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Advances in Brief

Repair of 8-Oxodeoxyguanosine Lesions in Mitochondrial DNA Depends on the Oxoguanine DNA Glycosylase (*OGG1*) Gene and 8-Oxoguanine Accumulates in the Mitochondrial DNA of OGG1-defective Mice

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Abstract

Mitochondria are not only the major site for generation of reactive oxygen species, but also one of the main targets of oxidative damage. One of the major products of DNA oxidation, 8-oxodeoxyguanosine (8-oxodG), accumulates in mitochondrial DNA (mtDNA) at levels three times higher than in nuclear DNA. The main pathway for the repair of 8-oxodG is the base excision repair pathway initiated by oxoguanine DNA glycosylase (OGG1). We previously demonstrated that mammalian mitochondria from mice efficiently remove 8-oxodG from their genomes and isolated a protein from rat liver mitochondria with 8-oxoguanine (8-oxodG) DNA glycosylase/apurinic DNA lyase activity. In the present study, we demonstrated that the mitochondrial 8-oxodG DNA glycosylase/apurinic DNA lyase activity is the mitochondrial isoform of OGG1. Using mouse liver mitochondria isolated from ogg1$^{-/-}$ mice, we showed that the *OGG1* gene encodes for the mitochondrial 8-oxodG glycosylase because these extracts have no incision activity toward an oligonucleotide containing a single 8-oxodG DNA base lesion. Consistent with an important role for the OGG1 protein in the removal of 8-oxodG from the mitochondrial genome, we found that mtDNA isolated from liver from OGG1-null mutant animals contained 20-fold more 8-oxodG than mtDNA from wild-type animals.

Introduction

Mitochondria are the major cellular source of ROS.[2] Calculations based on early observations suggest that up to 5% of the oxygen consumed by the electron transport chain may be converted to ROS by incomplete oxygen reduction (1). Hence, mitochondria are also one of the main cellular targets of ROS-induced oxidative damage, and in fact relatively high levels of oxidized proteins, lipids, and nucleic acids are detected in mammalian mitochondria under normal metabolic conditions (2). The mtDNA is highly prone to oxidative damage because it sits on the inner mitochondrial membrane in close proximity to the electron transport chain, and indeed, the levels of oxidized bases in mtDNA are two to three times higher than in nuclear DNA (3).

8-oxodG is one of the most prevalent products of the oxidative attack of DNA. It accumulates in mtDNA with age and under certain pathological conditions, *e.g.*, associated with some types of cancer and neurodegenerative diseases. This adduct may be of particular biological relevance because, unless repaired, it induces G:C to T:A transversions with high frequencies (4). Aerobic organisms, from bacteria to humans, have developed sophisticated repair pathways to remove and prevent the formation of 8-oxodG from their genomes. In mammals, BER is the main pathway for the repair of 8-oxodG (5). BER is initiated by a DNA glycosylase, which recognizes and removes the base in a free form by cleavage of the glycosylic bond between the damaged base and the deoxyribose residue. The resulting abasic site is cleaved to generate a gap that, after appropriate processing of the termini, will be filled by DNA polymerase and ligated (6).

The OGG1 protein is the main DNA glycosylase for the repair of 8-oxodG lesions in DNA. This enzyme is a 38–44 KDa protein with functional homology to bacterial formamidopyrimidine glycosylase. OGG1 is a bifunctional DNA glycosylase, with an associated AP lyase activity that cleaves DNA at abasic sites through a β-elimination mechanism (7, 8).

Mammalian mitochondria efficiently remove 8-oxodG from their genome. Using a Southern blot technique that allows repair rates to be measured in specific parts of the genome, Taffe *et al.* (9) demonstrated that mitochondria from Chinese hamster ovary cells remove formamidopyrimidine glycosylase-sensitive sites from their DNA at rates comparable to an actively transcribed gene in nuclear DNA. Recently, our laboratory reported the isolation of an oxidative damage endonuclease from rat liver mitochondria, with a molecular mass estimated by gel-filtration chromatography to be between 25 and 30 kDa. This activity was specific for 8-oxodG, with a preference for 8-oxodG:C bp. We also demonstrated that this enzyme is a putative 8-oxodG glycosylase/AP lyase because it can be covalently linked to an 8-oxodG oligonucleotide by sodium borohydride reduction (10). Here, we demonstrate that the mitochondrial 8-oxodG glycosylase/AP lyase activity is attributable to an isoform of OGG1. Using knockout mice for the *OGG1* gene, we found that extracts prepared from liver mitochondria isolated from those animals have no incision activity toward an 8-oxodG-containing substrate and that DNA from the mitochondria of such mice has a 20-fold increase in the steady-state levels 8-oxodG.

Materials and Methods

Materials. HEPES, benzamidine HCl, DTT, BSA, and acrylamide/bisacrylamide (19:1) were from Sigma Chemical Co. (Saint Louis, MO). Protease inhibitors and UDG were from Boehringer Mannheim (Indianapolis, IN). Isotopes were from NEN Life Science Products (Wilmington, DE). G25 spin columns were from Pharmacia (Peapack, NJ). T4 polynucleotide kinase was from Stratagene (Austin, TX). All other reagents were ACS grade from Sigma Chemical Co.

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[2] The abbreviations used are: ROS, reactive oxygen species; mtDNA, mitochondrial DNA; 8-oxodG, 7,8-dihydro-8-oxodeoxyguanosine; BER, base excision repair; OGG1, oxoguanine DNA glycosylase; AP, apurinic DNA; UDG, uracil DNA glycosylase; MLM, mouse liver mitochondria; HPLC, high-performance liquid chromatography; EC, electrochemical; wt, wild type; mtUDG, mitochondrial UDG.

Animals. Male C57Bl/6J mice with or without a targeted mutation of the *OGG1* gene (11) were housed under standard conditions. For the isolation of mitochondria, the animals were killed by cervical dislocation. The livers were removed immediately, washed free of blood with isolation buffer, and processed as described below.

Isolation of Liver Mitochondria. MLM were isolated by a combination of differential centrifugation and Percoll gradient centrifugation as described previously (10). Protein concentrations were determined by the Lowry method with BSA as standard.

Oligonucleotides. All oligonucleotides used in this study were from Midland Certified Reagent Co. (Midland, TX); their sequences are presented in Table 1. Oligonucleotides containing either the DNA lesion or an unmodified base were 5′-end labeled using T4 polynucleotide kinase and [γ-^{32}P]ATP, as described previously (12).

Measurement of 8-oxodG Incision Activity. 8-oxodG glycosylase/AP lyase activity was measured by an oligonucleotide incision assay, as described previously (12). Briefly, intact mitochondria were suspended in a buffer containing 20 mM HEPES-KOH (pH 7.6), 1 mM EDTA, 2 mM DTT, 300 mM KCl, 5% glycerol, and 0.05% Triton X-100. The samples were diluted to a final protein concentration of 10 μg/μl, and the KCl concentration was adjusted to 100 mM. Incision reactions (20 μl) contained 40 mM HEPES-KOH (pH 7.6), 5 mM EDTA, 2 mM DTT, 75 mM KCl, 10% glycerol, 88.7 fmol of ^{32}P-labeled duplex oligonucleotide (Table 1), and 100 μg of mitochondrial protein. The reactions were incubated at 32°C for 16 h and terminated by the addition of 0.8 μl each of 5 mg/ml proteinase K and 10% SDS and incubation at 55°C for 15 min. The DNA was ethanol precipitated and resolved in a denaturing 20% polyacrylamide gel containing 7 M urea. The gels were visualized by a Molecular Dynamics Phosphoimager. Quantification used the ImageQuant NT software. Incision activity was calculated as the amount of radioactivity in the band corresponding to the damage-specific cleavage product over the total radioactivity in the lane.

Measurement of UDG Activity. UDG activity was also measured using a 28mer oligonucleotide containing a single deoxyuracil at position 11 (Table 1), as described elsewhere (12).

Measurement of 8-oxodG in mtDNA. The 8-oxodG content was determined using HPLC with EC detection. Livers from wt and ogg1$^{-/-}$ mice 4 months of age were removed and immediately frozen at −80°C. Liver mitochondria were then isolated according to the method described by Croteau *et al.* (10). mtDNA was further isolated and digested with nuclease according to Hofer and Möller (13). HPLC/EC was carried out as described previously (11).

Statistical Analysis. The results are reported as mean ± SD of six different mitochondrial preparations for each genotype, assayed twice in duplicate. For each experiment, two different gels were run. The differences between groups were analyzed by the Student's *t* test, and $P \leq 0.05$ was considered statistically significant.

Results and Discussion

The observation that exon sequences of the *OGG1* gene encode both mitochondrial and nuclear targeting sequences suggested that this gene might encode both nuclear and mitochondrial isoforms. Studies with GFP constructs transfected into mammalian cells have confirmed sorting to both the nucleus and the mitochondria (14, 15). It appears that there is an alternative last exon of the *OGG1* gene that upon differential splicing directs OGG1 to the nucleus (exon 7) or to the mitochondria (exon 8; Ref. 15). However, in these studies, OGG1 protein expression was driven by strong promoters, which may produce abnormal cellular distribution that differs from the *in vivo* distribution because of excessive amounts of protein. The generation of an *ogg1*-null mouse by Klungland *et al.* (11) allowed us to investigate whether the 8-oxodG glycosylase activity we previously detected in rat and mouse liver mitochondria is indeed attributable to an OGG1 isoform. We isolated liver mitochondria from wt and *ogg1*$^{-/-}$ mice and tested the capacity of the mitochondria extracts to incise a 28mer oligonucleotide containing a single 8-oxodG, at position 11. The autoradiogram presented in Fig. 1 shows that incubation of OG oligonucleotides with increasing concentrations of MLM from wt mice extracts (Fig. 1, *Lanes 3–5*) resulted in a concentration-dependent formation of a 10mer incision product as a result of cleavage at the site of the lesion. This incision activity was specific for the 8-oxodG lesions: incubation of a control undamaged oligonucleotide with either wt or *ogg1*$^{-/-}$ extracts (Fig. 1, *Lanes 1* and *2*, respectively) did not produce any incision product. In contrast, increasing concentrations of *ogg1*$^{-/-}$ MLM extracts exhibited no incision activity toward the OG oligonucleotide (Fig. 1, *Lanes 6–8*). Interestingly, we observed that the incubation of the 28mer substrates, both control and damaged oligonucleotides, resulted in the formation of two smaller products (Fig. 1, *Lanes 2* and *6–8*), suggesting that it is possible that the knockout of OGG1 led to the up-regulation of other enzymes involved in DNA metabolism in those animals, such as exonucleases. It is important to point out that MLM isolated under our conditions are free of nuclear contamination. We ascertained the purity of the mitochondrial preparations by Western blots and found that our extracts showed no contamination with lamin B, a very abundant nuclear protein (not shown).

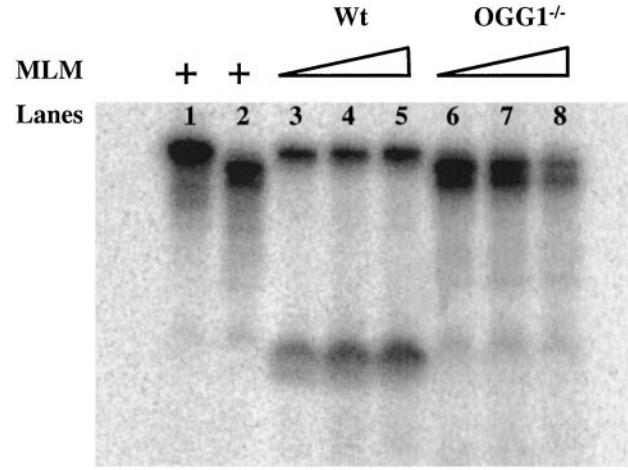

Fig. 1. Incision of an 8-oxodG oligonucleotide by wt and *ogg1*$^{-/-}$ MLM extracts. OG nucleotide (88.7 fmol; Table 1) was incubated with increasing concentrations (50–150 μg) of mitochondrial extracts from wt (*Lanes 3–5*) or knockout mice (*Lanes 6–8*) for 16 h. The reactions were resolved as described in the text. *Lanes 1* and *2*, control oligonucleotides (Table 1) incubated with 150 μg of extracts from wt (*Lane 1*) and *ogg1*$^{-/-}$ (*Lane 2*) animals.

Table 1 *Oligonucleotide substrates used*

Name	Sequence[a]
OGcon	5′-GAA CGA CTG T**G**A CTT GAC TGC TAC TGA T-3′ 3′-CTT GCT GAC ACT GAA CTG ACG ATG ACT A-5′
OG	5′-GAA CGA CTG T**oxoG**A CTT GAC TGC TAC TGA T-3′ 3′-CTT GCT GAC ACT GAA CTG ACG ATG ACT A-5′
UC	5′-GAA CGA CTG T**T**A CTT GAC TGC TAC TGA T-3′ 3′-CTT GCT GAC AAT GAA CTG ACG ATG ACT A-5′
UU	5′-GAA CGA CTG T**U**A CTT GAC TGC TAC TGA T-3′ 3′-CTT GCT GAC AAT GAA CTG ACG ATG ACT A-5′

[a] The important features are underlined and in bold.

We measured 8-oxodG incision activity in six wt and six *ogg1*$^{-/-}$ mice. The results are presented in Fig. 2. MLM extracts from wt mice incised 63.2 ± 2.3 fmol/100 μg/16 h, whereas *ogg1*$^{-/-}$ MLM extracts incised 4.8 ± 2.3 fmol/100 μg/16 h. This latter value is similar to the incision found in the absence of mitochondrial protein (3.2 ± 1.3 fmol/100 μg/16 h; not shown), which is attributable to spontaneous degradation of the oligonucleotide. These results show that mitochondria from the *ogg1*$^{-/-}$ mice have no 8-oxodG glycosylase. Similarly, Klungland *et al.* (11), using liver whole cell extracts from the same mice, found no incision activity toward an 8-oxodG oligonucleotide. In addition, these results also show that OGG1 appears to be the only 8-oxodG glycosylase in mouse mitochondria. The

apparent discrepancy between the size of the native mitochondrial enzyme and that of OGG1 deduced from sequence calculations can be ascribed to possible protein cleavage and removal of an NH_2-terminal domain during entry into the mitochondria.

To ascertain that the lack of activity in *ogg1*$^{-/-}$ extracts was not attributable to a more general mitochondrial dysfunction or loss of integrity, we measured another DNA repair activity in those extracts. We chose mtUDG because this is an abundant enzyme that is important in mitochondrial DNA repair (16). We found that mtUDG activity was similar in extracts from wt and *ogg1*$^{-/-}$ mice (Fig. 3); wt and knockout mouse groups incised 13 ± 2 and 15 ± 3 fmol/10 μg/h, respectively. It is important to point out that this assay measures the combined activities of mtUDG and AP endonucleases because UDG is a monofunctional glycosylase. Thus, it is clear that *ogg1*$^{-/-}$ mitochondria have normal activity for at least some other DNA repair activities and that they may be defective only in 8-oxodG glycosylase activity.

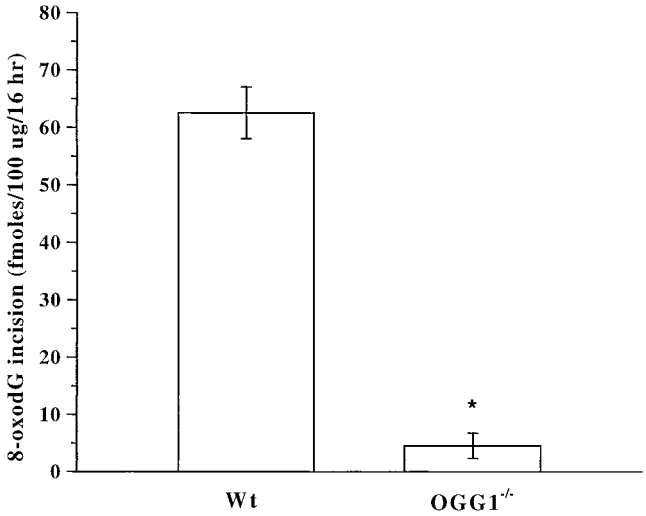

Fig. 2. Quantification of 8-oxodG incision by MLM extracts from wt and OGG1 knockout animals. OG oligonucleotide (88.7 fmol) was incubated with 100 μg of mitochondrial extracts for 16 h at 32°C. Incision activity was quantified as described in "Materials and Methods." Results are presented as mean ± SD (*bars*). $n = 6$ for each group. *, $P = 0.01$.

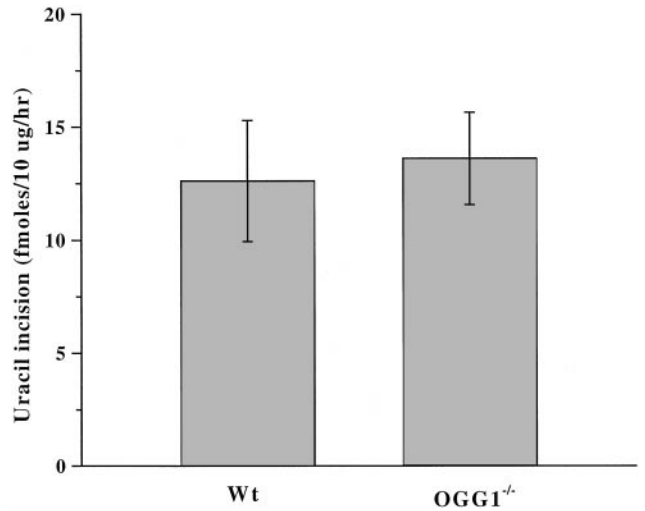

Fig. 3. Quantification of uracil incision by MLM extracts from wt and OGG1 knockout animals. UU oligonucleotide (88.7 fmol; Table 1) was incubated with 10 μg of mitochondrial extracts for 1 h at 37°C. Incision activity was quantified as described in "Materials and Methods." Results are presented as mean ± SD (*bars*) of two different experiments, performed in duplicate. $n = 6$ for each group.

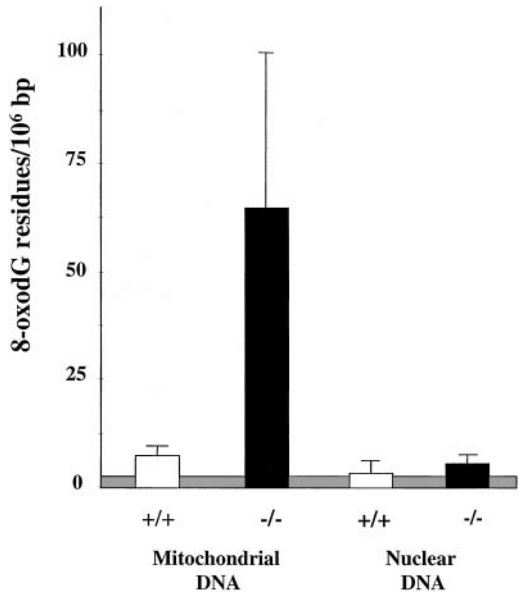

Fig. 4. Quantification of 8-oxodG in mtDNA from wt and OGG1 knockout animals. 8-oxodG levels were quantified in mtDNA extracted from livers of wt and ogg1$^{-/-}$ mice, using an HPLC system with EC detection. The results are presented as mean ± SD (*bars*) from two different experiments. Three to four livers for each group were collected for each experiment. Results from measurements of 8-oxodG levels in nuclear DNA from liver are shown for comparison. The *shaded area* along the *X axis* indicates artificial yields of 8-oxodG induced during DNA extraction and purification.

The activity measurements indicated that OGG1 is the only glycosylase for 8-oxodG removal in mouse mitochondria that is present in appreciable amounts. It was therefore of interest to investigate the endogenous levels of 8-oxodG in mtDNA from the null mutant mice. Using the standard technology of HPLC separation and EC detection, we found that mtDNA extracted from ogg1$^{-/-}$ mice contained almost nine times more 8-oxodG than mtDNA from wt animals (Fig. 4). Taking into account the problem of artificial introduction of 8-oxodG during DNA extraction and purification, it can be estimated that the factual increase of 8-oxodG is ~20-fold. These values compare with the observed increase of ~2-fold and a calculated increase of ~10-fold in the nuclear DNA (11). However, there is a striking increase in the levels of 8-oxodG in DNA from the mitochondria *versus* the nucleus in the null mutant mice (Fig. 4), indicating that OGG1 could be much more important for the removal of 8-oxodG from the mtDNA than from the nuclear DNA. It has been shown previously that a backup mechanism exists for 8-oxodG removal from nuclear DNA in ogg1$^{-/-}$ mice (11). This may represent some form of transcription-coupled repair (17). It seems possible that such a backup may not exist in the mitochondria and that BER initiated by OGG1 is the only pathway for the repair of 8-oxodG in mouse mitochondria.

We therefore conclude that the OGG1 protein exerts a pivotal role in the repair of oxidative damage in mammalian mitochondria.

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