Protection of Human Lung Cells against Hyperoxia Using the DNA Base Excision Repair Genes hOgg1 and Fpg

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Hyperoxia causes pulmonary toxicity in part by injuring alveolar epithelial cells. Previous studies have shown that toxic oxygen-derived species damage DNA and this damage is recognized and repaired by either human enzyme 8-oxoguanine DNA glycosylase (hOgg1) or *Escherichia coli* enzyme formamidopyrimidine DNA glycosylase (Fpg). To determine whether these DNA repair proteins can reduce O_2-mediated DNA damage in lung cells, A549 lung epithelial cells were transduced with either hOgg1 or Fpg using a retroviral vector containing enhanced green fluorescent protein. Expression of each gene in the transduced cells was confirmed by fluorescent microscopy, Northern blotting, Western blotting, and an enzymatic oligonucleotide cleavage assay. A549 cells expressing either hOgg1 or Fpg were protected from hyperoxia as evidenced by a decrease in DNA damage and a corresponding increase in cell survival. Further, we determined that overexpression of hOgg1 or Fpg partially mitigated the toxic effects of hydrogen peroxide in lung cells. Our data suggest that increased expression of DNA base excision repair genes might represent a new approach for protecting critical lung cells from the toxic effects of hyperoxia.

Keywords: oxygen toxicity; DNA damage; DNA repair; hydrogen peroxide

High concentrations of oxygen are often required to maintain normoxemia in acute lung injury; yet, hyperoxia is also a common cause of acute lung injury (1). Current evidence indicates that the damaging effects of oxygen are mediated by reactive oxygen species (ROS) such as the superoxide anion (O_2^-) (2–4). Oxygen and ROS can damage alveolar epithelial cells, leading to loss of the alveolar capillary barrier function (5). Mechanisms of oxygen-mediated lung injury include lipid peroxidation of cell membrane, structural damage of key proteins, and depletion of cellular reducing agents such as the reduced form of nicotinamide adenine dinucleotide phosphate (6).

Hyperoxia has been thought to be a cause of DNA damage and mutagenesis for decades (7, 8). More recent studies indicate that oxidant-mediated lung injury is associated with DNA damage as evidenced by an increase in p53 and p53-related gene products (9–11). Nonetheless, it is not clear how DNA damage and acute lung injury are linked and whether repair of acute DNA damage will reduce lung cell injury.

Oxygen and other oxidizing agents cause DNA single- and double-strand breaks that may result in cell death (12, 13). The main products of oxygen-induced DNA damage are 8-oxo-7,8-dihydro-2′-deoxyguanosine and imidazole ring–opened guanine (Fapy-Gua), followed by imidazole ring–opened adenine (Fapy-Ade) and cytosine glycol (14). Generation of oxidative DNA damage is prevented by antioxidant enzymes such as catalase (14) and superoxide dismutase (14) by reducing the amount and toxicity of ROS.

Eukaryotes possess a number of DNA repair enzymes that can rapidly reverse DNA damage. One of the key DNA repair enzymes involved in the base excision repair (BER) pathway in human cells to reverse oxidant-mediated DNA damage is 8-oxoguanine DNA glycosylase (hOgg1) (15, 16). hOgg1 is a functional, but not structural, analog of the *Escherichia coli* formamidopyrimidine-DNA glycosylase (Fpg) (17, 18). hOgg1 removes 7,8-dihydro-8-oxoguanine (8-oxoG) and other oxidative guanine modifications from nuclear and mitochondrial DNA. hOgg1 is present in mitochondria as well as in nuclei of human cells (19), as mitochondrial DNA is even more prone to oxidant-mediated damage than nuclear DNA is (20, 21). Wilson and colleagues have reported that BER protein Ogg1 can repair mtDNA damage in human cells (22). Deletion of the Ogg1 gene results in accumulation of 8-oxoG and an increase in mutational risk (23, 24). Fpg repairs 8-oxo purines, FaPy purines, and the imidazole ring–opened aminoethyl purines (25). Ogg1 deficiency in yeast, as well as Fpg deficiency in bacteria, results in a spontaneous mutator phenotype (26).

Although hOgg1 and Fpg are considered prime candidates for repair of oxidant-mediated DNA damage (27–31), it is not known whether these DNA repair proteins can protect mammalian lung cells from hyperoxia. Thus, we hypothesized that overexpression of hOgg1 or Fpg in lung cells might enhance resistance to hyperoxic injury. hOgg1 or Fpg was transduced into A549 alveolar epithelial cells using a retroviral vector pSF91. The cells transduced with either hOgg1 or Fpg were more resistant to hyperoxia when compared with vector control cells with less DNA damage, and enhanced cell survival. Further, overexpression of either hOgg1 or Fpg protected human lung cells from H_2O_2-induced DNA damage. These data suggest that rapid reversal of oxidant-mediated DNA damage may enhance resistance of lung cells to the toxic effects of hyperoxia.

METHODS

Construction of Vectors

The retroviral vector pSF91N, based on murine stem cell virus backbone, was a gift of Dr. C. Baum, University of Hamburg, Germany (32). The bicistronic retroviral vector pSF91 was essentially constructed as described previously (33), with an internal ribosome entry site (IRES) upstream to the gene expressing enhanced green fluorescence protein (EGFP).

hOgg1 was a gift from Dr. S. Mitra (University of Texas Medical School, Galveston, TX). The hOgg1–6 cDNA was amplified by polymerase chain reaction (PCR) by primers (5′-ATCGAATTCCAC-CATHCCTGCCTGCCCGCGCGCTTCTGCCCA-3′ and 5′-ATC-GTCGACTTAGCCTTACGGCCCTTTGGA-3′) to introduce a Kozak

sequence at the 5′ end of the gene to increase translation efficiency (34). This product was digested with *Eco*RI and *Not*I and then ligated, using T4 DNA ligase, into pSF91. After transformation into DH5α competent cells (Life Technologies, Gaithersburg, MD), colonies containing pSF91-hOgg1 were identified by PCR with the 5′-SF91 primer and the 3′-hOgg1–6 primer. The inserts were sequenced to confirm the integrity of the hOgg1–6 gene. The hOgg1–6 sequence is identical to that previously reported (15, 16), except that it lacks the 6 C-terminal codons (amino acids 317–322) of the wild-type gene. Eight micrograms of purified vector DNA was mixed with LipofectAMINE (Life Technologies) and transfected into phoenix-AMPHO cells. The viral supernatant was then used to infect the GP+E86 packaging cells. GP+E86 cells exhibiting high fluorescent intensity were selected as stable producer populations. Virus supernatant (titers $> 1 \times 10^6$ particles/ml) was filtered through a 0.45-μm Acrodisc filter (Gelman Sciences, Ann Arbor, MI), and the filtered supernatant was incubated overnight with A549 cells in a solution containing 10 μg/ml polybrene (Sigma, St. Louis, MO). After 36 hours, the infected cells were selected by fluorescence-activated cell sorting.

The Fpg gene was cloned from HB101 *E. coli* cells using PCR primers that introduced *Eco*RI and *Sal*I sites at the 5′ and 3′ termini of the gene, respectively (31, 33). The reaction conditions used for PCR were as described in the *Tfl* polymerase (Promega, Madison, WI) protocol. The PCR product and vector pGEX 4T-1 (Amersham Pharmacia Biotech, Piscataway, NJ) were digested with *Eco*RI and *Sal*I, and the purified DNAs were ligated using T4 ligase (Life Technologies). After confirming the DNA sequence of the insert, the Fpg cDNA within the pGEX4T-1 vector was amplified in another round of PCR to introduce a Kozak sequence at the 5′ end of the gene (primers as in the foregoing). This PCR product was digested with *Eco*RI and *Sal*I and ligated to linearized pSF91 vector using T4 ligase. The transient and stable producer cells were constructed and used to transduce A549 cells as in the foregoing.

Northern Blot Analysis

Total cellular RNA was isolated from cells (5×10^6) using the RNeasy Mini Kit (Qiagen, Valencia, CA) as per the manufacturer's instruc-tions. Total cellular RNA (15 μg) was separated in a 1.2% formamide, agarose gel containing 18% formaldehyde and transferred onto Hybond-N nylon membrane (Amersham Pharmacia Biotech) using a 10× standard saline citrate solution (1.5 M NaCl, 0.15 M sodium citrate). The membrane was prehybridized in 10 ml of Hyb-9 solution (Gentra Systems, Minneapolis, MN) for 1 hour. Full-length cDNA fragments of each gene were labeled with [α-^{32}P]dCTP (Amersham Pharmacia Biotech) using the Megaprime DNA Labeling System (Amersham Pharmacia Biotech) as per the manufacturer's protocol. After a 24-hour hybridization, the membrane was washed with low, medium, and high stringency washes, and visualized by autoradiography on Hyperfilm MP (Amersham Pharmacia Biotech).

Western Blot

Cell lysates were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10%), and then electroblotted to presoaked Immobilon-P membranes (Millipore, Bedford, MA) for 18 hours at 40 mA constant current in transfer buffer (192 mM glycine, 20 mM Tris–HCl, and 15% methanol). The membranes were blocked by soaking in 1.5% bovine serum albumin in TBS-T (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween-20) solution for 2 hours at room temperature. Each blot was incubated with rabbit polyclonal antibodies to hOgg1 (Novus Biologicals, Littleton, CO) at a dilution of 1:500 at room temperature for 1–1.5 hours. The membranes were briefly rinsed with deionized water and then washed five times with TBS-T (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 0.5% Tween 20) for 10 minutes per wash (35, 36). Secondary anti-rabbit peroxidase-conjugated antibodies (Sigma–Aldrich, St. Louis, MO) at 1:1,000 were then added and incubated with the membrane at room temperature for 45 minutes. Finally, the membrane was extensively washed as previously described and exposed on Kodak OMAT film using a chemiluminescence detection kit (Pierce, Rockford, IL).

Enzyme Activity Assay

The hOgg1 activity of transduced cells was quantified by detecting the cleavage of the 23-bp oligonucleotide substrate, containing a single 8-oxoguanine lesion 5′-GAA-CTA-GTGOATC-CCC-CGG-
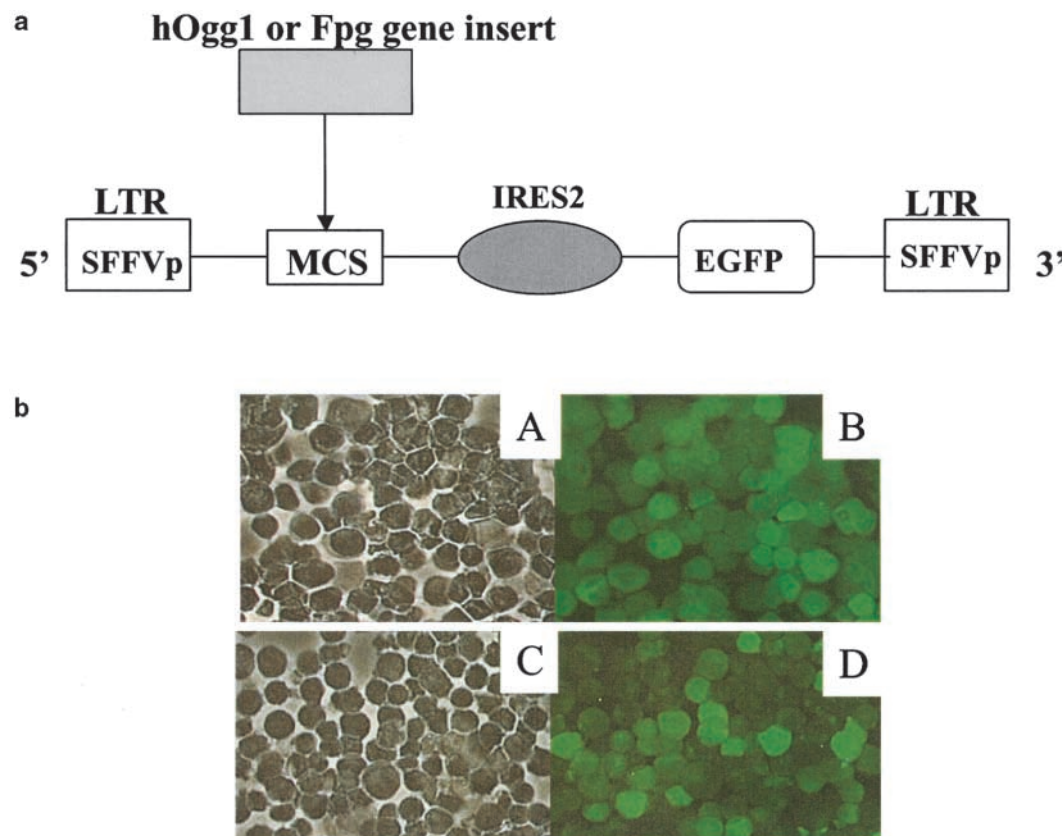

Figure 1. Construction and expression of retroviral vector containing the hOgg1 or *E. coli* Fpg coding regions. (*a*) Mammalian expression construct. From 5′-end: *LTR*, spleen focus-forming virus (*SFFV*), multiple clone sites with the mIFN-γ insert (*Eco*RI and *Sal*I sites), internal ribosome entry site, *EGFP*, and 3′-end *LTR*. The transcript contains EGFP, the vector's splice donor and splice acceptor sites, and BER gene. (*b*) Expression of EGFP in A549 cells. Phase contrast (*A, C*) and fluorescence (*B, D*) microscopy of retroviral-transduced A549 cells expressing EGFP/hOgg1 (*A, B*—population; *C, D*—Clone 10) (×400).

GCT-GC-3′ (Trevigen, Gaithersburg, MD). Cell lysates from control cells and cells transduced with hOgg1 (10-μg protein samples) were reacted separately with 0.1 pmol of γ-[^{32}P]-dATP end-labeled 23-bp substrate at 37°C for 1 hour in 150 μl of analysis buffer (10 mM *N*-2-hydroxythylpiperazine-*N*′-2-ethane sulfonic acid–KOH, pH 7.4, 10 mM KCl, 10 mM ethylenediamine tetraacetic acid [EDTA], and 0.1 mg/ml bovine serum albumin). The reaction was terminated by addition of 90% formamide loading buffer, and the samples were separated by denaturing sodium dodecyl sulfate polyacrylamide gel (20%) electrophoresis. The gel was dried and exposed on X-ray film. The relative fractions of full length and cut substrate were determined by densitometric analysis using Kodak Analysis Software (Kodak, Rochester, NY). Fpg activity was detected as described previously using a 26-bp 8-oxoG–containing oligonucleotide, 5′-AATTCACCGGTAC-COGTCTAGAATTCG-3′ (33, 37).

Hyperoxic Exposure

A549 cells were grown in Dulbecco's modified Eagle's medium supplemented with penicillin (100 U/ml)/streptomycin (100 μg/ml) and 10% fetal bovine serum, counted, and seeded on 100–mm^2 tissue culture dishes at a concentration of 8×10^4 cells/ml. Next day, the cells were subjected to hyperoxia in a NuAire Nu-3500 cell culture incubator (NuAire Inc., Plymouth, MN). The incubator was infused with 95% O_2–5% CO_2 for 12, 24, 48, 72, and 96 hours. Control A549 cells were incubated in room air–5% CO_2 for the same time intervals. O_2 concentrations were monitored with a mini Oxygen Ted 60T meter (Teledyne Analytical Instruments, City of Industry, CA). O_2 tension in culture medium was 647 ± 9 mm Hg (38). At each time point, the cells were harvested for assessment of DNA damage (Comet assay) and cell survival (colony forming assay).

H$_2$O$_2$ Exposure

A549 cells were plated into 60-mm dishes (five dishes) at a density of 3×10^5 cells/dish overnight. Next day, the cells were washed with phosphate-buffered saline (PBS) and incubated in the presence of H_2O_2 (0, 100, 200, 400, and 1,000 μM) at room temperature for 20 minutes. The cells were washed with PBS and used for assessment of DNA damage (Comet assay) and cell survival (colony forming assay).

Comet Assay

The Comet assay was performed according to the manufacturer's instructions using a CometAssay kit (Trevigen). Briefly, after exposure of cells to hyperoxia or H_2O_2, cells were washed with Ca^{2+}- and Mg^{2+}-free PBS (Trevigen) at a concentration of 3×10^5 cells/ml. The cell suspension was mixed with liquefied agarose at a 1:10 (vol/vol) ratio. A small aliquot of this mixture was immediately transferred onto the slide provided. After cell-lysis at 4°C, slides were treated with alkali solution (0.3 M NaOH, 1 mM EDTA) for 60 minutes to unwind the double-stranded DNA. Slides were electrophoresed at 1 volt/cm for 10 minutes. After staining with SYBR green dye, samples were visualized and photographed by fluorescent microscopy using an Olympus BX60 Microscope System and Paxit software (MIS, Franklin Park, IL). Tail length was defined as the distance between the leading edge of the nucleus and the end of the tail. Seventy-five determinations were made for each sample using Adobe Photoshop software (Adobe System Inc., San Jose, CA).

Colony Forming Assays

After exposure of cells to hyperoxia or H_2O_2 as described in previous sections, 1 ml of trypsin–EDTA (0.25%:1 mM) was added to each plate and incubated for 1 minute at 37°C. After the incubation, 5 ml of media containing fetal bovine serum was added and the cells were resuspended. Viability of the cells was determined by trypan blue (0.4% wt/vol) exclusion. The viable cells were then counted and plated at various concentrations in triplicate on 100-mm^2 tissue culture plates. After 10–14 days, the colonies were stained with 1% methylene blue in 50% ethanol and enumerated.

Statistical Analysis

Experiments were performed in triplicate and repeated at least three times. Student's *t* test and analysis of variance were performed, and significant difference was accepted at p < 0.05.

RESULTS

Analysis of Transgene Expression

To express DNA repair proteins in host cells, we subcloned the *E. coli* Fpg gene into pSF91 (Figure 1a) from pGEX 4T-1 Fpg. Vector pSF91, derived from murine stem cell virus backbone and LN-based retroviral vectors, was used for transduction of A549 cells and hematopoietic progenitor cells (31, 33) (Figure 1a). After selection for EGFP with fluorescence-activated cell sorting, more than 95% of the cells expressed EGFP (Figure 1b). The A549 cell population expressing EGFP (Figure 1b) was serially diluted to isolate single clones. Clone 10 is shown as representative of 27 clones selected (Figure 1b).

Northern blot analysis was performed to determine which clones contained actively transcribed transgenes and the relative expression level for each colony (Figure 2). hOgg1 transgene transcript is approximately 2 kb, which includes the vector's splice donor and splice acceptor sites (Figure 2a). hOgg1 expression was also confirmed by Western blotting (Figure 2b). Various amounts of hOgg1 expression were demonstrated, as expected, at a molecular weight of 39 kD. Finally, the successful expression of hOgg1 was displayed in an enzymatic cleavage activity assay (Figure 2c). Similarly, the expression of Fpg in A549 cells was detected by Northern blot (Figure 2d). Enhanced Fpg expression in A549 cells was also confirmed by enzyme cleavage activity assay (data not shown).

Protection from Hyperoxia

A population of hOgg1-expressing A549 cells showed less DNA damage compared with vector-transduced control cells (Figure 3A). As shown in a representative picture of the Comet assay, exposure to hyperoxia for 3 days increased the tail length in vector-transduced cells, but this was not seen in hOgg1-transduced cells (Figure 3B). Similarly, a population of A549 cells expressing Fpg was protected from hyperoxia (Figure
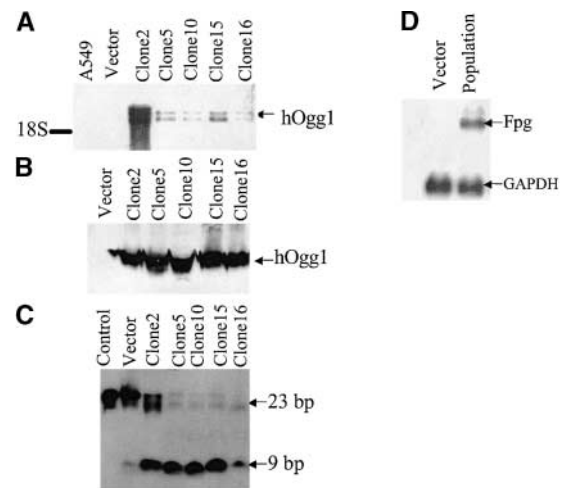

Figure 2. Expression and activity analysis of hOgg1-transduced A549 cells. (*A*) Northern blot analysis of hOgg1 mRNA. Clones 2, 5, 10, 15, and 16 expressing different amounts of hOgg1 mRNA. (*B*) Western blotting analysis of hOgg1 protein. Clones 2, 5, 10, 15, and 16 expressing different amounts of hOgg1 protein. (*C*) hOgg1 enzyme activity detected by oligonucleotide cleavage assay. *Control*, oligonucleotide without cleavages; Clones 2, 5, 10, 15, and 16 expressing different amounts of enzyme activity. Ten micrograms of cell-free extract reacted with 2.5 pM γ-[^{32}P]-dATP–labeled 8-oxoguanine–containing 23-bp oligonucleotide for 60 minutes at 37°C. The cleavage product of reaction was a 9-bp oligonucleotide. (*D*) Nouthern blot analysis of Fpg DNA expression.
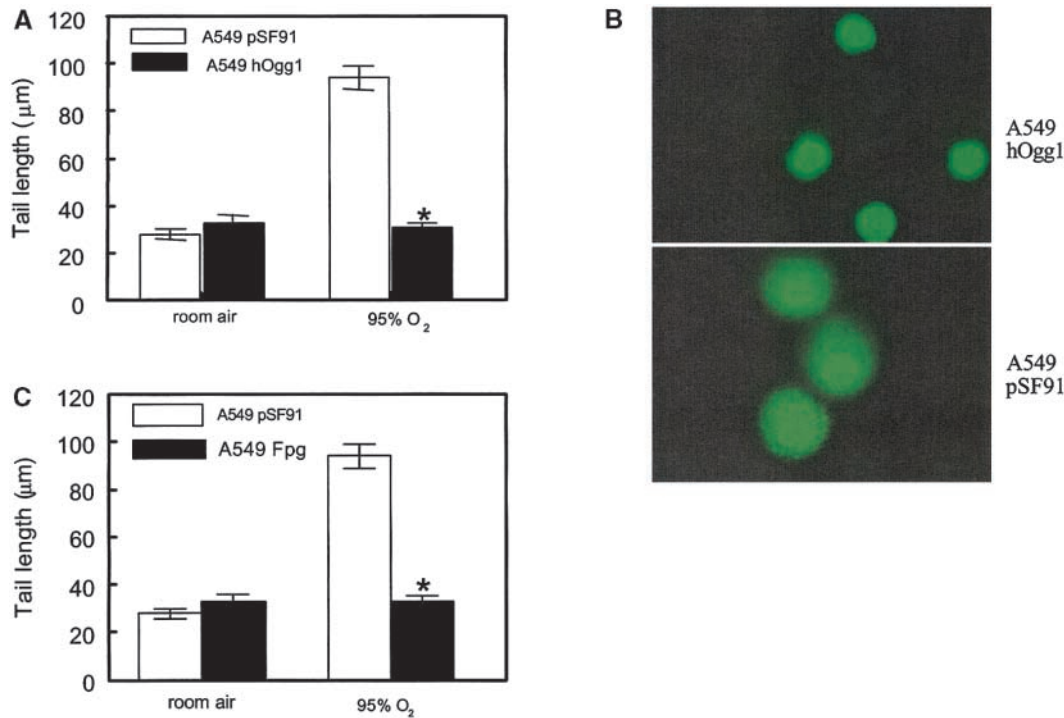
Figure 3. Effect of DNA BER protein expression in A549 cell population on O_2-induced DNA damage. A549 cells expressing either hOgg1 or Fpg were incubated in 95% O_2 or room air for 72 hours. DNA damage was measured by Comet assay, and tail length was defined as the distance between the leading edge of the nucleus and the end of the tail. (*A*) A549 cell population expressing hOgg1. (*B*) Comet assay showing decreased DNA damage in hOgg1-transduced A549 population. (*C*) A549 cell population expressing Fpg. *p < 0.05, compared with the vector control.
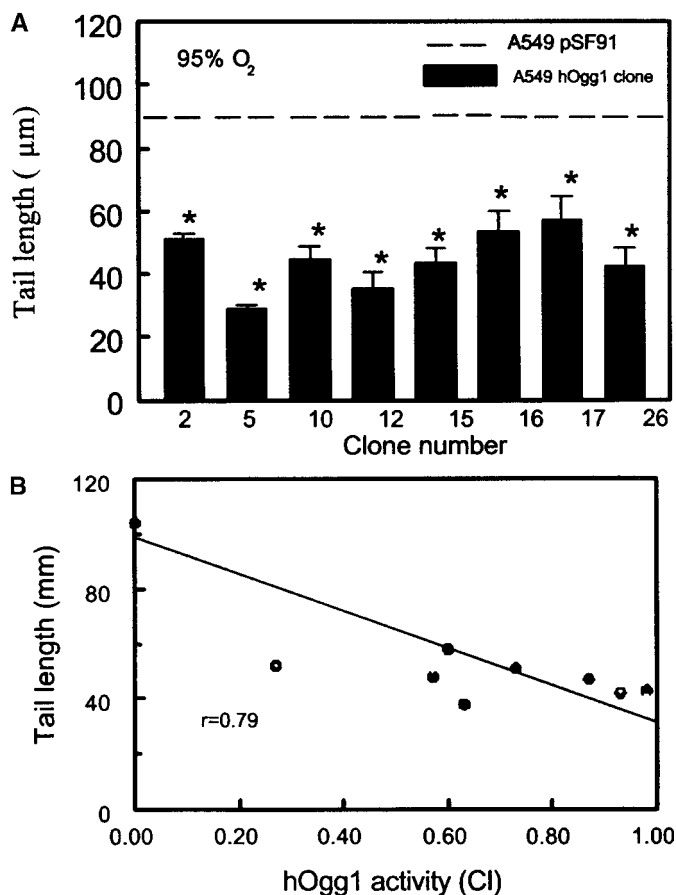

3C). We generated 27 A549/hOgg1 clones that variably expressed the transgene hOgg1. Eight clones (2, 5, 10, 12, 15–17, and 26) were selected that expressed different levels of the hOgg1 transgene; yet, each clone demonstrated significant protection from DNA damage when incubated in hyperoxia (Figure 4A).

We assessed the relationship between hOgg1 activity and resistance to DNA damage during hyperoxia. The results showed that the levels of hOgg1 DNA repair activity in various A549 cell clones correlated with resistance to hyperoxic DNA damage in A549 cells ($r = 0.79$, p < 0.05) (Figure 4B). Although a correlation exists, hOgg1 activity in an individual A549 cell clone does not reliably predict the degree of protection from hyperoxia.

A549 cells expressing either hOgg1 or Fpg demonstrated longer survival compared with the vector-transduced control cells (p < 0.05, Figures 5A and 5B). However, there were no significant differences between Fpg and hOgg1 in the ability to protect the cells from hyperoxia. Similarly, five hOgg1 clones (2, 5, 10, 15, and 16) expressing significantly different levels of the hOgg1 transgene demonstrated a similar ability to sustain cell survival during hyperoxia (Figure 6A). The relationship between hOgg1 activity and cell survival demonstrated only a marginal correlation ($r = 0.55$, p < 0.05) (Figure 6B). Further, the correlation between DNA damage and cell survival in the hOgg1 clones was also marginal ($r = 0.54$, p < 0.05).

Protection from H_2O_2

To assess whether overexpression of BER genes would afford protection from H_2O_2, DNA damage and cell survival were assayed in lung cells overexpressing hOgg1 or Fpg in the pres-

Figure 4. Effect of variable hOgg1 expression on DNA damage in hyperoxia. (*A*) Various A549 cell clones expressing different amounts of hOgg1 activity were incubated in 95% O_2 for 72 hours, and DNA damage was detected by Comet assay (*p < 0.05, compared with the vector control). (*B*) Relationship between hOgg1 enzymatic activity and resistance to hyperoxia. hOgg1 activity (oligonucleotide cleavage assay)

and DNA damage (tail length measured by the Comet assay) were compared in A549 cell clones with variable expression of hOgg1 proteins. The hOgg1 activity was expressed as a cleavage index (*CI*) indicating the ratio of cleaved oligonucleotide to total oligonucleotide.

ence or absence of H_2O_2. Overexpression of hOgg1 or Fpg significantly protected against DNA damage by H_2O_2 (0–1,000 μM) compared with the vector-transduced control cells (p < 0.05, Figures 7A and 7B). Further, overexpression of hOgg1 partially enhanced cell survival in the presence of H_2O_2 (200 and 1,000 μM) compared with the vector-transduced control cells (p < 0.05), whereas overexpression of Fpg did not enhance cell survival (Figures 7C and 7D). These data suggest that H_2O_2 might injure DNA of lung cells in a manner different from hyperoxia, and that the protective effects of BER proteins such as hOgg1 or Fpg on cell survival also differ.

DISCUSSION

We report a successful reduction in oxygen toxicity to lung cells by overexpression of BER repair proteins in these cells. Using an improved bicistronic retroviral vector (33, 39), we were able to transduce lung epithelial cells with either hOgg1 or Fpg DNA to achieve protein overexpression with each transgene. Overexpression of hOgg1 or Fpg protected the lung cells from hyperoxia-induced injury as demonstrated by either DNA damage or cell survival assays. This is the first study to show that overexpression of DNA BER proteins can be protective from oxygen toxicity in lung cells.

High concentrations of oxygen likely damage DNA of lung cells by forming an array of reduced ROS (9, 13). Evidence that DNA is a target for this damage is provided by the increase in DNA damage–inducible proteins such as p53 and p21 in the lung during hyperoxia (10, 13, 40). In a manner similar to hyperoxia, bleomycin induces p53 and p21 expression, and this has been localized to type II alveolar epithelial cells in the lung (11). Of interest, hyperoxia is a frequently suspected

cofactor in clinical bleomycin toxicity (41). Bleomycin generates DNA lesions typical of the injury/repair cycle associated with oxidant damage (42). Widespread injury to the alveolar epithelium by hyperoxia or drugs may jeopardize the integrity of the lung. Type II cell injury and death is a catastrophic event for the alveolar unit as the type II cell is the progenitor of the type I alveolar epithelial cell (43) and is the major source of pulmonary surfactant proteins (44). New strategies are needed to protect alveolar epithelial cells from O_2 toxicity. Our study suggests one approach to achieving protection is to rapidly reverse O_2-induced DNA damage (Figure 8).

Recognition and removal of 8-oxoguanine residues from DNA is dependent on similar enzymes in bacterial and eukaryotic cells. The Fpg and hOgg1 DNA glycosylases recognize and initiate repair of 8-oxoguanine and formamidopyrimidine lesions produced by oxidative and alkylative DNA damage, respectively. The BER genes release the modified base from the sugar molecule of a nucleotide leaving an apurinic/apyrimidinic (AP) site in the DNA (45). After release of the damaged bases, downstream enzymes, including AP endonucleases (Ape1), DNA polymerase-β, and DNA ligase, continue the repairing process by filling and sealing the gap (26, 45).

Both hOgg1 and Fpg have AP lyase activity for removing 5'-terminal deoxyribose phosphate flanking the DNA strand breaks next to abasic sites (46), although Fpg is more active in this respect than hOgg1 (47). Recent studies have shown that hOgg1 is a strong glycosylase but a much weaker AP lyase (47). It has been suggested that hOgg1 acts with other members of the BER pathway, namely the major AP endonuclease (Ape1/ref-1) or β-polymerase, to augment its inherent low lyase activity during hydrolytic processing of the AP site (47). This has been confirmed for the human T–G mismatch glycosylase that is dislocated by Ape1/ref-1 (48). However, enzymes downstream to hOgg1 may be rate-limiting in human
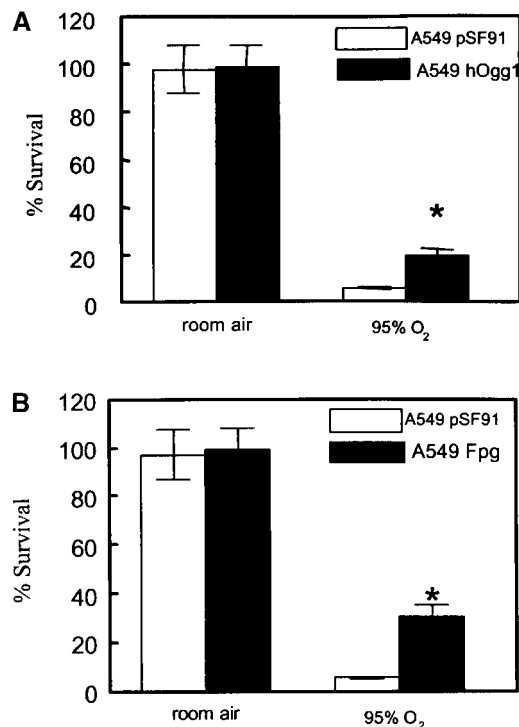

Figure 5. Effect of DNA BER protein expression on cell survival. A549 cells expressing hOgg1 or Fpg were incubated in 95% O_2, and cell survival was measured by colony forming assay. Cell survival was expressed as percentage of surviving colonies in hyperoxia to surviving colonies in room air. (A) A549 cell population expressing hOgg1. (B) A549 cell population expressing Fpg. *p < 0.05, compared with the vector control.
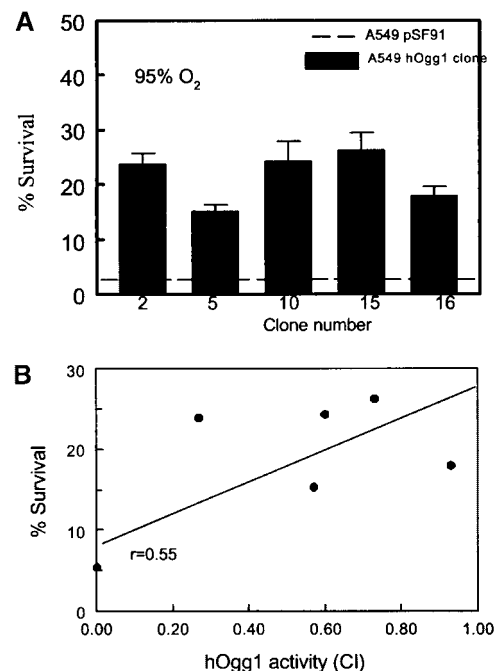

Figure 6. Effect of variable hOgg1 expression on cell survival in hyperoxia. (A) A549 cell clones expressing different amounts of hOgg1 were incubated in hyperoxia, and cell survival was measured by colony forming assay (*p < 0.05, compared with the vector control). (B) Relationship between hOgg1 enzymatic activity and resistance to hyperoxia-induced toxicity (r = 0.55, p < 0.05).
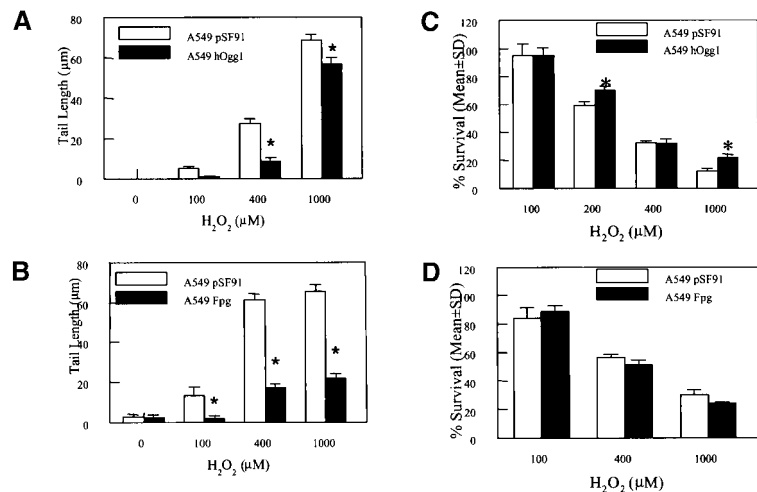
Figure 7. Protection from hydrogen peroxide–induced toxicity. Detection of DNA damage by the Comet assay: (*A*) A549 cell population expressing hOgg1. (*B*) A549 cell population expressing Fpg. Detection of cell survival by the colony forming assay: (*C*) A549 cell population expressing hOgg1. (*D*) A549 cell population expressing Fpg. *$p < 0.05$, compared with the vector control.

cells (31, 49). It would be interesting to determine if increased expression of these downstream enzymes (Ape1/ref-1 and/or β-polymerase) will augment cell survival with coexpression of either hOgg1 or Fpg (31).

Our studies also demonstrate that cells overexpressing hOgg1 or Fpg were protected from the cytotoxic effects of exposure to H_2O_2. Among various lesions associated with oxidative DNA damage, 8-oxoG is the most important because of its abundance and mutagenicity. 8-oxoG and formamidopyrimidine are both substrates for Fpg and hOgg1 (50); however, recent studies have shown that the rate of excision of 7-methyl-formamidopyrimidine by hOgg1 is less than that of Fpg (51). hOgg1 and Fpg exhibit both *N*-glycosylase and 3′ and 5′ β-lyase activities for the removal of deoxyribose phosphate. Therefore, protection against H_2O_2 as evidenced in our study might be mediated by these other activities.

Although exposure to H_2O_2 is often used as a surrogate for ROS damage generated during hyperoxia, it is unclear if H_2O_2 causes a similar type of DNA damage. Studies in Chinese hamster ovary cells revealed that H_2O_2 produces mutagenic single-strand DNA damage, whereas hyperoxia causes sister chromatid exchanges and other chromosome aberrations (52). Iron chelators enhance survival of cells exposed to H_2O_2, but not when exposed to hyperoxia (53). The toxicity of H_2O_2 is probably dependent on the formation of hydroxyl radicals ($^\bullet$OH) through the Fenton reaction, as scavengers of ROS reduce $^\bullet$OH formation and prevent the lethal action of H_2O_2 (13, 54). However, hydrogen peroxide has direct effects on redox-signaling pathways, independent of hydroxyl radical formation (55).

Although overexpression of BER proteins in lung cells protected from hyperoxia-induced DNA damage, it appears that BER proteins were less effective in protecting the cells from cell death. These findings are consistent with a role for DNA damage to be a contributing but not sole mechanism of hyperoxia-induced cell death. Apoptosis is generally considered to be the predominant mode of cell death from oxidative insults (56), which is controlled by complex signaling involving many regulatory proteins such as DNA damage–inducible proteins, proinflammatory factors, and cell cycle regulation kinases (57). However, necrosis during acute lung injury is also observed, particularly in A549 cells under hyperoxic condition (58), suggesting a dual mode of cell death (59). Other factors such as cell types and confluence state may determine the mode of cell death (60). Thus, we speculate that enhanced expression of BER proteins in A549 cells may protect from one type of hyperoxia-induced cell injury but not another. A limitation to the use of A549 cells is that they might also not accurately reflect the degree of DNA damage or repair that occurs to alveolar epithelial cells *in vivo*.

In summary, we have been able to successfully reduce hyperoxia-induced DNA damage to lung cells by overexpression of DNA BER protein hOgg1 or Fpg. This is the first study to demonstrate that DNA BER proteins have protective effects on reducing O_2 toxicity in lung cells. Our data suggest that increased expression of DNA BER genes might represent a new approach for protecting critical lung cells from the toxic effects of hyperoxia.
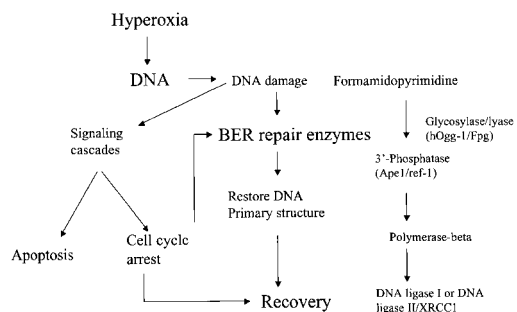
Figure 8. Hyperoxia: possible mechanisms of DNA damage and repair.

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