and reticulum cell sarcomas in SJL/J mice have not been elucidated yet. There is a possibility that the mutant *Ogg1* has some roles in pathogenesis of these tumors. High 8-oxoguanine content due to mutant *Ogg1*, for example, may induce high rate double-strand breaks in genomic DNA, leading to illegitimate recombination and deletion of tumor-suppressor gene. In *E. coli*, it is suspected that 8-oxoguanine residues introduce double-strand breaks into DNA, while the bacterial homologue of OGG1, MutM, counteracts to suppress illegitimate

recombination induced by oxidative stress [39]. Consistent with this, SAM mice also show age-dependent acceleration of chromosomal abnormality, such as extrachromosomal, small, circular DNA [40]; chromosome aberrations [41]; and DNA single-strand break [42,43]. The association between mutant *Ogg1* and pathologic conditions in these mouse strains deserves further study.

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ABBREVIATIONS

AP lyase—apurinic/apyrimidinic lyase
8-OHdG—8-hydroxy-2′deoxyguanosine
GFP—green fluorescent protein
OGG1—8-oxoguanine DNA glycosylase
SAM—Senescence-Accelerated Mouse