

## Age-associated Decrease of Oxidative Repair Enzymes, Human 8-Oxoguanine DNA Glycosylases (hOgg1), in Human Aging

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### Ogg1/Aging/Polymorphism/8-Oxoguanine/Glycosylase.

8-Oxoguanine has been shown to be a dominant cause of oxidative DNA damage by oxygen free radicals in eukaryotic cells. The 8-oxoguanine repair-specific enzyme 8-oxoguanine-DNA glycosylase (hOgg1) was recently cloned and was observed to conduct mainly short-patch base-excision repair. It has also been suggested that reactive oxygen species play an important role in the cellular aging process. We explored the association between the hOgg1 enzyme activity in somatic cells of human subjects of various ages and the role of *hOgg1*<sup>326</sup> genetic polymorphism. An 8-oxoguanine-containing 28 mer oligonucleotide was end-labeled with  $\gamma$ -<sup>32</sup>P ATP and incubated with protein extracts from peripheral blood lymphocytes (PBL) from 78 healthy individuals ranging in age from newborn to 91 years old. The hOgg1 repair activity toward the radiolabelled 8-oxoguanine-containing DNA was determined, and the results indicated a significant age-dependent decrease in the hOgg1 activity in their lymphocytes. Significantly reduced activity was also shown in those with Cysteine/Cysteine genotypes. The genders of the subjects were not shown to be associated. These results provide an important observation regarding the cellular hOgg1 activity in somatic cells during the normal human aging processes.

### INTRODUCTION

The accumulation of reactive oxygen species (ROS) or increased levels of DNA damage has been shown to be associated with aging or cellular senescence in mammalian cells<sup>1</sup>. One of the major forms of oxidized DNA damage is the formation of 8-oxoguanine (7,8-dihydro-8-oxoguanine, 8-hydroxyguanine), which has been shown to be a highly mutagenic base modification produced by oxidative stress<sup>2,3</sup>. 8-oxoguanine residue, if unrepaired, may mispair with adenine during DNA replication and induce G:C→A:T transversion mutations<sup>4</sup>. The 8-oxoguanine-specific repair enzyme 8-oxoguanine DNA glycosylase 1 (hOgg1) was recently cloned, and observed to function mainly by short-patch base-excision repair (BER)<sup>5,6</sup>, which was initiated by DNA glycosylase that recognized the damaged site and

generated an apurinic/ apyrimidinic (AP) site on DNA. Subsequently, the AP site was able to be incised and repaired by AP endonuclease and ligase<sup>7,8</sup>.

Several laboratories have reported observations in changes of the 8-oxoguanine contents with aging in a variety of different tissues<sup>9</sup>, while oxidative DNA damage is generally due to continuous exposure to oxidants in the aging process<sup>10</sup>. It was also indicated the accumulation of oxidation-related enzyme, e.g. catalase activity, decreased human skin fibroblasts during the cellular aging processes, resulting in an accumulation of 8-oxoguanine in cells of late passages<sup>11</sup>. Therefore, it is of interest to evaluate the hOgg1 repair capacity toward 8-oxoguanine and the molecular mechanism involved in the cellular responses to oxidative stress in human individuals with normal aging.

The genetic polymorphism of hOgg1 at codon 326 was recently shown to encode hOgg1-Ser<sup>326</sup> and hOgg1-Cys<sup>326</sup> proteins, while hOgg1-Ser<sup>326</sup> was shown with a higher repair activity toward 8-oxoG than the hOgg1-Cys<sup>326</sup><sup>12</sup>. On the contrary the hOgg1 activities of these two phenotypes were not shown to differ in the hOgg1-GST fusion proteins expressed in the pPR71 plasmid<sup>13</sup>. Until now, an association between hOgg1 polymorphism and decreased hOgg1 activity in human cells in vivo has not been fully demonstrated<sup>14</sup>. Therefore, it will be of importance to demonstrate an association between the hOgg1 activity and the genetic polymorphisms involved in human cellular aging.

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## MATERIALS AND METHODS

### Sample population and cellular cultivation

First, 55 adult individuals were invited to participate in this study at a local health clinic in Taipei city, while 23 mothers were asked to provide cord blood samples after delivery in the same community hospital; 10 ml of vacuumed blood was collected after informed consent via venepuncture. Peripheral blood lymphocytes (PBL) were isolated immediately via Ficoll-pague gradient centrifugation (Pharmacia) and incubated with RPMI-1640 medium, supplemented with 10% fetal calf serum and 1% phytohemagglutinin (PHA-M; Gibco), and sufficient concentrations of antibiotics<sup>15</sup>. These primed lymphocytes were then maintained at 37°C in an incubator for 3 days before protein extraction to have the maximal levels of expression of hOgg1, which were observed via Western blotting in several studies throughout this lab (data not shown).

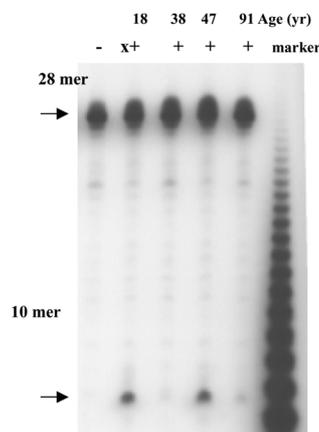
### Preparation of PBL nuclear extracts

After 3 days of cultivation, the PBL cellular suspension was centrifuged, and then cellular pellets were resuspended in 0.5 ml of 25 mM Tris-HCl (pH 7.6), 2 mM Na<sub>2</sub>-EDTA and 50 mM KCl<sup>14</sup>. The suspension was sonicated by 25 × 0.5 s pulses of 20 kHz on ice, and then being centrifuged at 12,000 r.p.m. before use. The supernatant was determined for its protein concentration by a Bio-Rad protein assay (Bio-Rad Lab) and aliquated for storage at -80°C.

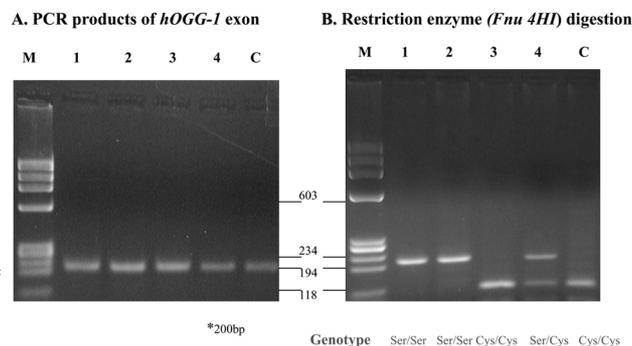
### Preparation of DNA substrates

A random-designed 28-base DNA fragment, 5'-GAAC-GACTGT(G\*)ACTTGACTGCTACTGAT-3' and its complementary sequence with a cytosine opposite 8-oxodG was generated by the Midland Certified Reagent Co. (Texas, USA). DNA fragments containing 8-oxodG were then 5'-end labeled with [ $\gamma$ -<sup>32</sup>P]ATP (Amersham) by T4 polynucleotide kinase (New England BioLabs). These end-labeled oligonucleotides were annealed with its complementary sequence and used as the substrate DNA<sup>16</sup>. The remaining unincorporated radioactivity was removed by a QIAquick Nucleotide Removal Kit (QIAGEN). The manufacturer indicated that 17–40 mer oligonucleotides were with 80% recovery after purification.

First, 15  $\mu$ g each of a PBL supernatant was mixed with 100 fmol of <sup>32</sup>P-labeled DNA substrates at 37°C for 20 h in a reaction mixture containing 25 mM Tris-HCl (pH 7.6), 2 mM Na<sub>2</sub>-EDTA and 50 mM KCl. The reaction samples were mixed with a loading dye, heated at 95°C for 5 min and run on 20% polyacrylamide gels containing 7 M urea at 20 W for 3 h. The gels were dried and exposed to radiographic film (Fig. 1; X-OMAT; Kodak), then analyzed using an autoradiograph (Molecular Dynamics). After incubation with PBL extracts, radiolabeled oligomers were separated as intact band of 28 mers and cleavage bands of 10 mers (Fig. 1). The repair activity was quantified as



**Fig. 1.** Assay for hOgg1 repair activity for 8-oxodG-containing oligonucleotide in vitro. Polyacrylamide gel of 8-oxodG containing 28mer oligonucleotides incubated with 15  $\mu$ g PBL nuclear extracts from subjects of different ages. Arrows indicate intact and cleavage DNA products.



**Fig. 2.** Evaluation of *hOgg1* codon 326 genetic polymorphism. (A) Genomic DNA was purified and amplified by PCR for exon 7 of *hOgg1*. (B) The polymorphic site of codon 326 was detected by *Fnu4HI* restriction enzyme digestion.

incised from the radioactivity of the original intact 28 mer oligonucleotide in each sample.

### Determination of genetic polymorphism

Genomic DNA was purified using QIAamp DNA Kit (QIAGEN Co.) and amplified by PCR for exon 7 of *hOgg1*. Oligodeoxynucleotide primers and thermocycle PCR conditions have been reported in other studies (Sugimura *et al.*, 1999). The polymorphic site of codon 326 was detected by *Fnu4HI* restriction enzyme digestion (recognition site, GCNGC; New England Biolabs, Beverly, MA) for 5 h at 37°C. After electrophoresis in 2% agarose gel and staining with ethidium bromide, the genotype of codon 326 was determined (Fig. 2). The DNA collected first underwent PCR amplification on the *hOgg1* exon 7 (~200 bp), then *Fnu4HI* restriction enzyme digestion, and confirmed by sequence analysis.

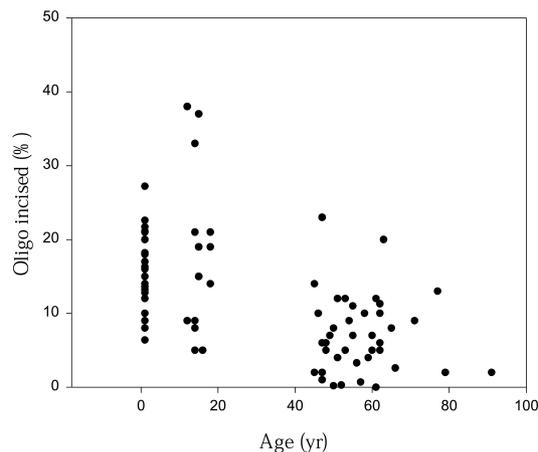
### Statistical analysis

A multiple factorial linear-regression analysis was employed while variables like age, gender, and the genotype (as Cys/Cys, Ser/Cys, or Ser/Ser) were considered and analyzed by the SAS statistical software (version 6.12; SAS Institute Inc., Cary, NC, 1989). A test was considered to be statistically significant if its *p* value was less than 0.05, and borderline significant if its *p* value was between 0.1 and 0.05.

## RESULTS

The incised oligo (%) of cleaved hOgg1 oligonucleotides, as compared with intact oligomers in PBL extracts of these 78 subjects, ranged from 0 to 0.38 (Fig. 3). In general, the incised oligo (%) of cleaved hOgg1 or the hOgg1 repair activity decreased in the lymphocytes when the ages of the study subjects increased.

The distribution of genetic polymorphism in these 78 subjects was 27 subjects as Ser/Ser (34.6%), 35 as Ser/Cys (44.9%), and 16 as Cys/Cys (20.5%). The total allele frequencies of Ser and Cys were 57% and 43%, respectively. The hOgg1 repair activity in these subjects was analyzed by multiple linear-regression analysis, which included variables such as gender, age, and



**Fig. 3.** Distribution of hOgg1 repair activity and ages of 78 human subjects.

*hOgg1*<sup>326</sup> genotypes (Table 1). The results indicated a significant age-dependent decrease in the hOgg1 activity ( $p < 0.0001$ ). The lymphocytes hOgg1 activity was also shown to be significantly lower in those with the Cys/Cys genotype ( $p = 0.02$ ), and with a borderline significant decrease in those with Ser/Cys ( $p = 0.05$ ), as compared with those with the Ser/Ser genotype. No difference was shown between the female and male subjects.

## DISCUSSION

We examined the ex vivo hOgg1 repair activity of PBLs in subjects ranging in age from newborns to 91 years old. The results showed that the hOgg1 repair activity decreased as the ages of the subjects increased. Similar observations of aging-associated decrease in the DNA damage repair activity were noted in primary dermal fibroblasts derived from donors of different ages<sup>11,17</sup>. Furthermore, a significant decrease in the nucleotide excision repair of thymine dimers and photoproducts of human skin fibroblasts from 20 healthy subjects 3 to 96 years old was observed<sup>17</sup>. The accumulation of oxidation-related enzyme, e.g. catalase activity, was shown to decrease in human skin fibroblasts during the cell aging process, resulting in an accumulation of 8-oxoguanine in cellular passages<sup>11</sup>. These results suggested that the oxidation-damage related DNA repair enzyme activity might be correlated with cellular aging. The DNA repair activities of hOgg1 in cryopreserved human lymphocytes were also shown to vary over 2 folds in an adult Caucasian population<sup>14</sup>. However, we observed a much larger inter-individual variation in the hOgg1 activities in this study population than those observed by a similar study<sup>14</sup>, probably due to larger differences in the age distribution in this study. Moreover, the collected cells underwent three days of cultivation before being assayed for their activities, which may also contribute to the large difference in the selection for cellular proliferation in the assayed cells, as compared with the cryopreserved cell stocks in a similar study<sup>14</sup>.

The genetic background concerning the DNA repair activity is thought to be important determinant of both the population and individual risk<sup>13,18</sup>. Therefore, we examined the distribution of

**Table 1.** The hOgg1 repair activity in 78 subjects and their associations with gender, age, and genotype by multiple linear regression analysis.

Parameter	Number of subjects (%)	Oligoincised	Estimate	Standard error	<i>p</i> values
Gender					
Male	34 (43.6)	0.12 + 0.09	0.0124	0.0153	0.42
Female	44 (56.4)	0.12 + 0.07	0.0000	0.0000	–
Genotype					
Cys/Cys	16 (20.5)	0.10 + 0.05	–0.0500	0.0210	0.02**
Ser/Cys	35 (44.9)	0.11 + 0.07	–0.0335	0.0170	0.05*
Ser/Ser	27 (34.6)	0.14 + 0.10	0.0000	0.0000	–
Age	78	0.12 + 0.08	–0.0017	0.0003	<0.0001**

\*0.05 < *p* < 0.1, \*\* < *p* 0.05.

genetic polymorphism in these 78 subjects, showing 27 subjects as Ser/Ser (34.6%), 35 as Ser/Cys (44.9%), and 16 as Cys/Cys (20.5%). The hOgg1 repair activity was significantly lower in subjects with Cys/Cys and with a borderline significant decrease in those with Ser/Cys genotypes (Table 1). The *hOgg1*<sup>326</sup> polymorphism has been reported to be related to a slightly lower hOgg1 activity in *E. coli*<sup>12</sup>. On the contrary, the hOgg1 repair activity in human lymphocytes was not shown to be related to Ser<sup>326</sup>/Cys<sup>326</sup> genetic polymorphism<sup>14</sup>. This discrepancy may be due to the limited number of individuals or quite different distributions of genetic polymorphism in these investigations.

The genotype distribution of *hOgg1* codon 326 polymorphism was shown in the Taiwanese population in this preliminary analysis, and the genotype frequencies in 78 Taiwanese subjects were 0.57 (Ser) and 0.43 (Cys). No difference was shown between the distribution of these subjects and those reported as 0.59 : 0.41 in a Japanese population ( $p = 0.97$ )<sup>12</sup>. However, the ratio in a Caucasian population in the Germany was observed to be 0.75 : 0.25 ( $p = 0.07$ )<sup>14</sup>. These results suggested that the *hOgg1* Cys allele was found to be more common in Taiwanese (43%) and Japanese (41%) than in Caucasians (25%).

As a result, the hOgg1 protein may play a role in the aging process, and subjects with the Cys/Cys genotype might be less effective in the repair of 8-oxoguanine. However, the biological mechanism between the hOgg1 repair activity and the *hOgg1*<sup>326</sup> genotype remains unclear. It is proposed that the glycosylase activity of hOgg1 could be stimulated by a major human AP endonuclease, HAP1<sup>19</sup>. In the presence of HAP1, the BER of 8-oxoguanine residues can be highly efficient in by passing the AP lyase activity of the hOgg1, and thus excluding a potentially rate-limiting step<sup>19</sup>. On the other hand, residue 326 lies within a short disordered peptide segment at the C terminus of the structure, and hence its functional role is unclear<sup>20</sup>. It is suggested that the Cys 326 residue of hOgg1 may alter its configuration and reduce the recognition ability toward 8-oxoguanine.

Although more study is needed to explore the mechanisms of the aging processes, in human somatic cells, this study appears to provide essential information on the hOgg1 repair activity in the human aging process.

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