Oxidation Status of Human OGG1-S326C Polymorphic Variant Determines Cellular DNA Repair Capacity

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Abstract

The *hOGG1* gene encodes the DNA glycosylase that removes the mutagenic lesion 7,8-dihyro-8-oxoguanine (8-oxoG) from DNA. A frequently found polymorphism resulting in a serine to cysteine substitution at position 326 of the OGG1 protein has been associated in several molecular epidemiologic studies with cancer development. To investigate whether the variant allele encodes a protein with altered OGG1 function, we compared the 8-oxoG repair activity, both *in vivo* and in cell extracts, of lymphoblastoid cell lines established from individuals carrying either Ser/Ser or Cys/Cys genotypes. We show that cells homozygous for the Cys variant display increased genetic instability and reduced *in vivo* 8-oxoG repair rates. Consistently, their extracts have an almost 2-fold lower basal 8-oxoG DNA glycosylase activity when compared with the Ser variant. Treatment with reducing agents of either the Cys variant cells directly or of protein extracts from these cells increases the repair capacity to the level of the Ser variant, whereas it does not affect the activity in cells or extracts from the latter. Furthermore, the DNA glycosylase activity of cells carrying the Cys/Cys alleles is more sensitive to inactivation by oxidizing agents when compared with that of the Ser/Ser cells. Analysis of the redox status of the OGG1 protein in the cells confirms that the lower activity of OGG1-Cys326 is associated with the oxidation of Cys326 to form a disulfide bond. Our findings support the idea that individuals homozygous for the OGG1-Cys variant could more readily accumulate mutations under conditions of oxidative stress. [Cancer Res 2009;69(8):3642–9]

Introduction

Oxidative DNA damage is continuously produced in aerobic cells through the generation of endogenous metabolic reactive oxygen species. One of the most frequent DNA modifications generated is 7,8-dihydro-8-oxoguanine (8-oxoG). If unrepaired, this oxidized guanine leads to the accumulation of GC to TA mutations (1). 8-oxoG is repaired via the base excision repair process initiated by a specific DNA glycosylase–Fpg in bacteria, OGG1 in eukaryotes. Absence of these proteins in a variety of model systems, such as bacteria (2), yeast (3), and mammals (4–6), was shown to favor the accumulation of 8-oxoG and, as a consequence, of G/C to T/A mutations. Thus, in mammalian cells, an alteration in 8-oxoG DNA glycosylase repair activity could favor cancer development through increased mutation rates. Consistently, mice in which the genes coding for OGG1 and MYH, another DNA glycosylase involved in avoidance of mutations induced by 8-oxoG, are inactivated show a predisposition to develop tumors (7, 8).

Within the human *OGG1* gene, the single nucleotide polymorphism at position 1245 in exon 7 results in a serine to cysteine amino acid substitution at position 326 of the protein (C>G, Ser326Cys). The Cys-326 allele frequency ranges from 23% to 41% in Caucasians to 40% to 60% in Asians (9). Molecular epidemiologic case-control studies suggest that the hOGG1-Cys326 allele is associated with a higher risk of developing several types of cancer, including lung (9, 10), gastric (11), prostate (12), and orolaryngeal (13) cancers. The underlying molecular mechanism of this increased risk remains to be established, but it has been hypothesized that it could be due to the hOGG1-Cys326 protein having an impaired repair activity allowing 8-oxoG to accumulate in the DNA with a consequent increase in mutation rates. Biochemical assays with purified proteins (14, 15), ectopic expression of the alleles in human cells (15), and complementation tests in bacterial (16) and rodent (17) cells deficient in their 8-oxoG DNA glycosylase, although showing a tendency for a lower activity of the Cys326 variant compared with the Ser326 form, have not thus far provided a mechanistic basis by which this amino acid substitution affects the enzyme's function. Furthermore, no clues can be drawn from the crystal structures available for OGG1 because none of them includes amino acids beyond position 325. More importantly, whether the endogenous 8-oxoG DNA glycosylase activity in human cells is affected by the 326 allele status remains controversial. No allele-specific differences were found by Janssen and colleagues (18), whereas Chen and colleagues (19) reported 40% lower enzymatic activity in lymphocytes from homozygous Cys326 allele carriers. Several hypotheses have been proposed for the mechanism underlying a potential deficiency in the DNA repair capacity of the Cys326 OGG1 form. This variant, or its mRNA, could be less stable and, therefore, the steady-state level of OGG1 in the cell lower. Alternatively, phosphorylation of Ser326, shown to be important for the localization of the protein (20), could also play a role in the catalytic efficiency, either directly or through the interaction with other cellular factors. Results from Smart and colleagues (17) using the ectopic expression of the human allelic variants in rodent cells suggested that replacement of Ser326 by a redox-sensitive cysteine may result in altered DNA repair capacity of the cells after pro-oxidant treatment.

To evaluate the effect of the endogenous *OGG1* genotype on cell repair capacity, we compared human LCLs established from individuals homozygous for one or the other OGG1-326 allele and analyzed the biochemical basis of the observed differences.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Materials and Methods

Cells and genotype determination. Lymphoblastoid cell lines (LCL) used in the experiments were selected from a bank of lines established through a collaborative study with Drs. P. Romestaing and J.P. Gérard (21) based on their *OGG1* codon 326 (rs1052133) genotype. The expected codon 326 genotypes were confirmed. Wild-type alleles were detected at single nucleotide polymorphisms rs1801127 and rs4986999, two other *OGG1* single nucleotide polymorphisms reported to show variations in the SNP500 cancer population, in all the 10 cell lines.

Cell culture and treatments. Cells were grown in suspension in RPMI 1640-glutamax supplemented with 15% heat-inactivated fetal bovine serum and antibiotics (penicillin-streptomycin; Invitrogen). Cells were maintained in a humidified incubator at 5% CO_2 at 37°C and fed with fresh medium at 48-h intervals. Treatments with diamide [30 min in dulbecco's phosphate-buffered saline or reduced glutathione-ethyl-ester (18 h in culture medium)] were done at a density of 10^6 cells per milliliter. After treatment, cells were washed in Dulbecco's phosphate-buffered saline by centrifugation and stored as dried pellets at −80°C until protein extraction.

Quantification of micronuclei. Samples of 10^5 cells were fixed on a microscope slide by cytospin centrifugation and treated with methanol for 1 h at −20°C. Subsequently, the cells were stained with 1 μg/mL bisbenzimide (Hoechst 33258) in phosphate buffer for 30 s, and 3000 cells per blinded sample per experiment were examined for the presence of micronuclei with a fluorescence microscope.

Cellular repair kinetics. For the analysis of cellular repair rates, additional oxidative guanine modifications were generated in exponentially growing cells (1×10^6/mL) by irradiation in the presence of the photosensitizer Ro19-8022 (22). The removal of the induced modifications was then followed by means of an alkaline elution technique (23) in combination with the repair glycosylase Fpg as described previously (See Supplementary Materials and Methods; refs. 24, 25).

Protein extraction. Cell pellets were sonicated in 20 mmol/L Tris-HCl (pH 7.5), 250 mmol/L NaCl, 1 mmol/L EDTA containing a cocktail of apoprotinin, antipain, and leupeptin (0.8 μg/μL each). The homogenate was centrifuged at 20,000 *g* for 30 min at 4°C and aliquots of the supernatant were stored at −80°C. Protein content was measured using a Bio-Rad assay kit (Bio-Rad Laboratories) with bovine serum albumin (BSA) as a standard.

8-oxoG DNA glycosylase assay. A 34-mer oligonucleotide containing an 8-oxoG at position 16 and labeled at the 5′ end with Cy5 was hybridized to its complementary oligonucleotide containing a cytosine opposite the lesion. In a standard reaction, mixture protein extracts (4 μL final volume) were added to a 10 μL reaction mixture containing 150 fmoles of the 8-oxoG:C-labeled duplex in 20 mmol/L Tris-HCl (pH 7.1), 1 mmol/L EDTA, 200 mmol/L NaCl, 1 mg/mL BSA, and 5% glycerol. After 1 h at 37°C, NaOH (0.1N final concentration) was added, and the mixture was further incubated for 15 min at 37°C and stopped by adding 4 μL of formamide dye and heating for 5 min at 95°C. The products were resolved by denaturating 7M urea −20% PAGE. Gels were scanned and band intensities were quantified using a Storm PhosphoImager (Amersham Bioscience).

Western-blot analysis. Aliquots from cell extracts were denatured by heating at 95°C for 5 min, electrophoresed on a 12.5% SDS-polyacrylamide gel (SDS-PAGE), and transferred to nitrocellulose membranes. Membranes were blocked overnight with 5% nonfat dry milk in PBS with 0.1% Tween 20 and incubated with primary antibodies in 1% blocking reagent (Roche Diagnostic) for 2 h at room temperature. After three 15-min washes with PBS with 0.1% Tween 20, membranes were incubated at room temperature with horseradish peroxidase–conjugated secondary antibody diluted in 1% blocking reagent. After washing, the protein-antibody complexes were revealed with enhanced chemiluminescence (Amersham Biosciences), visualized, and analyzed using a G:BOX ChemiXL (Syngene) and associated softwares GeneSnap and GeneTools. Anti-hOGG1 (PA3) was obtained by rabbit immunization with full-length hOGG1 protein purified from *Escherichia coli*. PA3 antibodies were further purified against the protein cross-linked to a Hytrap-NHS column. Other antibodies used were anti–green fluorescent protein (GFP; Roche) or anti-Flag (Sigma).

Detection of reduced cysteines in hOGG1. Cell extracts were incubated for 90 min at 25°C with (Methyl-PEO12)3-PEO4-Maleimide (Pierce; MalPEG, molecular mass of 2,360 Da) for covalent modification of accessible cysteines. The addition of malPEG to accessible cysteines increases the molecular mass of hOGG1 by 2.3 kDa per modification. SDS-PAGE loading buffer was then added. Samples were boiled for 5 min at 100°C, separated by denaturing SDS-PAGE, and analyzed by Western blot as described above.

Results

Increased genetic instability and slower repair of induced oxidative guanine modifications in cells homozygous for the hOGG1-Cys326 allele. The formation of micronuclei is extensively used as a biomarker of chromosomal damage and genetic instability (26). Recent prospective analyses confirmed that elevated micronuclei frequencies in peripheral blood lymphocytes is predictive of increased cancer risk (27). We therefore determined the levels of micronuclei present in 10 EBV-transformed human LCLs homozygous for each of the 2 OGG1-326 alleles (5 lines per genotype; ref. 21). Cells harboring the OGG1-Cys326 variant have a 1.7-fold increase in their levels of micronuclei when compared with cell lines carrying the OGG1-Ser326 variant, with means of 42.0 ± 5.6 versus 24.7 ± 4.0 micronuclei/10^3 nuclei, respectively (Fig. 1*A*). These results indicate that cells harboring the Cys allele have a higher genetic instability.

To establish whether the OGG1-Cys/Ser326 genotypes could affect the 8-oxoG repair capacity under physiologic conditions, we investigated the *in vivo* repair kinetics of induced purine oxidative lesions. LCLs were exposed to the photosensitizer Ro19-8022 plus light. Under these conditions, oxidative guanine modifications, mostly 8-oxoG, are generated in large excess to other DNA lesions (22) with no significant influence on proliferation rates (data not shown). The removal of induced oxidative guanine modifications during the repair incubation was followed by alkaline elution using Fpg protein, the bacterial functional analogue of OGG1, as a probe. The repair rates of the five homozygous cysteine variants are clearly slower than those of the 5 serine variants (Supplementary Fig. S1*A*; Fig. 1*B*). The levels of modifications in untreated cells were not significantly different between the two genotypes (177 ± 28 and 174 ± 21 modifications per 10^9 bases for the Ser and Cys alleles respectively; Supplementary Fig. S2). Nor was the number of induced modifications significantly affected by the *OGG1* genotype (Supplementary Fig. S2): 466 ± 35 and 456 ± 36 per 10^9 bases in cells with the Ser and Cys alleles, respectively. Taken together, these results reflect an impaired *in vivo* repair capacity of induced oxidative DNA damage for cells carrying the hOGG1-Cys326 variant when compared with the Ser326 allele.

Cells carrying the homozygous *OGG1*-Cys326 allele have lower 8-oxoG DNA glycosylase activity. To determine whether differences in the enzymatic activities of the variant OGG1 proteins are at the origin of the phenotypes described above, we compared the 8-oxoG DNA glycosylase activity in cell extracts obtained from the 10 LCLs homozygous for either the Ser326 or the Cys326 (5 lines each) alleles. The enzymatic assays performed on the various extracts clearly show a lower activity for the extracts from cells homozygous for the Cys allele when compared with those from cells homozygous for the Ser allele (Fig. 2*A*). There is a significant difference between the two groups, with an average cleavage activity of 2.6 ± 0.2 fmoles/h/μg for the OGG1-Ser variant and 1.4 ± 0.2 fmoles/h/μg for the OGG1-Cys326 (Fig. 2*B*), thus establishing that the OGG1-Cys homozygous cell lines have a nearly 2-fold lower basal 8-oxoG DNA glycosylase activity than the OGG1-Ser326 lines. Time curve assays on extracts from Ser326 and Cys326 cell lines confirmed this result (Supplementary Fig. S3).

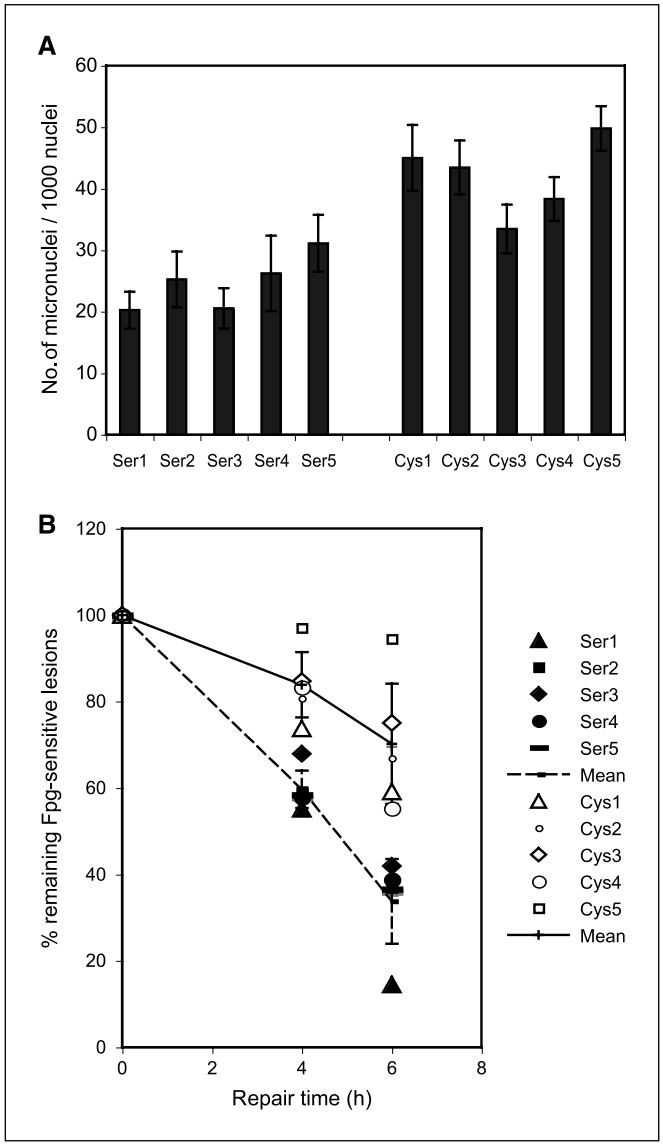

Figure 1. Increased genomic stability and reduced *in vivo* 8-oxoG DNA repair activity in hOGG1-Cys326 cell lines. *A,* micronuclei frequency in lymphoblastoid cells homozygous for one of the two position 326 alleles. *Columns,* mean of 3 independent experiments (3,000 cells each); *bars,* SD. *B, in vivo* repair kinetics. Cell lines were exposed to Ro19-8022 plus light and analyzed by alkaline elution for the repair of induced Fpg-sensitive sites after 4 and 6 h. *Points,* mean for triplicate experiments for each indicated cell line; *bars,* SD. Lines join the average values for each of the two allelic variants.

One explanation for such a difference could be that the reduced DNA glycosylase activity found in the OGG1-Cys326 cell lines is the consequence of altered expression of the *OGG1* gene. Quantitative reverse transcription-PCR in all 10 LCLs showed no differences in transcript levels (Supplementary Table S1). Western blot analysis of the same extracts used for the DNA glycosylase activity experiment showed that the levels of hOGG1 protein in the cell lines was not dependent on the *OGG1* genotype (Fig. 2*C*). These results suggest that the deficiency in 8-oxoG DNA glycosylase activity of the extracts is directly associated with the presence of a different amino acid residue at position 326 affecting the enzymatic activity of the protein rather than its concentrations.

DNA glycosylase activity in OGG1-Cys326 cells is recovered by reducing agents. Reversible inhibition of OGG1 activity by oxidation of cysteine residues has been shown to occur after oxidative stress (28). The presence of an additional cysteine residue in the OGG1-Cys326 variant could therefore sensitize the protein to oxidation. This led us to analyze whether the lower 8-oxoG DNA glycosylase activity in cells homozygous for the Cys326 allele could be linked to the oxidation of the extra cysteine residue. Protein extracts prepared from cells with either the Cys or Ser alleles were preincubated with DTT or reduced glutathione before performing the activity assay. DTT treatment had no effect on the activity of OGG1-Ser326 extracts, whereas it led to an increased OGG1 activity of OGG1-Cys326 extracts (Fig. 3*A*). Interestingly, after the reducing treatment, the glycosylase activity of the extracts from the Cys326 cells was comparable with that found in extracts from the Ser326 cells. Similar results were found after preincubation with reduced glutathione, although in this case, a slight induction of the activity from the Ser extracts was also observed (Fig. 3*B*). These results showing that reductive treatment is able to restore a "normal" activity to the OGG1-Cys326 protein—with marginal effect on the

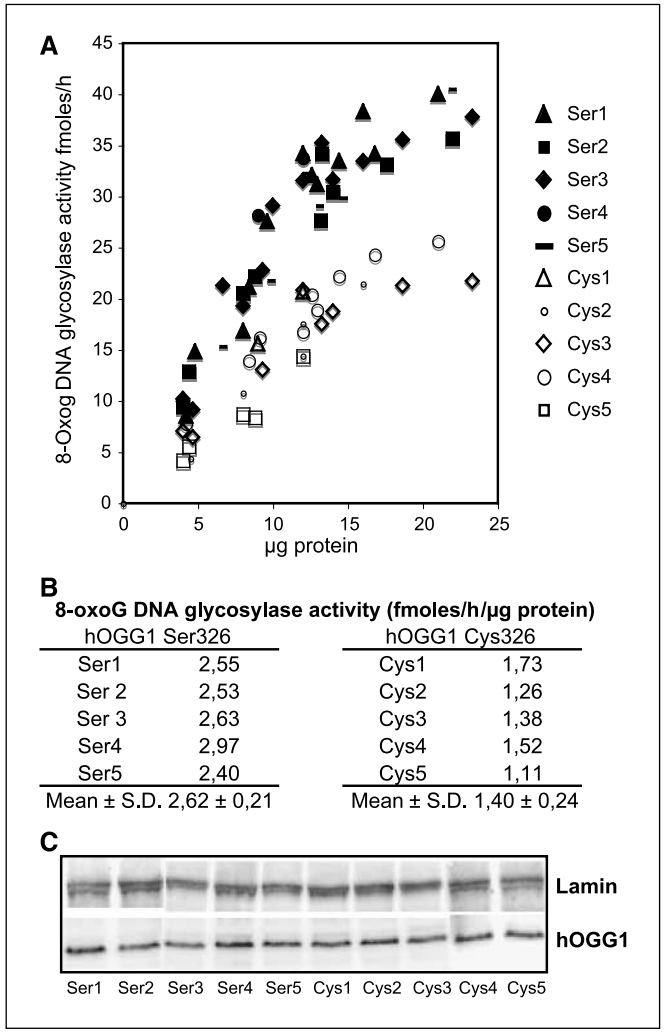

Figure 2. Reduced *in vitro* 8-oxoG DNA glycosylase activity in hOGG1-Cys326 cell lines. *A,* cell extracts from 5 hOGG1-Ser326 (*filled symbols*) and 5 hOGG1-Cys326 (*open symbols*) cell lines were assayed for 8-oxoG DNA glycosylase activity. *B,* 8-oxoG DNA glycosylase activity calculated for each cell line. *C,* cell extracts (25 μg) from OGG1-Ser326 and OGG1-Cys326–independent cell lines were analyzed by Western blotting with anti-hOGG1 (*bottom*) and anti-lamin (*top*).

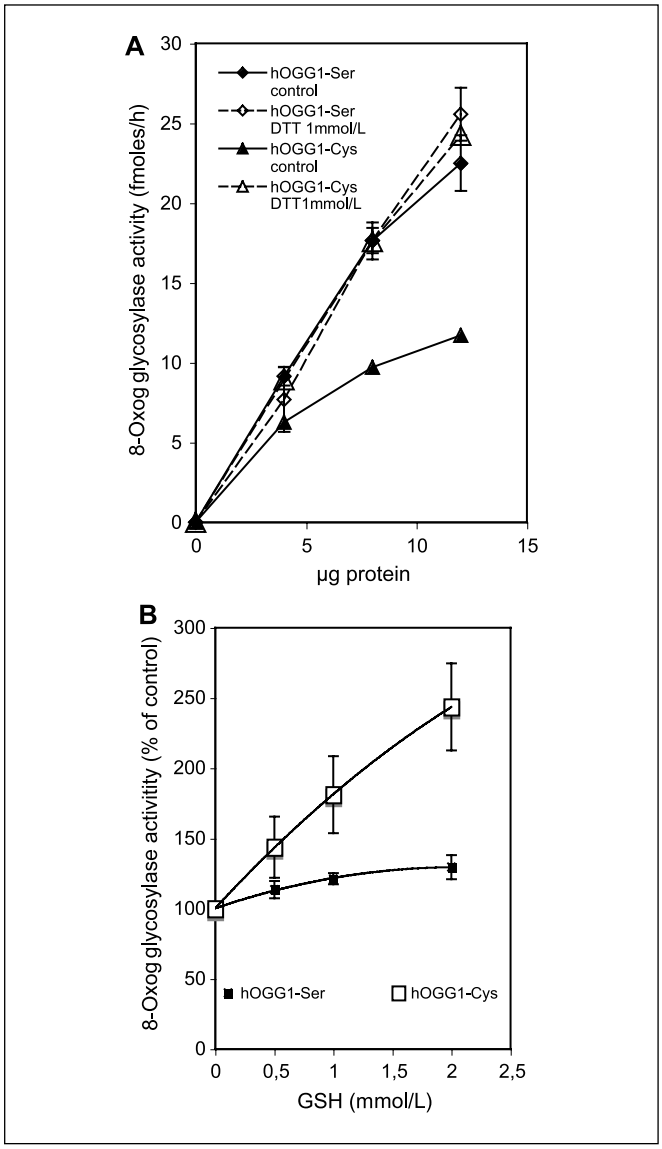

Figure 3. *In vitro* 8-oxoG DNA glycosylase activity in OGG1-Cys326 is recovered by reducing agents. *A,* cell extracts from hOGG1-Cys326 (Cys1, 3, 4, and 5) and hOGG1-Ser326 cell lines (Ser1, 2, 3, and 4) were preincubated for 20 min at 37°C with or without 1 mmol/L DTT before determination of their 8-oxoG DNA glycosylase activities. *Points,* mean for the four cell lines; *bars,* SD. *B,* cell extracts (12 µg) from the same cell lines were preincubated for 20 min at 37°C without or with reduced glutathione before determination of their 8-oxoG DNA glycosylase activities. *Points*, mean (n = 4 cell lines); *bars,* SD.

OGG1-Ser326 protein—strongly suggests that the impaired 8-oxoG DNA glycosylase activity of the OGG1-Cys326 cell extracts is a consequence of oxidation of the DNA glycosylase itself.

To confirm these results and establish whether the 8-oxoG DNA glycosylase activity of the OGG1-Cys326 variant could also be modulated by changes in the redox environment *in vivo*, we increased the cellular content of reduced glutathione by treating cells with reduced glutathione-ethyl-ester. This compound, after cleavage by cellular esterases, increases the intracellular glutathione content and protects cells against oxidative damage (29). As shown in Fig. 4*A*, increasing glutathione concentration in OGG1-Cys326 cells led to a 30% increase in 8-oxoG DNA glycosylase activity, whereas its effect on the activity of Ser326 cells remained marginal. Similarly, incubating cells with DTT and catalase during

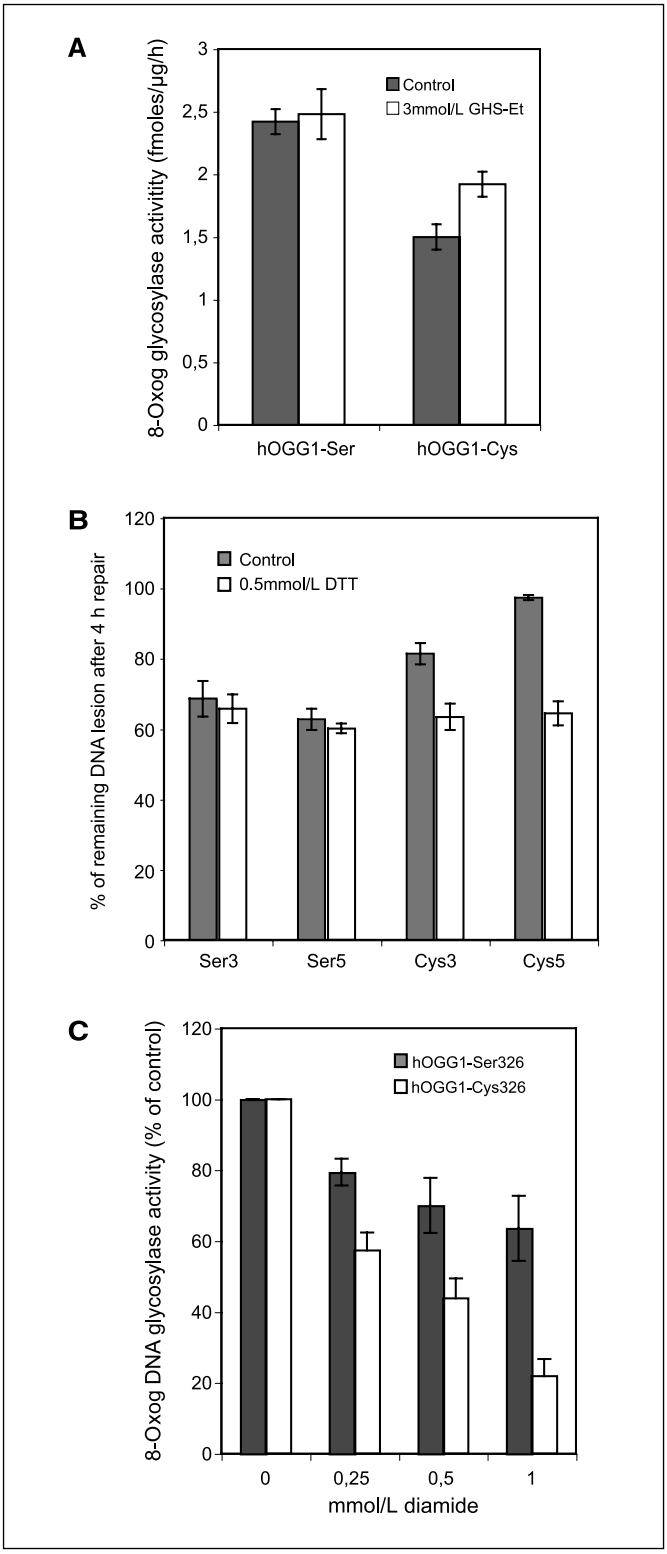

Figure 4. *In vivo* reductive or oxidizing treatments modulate 8-oxoG repair activity of hOGG1-Cys326 cells. *A,* cell lines were incubated for 17 h in normal culture medium or in medium containing 3 mmol/L reduced glutathione-ethyl-ester (*GSH-Et*) and harvested for 8-oxoG DNA glycosylase activity assay. *Columns,* mean for three cell lines; *bars,* SD. *B,* cells were incubated in the presence or absence of DTT and analyzed for repair of Fpg-sensitive sites. *Columns,* mean for triplicates for each line; *bars,* SD. *C,* hOGG1-Cys326 (Cys1, 2, and 5) and hOGG1-Ser326 (Ser1, 3, and 5) cell lines were incubated for 30 min with the indicated concentration of diamide and harvested for 8-oxoG DNA glycosylase activity assays. *Columns*, mean (n = 3 cell lines); *bars,* SD.

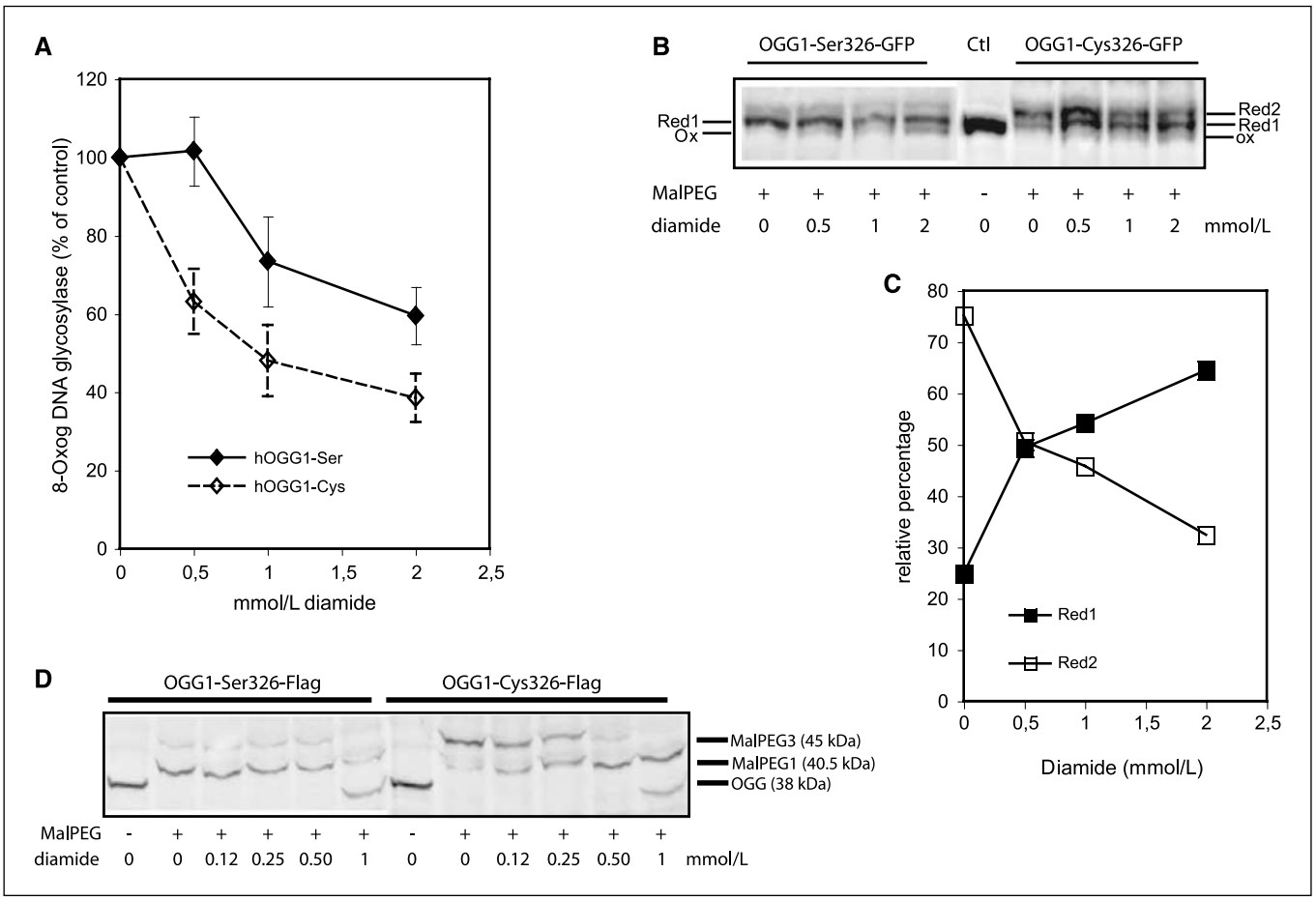

Figure 5. Diamide induced inhibition of hOGG1-Cys326 is related to oxidation of the extra cysteine residue. *A,* HeLa cells stably expressing hOGG1-GFP-Cys326 or hOGG1-GFP-Ser326 were incubated for 30 min with the indicated concentrations of diamide and harvested for 8-oxoG DNA glycosylase activity assays. *Points,* mean (n = 5); *bars,* SD. *B,* cell extracts (60 μg) from diamide-treated cells were incubated with MalPEG and analyzed by Western blot with anti-GFP antibody. *C,* relative intensities of band red 1 and red 2. *D,* cell extracts (60 μg) from HeLa cells transiently expressing hOGG1-Flag fusions were incubated at 37°C for 15 min with the indicated concentrations of diamide, incubated with MalPEG and analyzed by Western blot with anti-Flag antibody. Molecular weights for the different bands were calculated by using the *Gene Tools* software.

the repair period after induction of Fpg-sensitive modifications resulted in a net improvement of the *in vivo* repair kinetics for the OGG1-Cys326 cells without significantly altering the repair rate for the Ser326 cells (Fig. 4*B*). These results confirm that the OGG1-Cys326 level of activity is determined by the cellular redox environment.

hOGG1-Cys326 is more sensitive to cellular oxidative stress than hOGG1-Ser326. The results presented above suggest that the presence of Cys326 in OGG1 could result in a particularly high sensitivity of the protein to conditions of oxidative stress. To test this hypothesis, cells were incubated for 30 min in the presence of the cysteine oxidizing agent diamide and their 8-oxoG DNA glycosylase activity measured in whole cell extracts. Although incubation of the cells with diamide inhibited OGG1 activity both in the OGG1-Ser326 and OGG1-Cys326 cells, the latter are much more sensitive to the oxidizing treatment (Fig. 4*C*). After exposure to 1 mmol/L diamide, the Cys326 cells show only 20% of the activity of untreated cells compared with over 60% for the Ser326 cells. These data not only confirm our previous observations that the OGG1 protein activity is sensitive to oxidizing conditions (28) but also suggest that replacement of serine326 by a cysteine adds an additional target for oxidation within the protein, making it more susceptible to inactivation by oxidative stress.

hOGG1 inactivation is associated with Cys326 oxidation and disulfide bond formation. The results described above point to Cys326 as a target for oxidation leading to impaired OGG1 DNA glycosylase activity. To test this hypothesis, we analyzed the oxidation state of cysteine residues in GFP-hOGG1 fusion proteins expressed in HeLa cells. Comparison of the 8-oxoG DNA glycosylase activity in the extracts from these cells with that from the parental HeLa (Supplementary Fig. S4) shows that <3% of the DNA glycosylase activity is provided by the endogenous protein, which has a Ser residue at position 326 (data not shown). Therefore, all the effects observed in these experiments correspond to the effects on the exogenously expressed protein. As for the endogenous proteins, inactivation of hOGG1 activity by diamide treatment of the cells expressing GFP-hOGG1-Cys326 was greater than for those expressing the Ser326 variant (Fig. 5*A*). To assess the level of cysteine oxidation, we used MalPEG, which reacts with reduced thiol groups in cysteines increasing the molecular weight of the protein by 2.3 kDa per adduct. In extracts from the serine variant–expressing cells, MalPEG treatment generates a major OGG1 band (*Red1*), slightly higher than that found in control extracts without MalPEG treatment (Fig. 5*B*), indicating the presence of at least one reduced and accessible cysteine residue. In the case of the Cys variant, a slower migrating band (*Red2*),

corresponding to a protein containing extra modified cysteines, was also observed (Fig. 5*B*), showing that Cys326 provides additional residues accessible to MalPEG modification. We then analyzed MalPEG-treated extracts from cells exposed to diamide. Inactivation of OGG1-Ser at high diamide concentrations correlated with the appearance of a lower band corresponding to an oxidized form of the protein (*ox*), not modifiable by MalPEG (Fig. 5*B*). For extracts from cells expressing the Cys326 variant, the same oxidized form also appears at high diamide concentrations. However, in support of the link between oxidation of Cys326 and loss of the enzymatic activity, at lower concentrations of the oxidant, there is an enrichment for an intermediately reduced form of OGG1-Cys326 (*Red1*) at the expense of the more reduced form (*Red2*) in response to increasing concentrations of diamide (Fig. 5*B* and *C*).

We performed similar experiments on extracts obtained from cells expressing either the Cys or Ser326 forms of the protein fused to a FLAG tag instead of a GFP tag. With this less bulky tag, the treatment with MalPEG allowed the determination of the number of reduced cysteine accessible to the adduct in the hOGG1-Ser326 protein. The Western blot on Fig. 5*D* confirms the pattern obtained with the GFP-fusion proteins and allowed us to establish the number of adducts present in each of the OGG1 forms detected. Inactivation of the OGG1-Ser variant was associated with the oxidation of one cysteine residue (shift from 40.5–38 kDa band). In the case of the Cys326 variant, low diamide concentrations induced the shift from 3 (45 kDa) to 1 (40.5 kDa) reduced cysteines. The most straightforward explanation for this result is that Cys326 can be easily oxidized to form a disulfide bond, resulting in a loss of enzymatic activity.

To explore whether other cysteines within OGG1 can potentially interact with Cys326 to form a disulfide bond, cell extracts were treated with phenylarsine oxide (PAO), a reagent that specifically cross-links cysteines located in close proximity by forming stable dithioarsine ring bridge (30). Having a cysteine in position 326 rendered the OGG1 DNA glycosylase activity of lymphoblastoid cells more sensitive to PAO (Fig. 6*A*), supporting our hypothesis that Cys326 is capable of interacting with a nearby cysteine and that the resulting cross-link leads to inactivation of the enzyme. To confirm this hypothesis, we analyzed by MalPEG addition the free cysteines available after the PAO treatment. As in the case of the diamide treatment, exposure of the extracts from HeLa cells expressing the cysteine variant to increasing amounts of the cross-linking agent resulted in the appearance of the lower band (40.5 kDa) at the expense of an upper one (45 kDa; Fig. 6*B*). This result reflects the loss of accessibility of MalPEG to two cysteines (Cys326 and a closely located cysteine) due to their participation in an intramolecular cross-link, as found with an oxidative treatment (Fig. 5*D*).

Discussion

Oxidative stress has been shown to play a determinant role in cancer development, in part through the induction of DNA damage. The major DNA lesion formed by reactive oxygen species under physiologic conditions is the highly mutagenic 8-oxoG, the persistence of which can lead to GC to TA tranversions, a frequently found somatic mutation in cancers. In mammalian cells, the major defense against the mutagenic effect of 8-oxoG is provided by the base excision repair pathway initiated by the 8-oxoG–specific DNA glycosylase, OGG1. It has been shown that there is a correlation between the levels of 8-oxoG DNA glycosylase activity in lymphocytes and cancer predisposition (31, 32). Several studies have shown that the frequently found single nucleotide polymorphism in the human gene encoding OGG1, which results in a serine to cysteine replacement at position 326, is associated with an increased risk of cancer development (9, 33, 34). We show here that cells carrying the Cys variant present a higher genetic instability as determined by their levels of micronuclei. Moreover, the cell lines homozygous for the Cys allele also showed a lower DNA repair rate *in vivo*. Our results indicate that a serine at position 326 renders the protein extremely sensitive to oxidation, leading to an impairment of its DNA glycosylase activity, even under normal cell growth conditions.

Previous studies comparing the 8-oxoG DNA glycosylase activity of human OGG1 proteins purified from bacteria (14) or in nuclear extracts from HeLa cells overexpressing tagged OGG1 variants (15) suggested that the OGG1-Cys had slightly reduced activity. Our comparison of the endogenous 8-oxoG DNA glycosylase activity of 10 human LCLs homozygous for either the OGG1-Cys or the

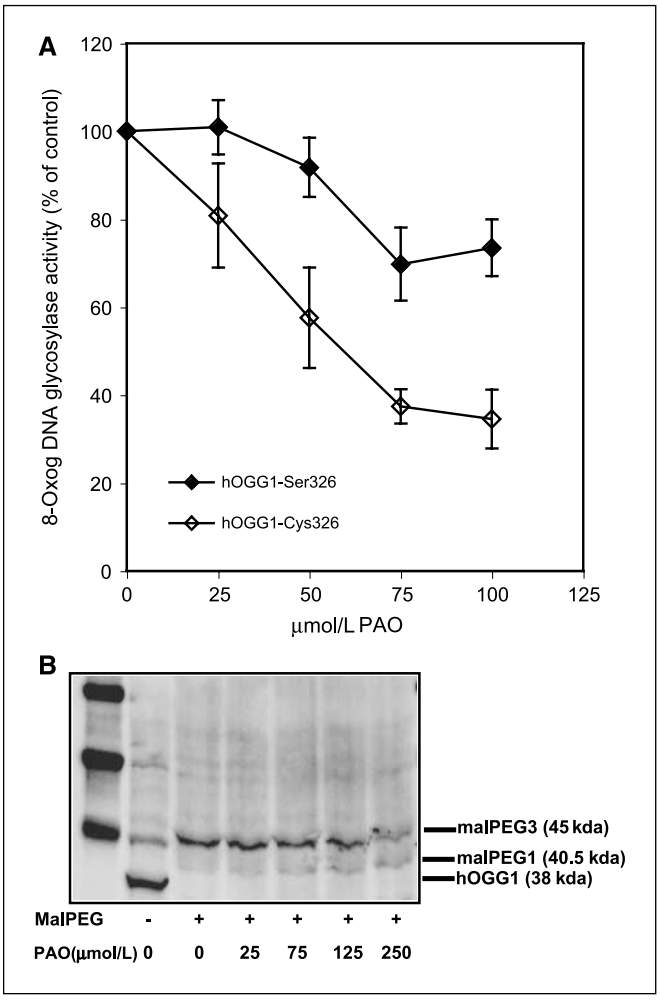

Figure 6. Inhibition of hOGG1-Cys326 is due to disulfide-bond formation. *A,* cell extracts from Cys 1, 3, 4, and 5 or Ser 1, 2, 3, and 4 LCLs were incubated for 10 min at room temperature with the indicated concentrations of PAO and analyzed for 8-oxoG DNA glycosylase activity. *Points,* mean ($n = 4$); *bars,* SD. *B,* cell extracts (70 μg) from HeLa cells expressing hOGG1-Cys326-Flag were incubated for 10 min at 37°C with the indicated concentration of PAO, incubated with MalPEG and analyzed by Western blot with anti-Flag antibody. Apparent molecular weights for the different bands were calculated using the *Gene Tools* software.

OGG1-Ser alleles clearly showed a nearly 2-fold lower enzymatic activity in the group of OGG1-Cys lines. The only previous study comparing the two allelic forms of OGG1 in the normal cellular context (18) concluded that the 8-oxoG DNA glycosylase activity in human lymphocytes was not dependent on the position 326 polymorphism. We have shown here that addition of a reducing agent to the reaction mixture with extracts from cells with the Cys326 genotype raises the DNA glycosylase activity to levels similar to those of cells with the Ser326 genotype (Fig. 3). Therefore, the discrepancy between the two data sets can be explained by the fact that Janssen and colleagues (18) performed a cleavage assay in the presence of 5 mmol/L DTT to measure OGG1 activity and thus probably increased artificially the activity of extracts from the Cys variant cells.

Our results clearly establish that the intrinsic 8-oxoG DNA glycosylase activity, and therefore repair capacity, of cells carrying the OGG1-Cys variant is particularly sensitive to the cellular redox status. Indeed, treating these cells with reductants such as DTT or reduced glutathione increases both the DNA glycosylase activity as measured in extracts and the rate of *in vivo* repair of Fpg-sensitive modifications. Conversely, exposure of cells or extracts to oxidizing agents results in a rapid inactivation of the enzyme. Our analysis of the redox status of the OGG1 cysteine residues indicates that oxidation of the extra cysteine residue present in the variant protein results in a reduction of the enzymatic activity as has been suggested by Lee and colleagues (35). Cys326 is in a highly positively charged sequence context [ADLRQ (Ser326Cys) RHAQ]. Cysteines in such environments are more likely to form a reactive thiolate anion that is particularly susceptible to oxidative modifications such as formation of disulfide bonds (36). Cross-linking experiments with PAO, showing the rapid inactivation concomitant with the inaccessibility of the extra cysteine to MalPEG (Fig. 6), support the idea that Cys326 can be easily involved in the formation of disulfide bonds. The resulting loss of enzymatic activity could be the consequence of conformational changes induced by the formation of a new Cys-Cys bond or via the sequestration through the new disulfur bond of a catalytically important cysteine within the OGG1 protein. It should be noted that oxidative stress also results in inactivation, although to a lesser extent, of the Ser326 form of OGG1, suggesting that one or more of the other eight cysteines found in OGG1 is required to be in a reduced form for full enzymatic activity of the protein.

Interestingly, the spontaneous levels of micronuclei in the cysteine variant cell lines are clearly higher than in the serine variant cell lines (Fig. 1*A*), although the basal levels of oxidative guanine modifications in the two variants are not significantly different (Supplementary Fig. S2). This could indicate that an aberrant generation of double-strand breaks during repair, which is believed to underlie micronucleus formation, is elevated in the case of repair by the OGG1-Cys326 variant.

The sensitivity of the cysteine variant to the redox environment predicts that phenotypic consequences should depend largely on cellular oxidative stress. This could explain the reported interindividual variability in OGG1 activity (18, 37) and the lack of a good correlation between OGG1 mRNA levels and DNA glycosylase activity found in population based studies (37). Age-associated loss of 8-oxoG DNA glycosylase activity (19, 38) or accumulation of 8-oxoG in DNA (39) might also reflect increased in oxidative stress over the life span of a tissue. In addition, this sensitivity may explain some of the variability in the results of association studies on the OGG1-326 genotype and cancer risk. The redox environment could be a potential confounding factor in such studies if it was different between cases and controls.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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