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Increased resistance to oxidative DNA damage of trabecular meshwork cells by *E. coli FPG* gene transfection

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Abstract

Oxidative damage plays a pathogenic role in various chronic degenerative diseases. Oxidative damage targeting trabecular meshwork (TM) cells as a consequence of mitochondrial damage is a pathogenic mechanism for glaucoma, the most common cause of irreversible blindness worldwide. Consequences of oxidative damage are attenuated by endocellular activities involved in scavenging reactive oxidative species and DNA repair. Selected bacterial genes are highly efficient at protecting cells from oxidative DNA damage. This situation occurs for *Escherichia coli* formamidopyrimidine DNA glycosylase (FPG), a major DNA glycosylase that repairs oxidatively damaged DNA. Accordingly, this study was aimed at transfecting human TM cells (HTMC) with Fpg in order to increase their resistance to oxidative damage. This study demonstrates that it is feasible to increase resistance of HTMC to endogenous oxidative damage by gene transfection. These findings bear relevance for primary and secondary prevention of degenerative glaucomas and other degenerative diseases where oxidative damage plays a pathogenic role.

Keywords: *Oxidative DNA damage, DNA repair, cell transfection, glaucoma, gene therapy*

Abbreviations: *AP, abasic; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; FapyA, 4,6-diamino-5-formamidopyrimidine; FPG, formamidopyrimidine DNA glycosylase; HTMC, human trabecular meshwork cell(s); IOP, intraocular pressure; OD, oxidative DNA damage; 5-OH-dC, 5-hydroxy-2'-deoxycytidine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; PBS, phosphate buffered saline; POAG, primary open-angle glaucoma; ROS, reactive oxygen species; TM, ocular trabecular meshwork.*

Introduction

Many degenerative diseases including cancer, cardiovascular and neurological diseases develop with age. Most are caused by oxidative stress, resulting in accumulation of mutations in nuclear and/or mitochondrial DNA that progressively cause cell loss through apoptosis or necrosis [1].

Oxidative DNA damage (OD) is an inevitable consequence of endogenous cellular metabolism in aerobic cells. Furthermore, exogenous exposure to toxic agents can contribute to its increase. Levels of oxidized nucleotides in DNA are a consequence of the balance between lesion induction by free radicals and repair. Pathological consequences of oxidative damage vary depending on the targeted cell type [2]. Whenever the target tissue is composed of proliferating cells, as occurs in epithelia, OD represents a pathogenic step towards cancer. Whenever the target tissue is composed of non-proliferating perennial cells, as occurs in neural and vascular tissues, OD represents a pathogenic step towards degenerative diseases.

Oxidative damage to the trabecular meshwork (TM) exerts a pathogenic role in glaucoma, inducing mitochondrial damage and triggering apoptosis and cell

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loss [3]. Glaucoma is a multi-factorial neurodegenerative disease characterized by loss of the retinal ganglion cell axons leading to progressive loss of vision. This disease causes loss of specific cell types through apoptosis in three separate areas: the anterior chamber of the eye, the retina and the central nervous system. Specific glaucoma targets are: (1) the trabecular meshwork endothelial cells in the ocular anterior chamber [4]; (2) the ganglion cells in the retina [5] and (3) the lateral geniculate nucleus, the intracranial optic tract and neurons of the visual cortex in the central nervous system [6]. Among these events, OD in the anterior segment of the eye has a leading role in glaucoma pathogenesis [7]. Aqueous humour is produced by the ciliary body and flows out from the eye through active drainage exerted by the TM, which is a target tissue for glaucoma pathogenesis. The TM consists of trabecular lamellae covered by endothelial cells in front of a resistor consisting of juxtacanalicular cells and the inner wall of Schlemm's canal. TM endothelial cells play a key role in regulating passage of aqueous humour from the anterior chamber to the venous district. The tri-dimensional shape of the TM reflects the need to increase the area of contact between TM endothelial cells and the aqueous humour in which they are immersed [8]. These cells have many functions, the most important being that, through a system of cytokines, they control permeability of the TM by regulating the function and shape of endothelial cells lining the lumen of Schlemm's canal [9]. In the case of TM endothelial cell malfunction, the regular outflow of aqueous humour through Schlemm's canal is impeded and consequently the intraocular pressure (IOP) may rise to abnormal levels [10]. Elevated IOP is a major risk factor for open-angle glaucoma [11].

Many factors contribute to TM malfunction. Cyclic mechanical stress as induced by significant IOP variations activates many genes that may influence the aqueous humour outflow facility, extracellular matrix synthesis, cytoskeletal organization and cell adhesion [12]. The level of deleted mitochondrial DNA in glaucomatous TM is remarkably high, thus contributing to TM degeneration in primary open-angle glaucoma (POAG) [13]. These factors result in the age-related decline of TM cellularity that specifically affects the filtering TM area in the glaucomatous eye [14]. It is likely that OD occurring in the TM has a pathogenic role in glaucoma. OD is dramatically increased in the TM of glaucomatous patients as compared to unaffected controls [4] and the TM has been demonstrated to possess the highest susceptibility to free radicals as compared to other tissues of the ocular anterior chamber [15]. From this perspective, it is noteworthy that some anti-glaucoma drugs bear antioxidant properties [8,16,17].

The main endogenous reactive oxygen species (ROS) source is mitochondria, which are also involved in activation of the intrinsic apoptotic pathway whenever OD reaches a critical level. In the TM of glaucomatous patients, DNA damage caused by ROS results in guanine oxidation giving rise to a dramatic increase in 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) [4]. This high OD level involves mitochondria causing dramatic increases in the level of mitochondrial DNA deletions (the common 4977 mtDNA deletion) resulting in apoptosis activation and cell loss [13]. Even if the defense mechanisms of HTMC against ROS are insufficiently characterized, the peculiar sensitivity of TM to oxidative damage likely represents a major mechanism contributing to age-related TM cell loss resulting in progressive anterior chamber outflow difficulties [15]. Hence, it would be useful to counteract the course of glaucoma by protecting TM endothelial cells from OD, which is the aim of the study presented here. *Escherichia coli* formamidopyrimidine DNA glycosylase (FPG) is a major DNA glycosylase repairing oxidatively damaged DNA [18]. This protein is a globular monomer that possesses combined DNA glycosylase-abasic (AP) site lyase activities. FPG rapidly releases oxidized purines such as 8-oxo-dG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 4,6-diamino-5-formamidopyrimidine (FapyA) from DNA and the resulting AP site is incised by the associated beta, delta-AP lyase activity [19]. FPG can repair oxidized pyrimidines such as 5-hydroxy-2'-deoxycytidine (5-OH-dC) and dihydrouracil as well, although at a lower rate when compared to oxidized purines [20]. Fpg transfection has been proposed as a possible tool to protect cells from OD [21]. The purpose of the present work is to verify the possibilities offered by Fpg transfection for making TM endothelial cells resistant to oxidative injury.

Materials and methods

Cells

Primary human trabecular meshwork cells (HTMC, ScienCell, San Diego, CA) were cultured in fibroblast medium (FM, ScienCell), 2% foetal bovine serum (FBS, ScienCell), fibroblast growth supplement (FGS, 1%, ScienCell) and penicillin/streptomycin solution (P/S, 1%, ScienCell) using poly-L-lysine-coated flasks. HTMC transfection efficiency was compared with that of T24 cells (American Type Culture Collection, Manassas, VA) derived from a bladder carcinoma; these cells were selected due to their established ability to be transfected by Fpg [22]. T24 cells were cultured in D-MEM containing 10% foetal calf serum. Medium for transfected cells was supplemented with 800 μg/ml geneticin (G418).

EGFP-FPG expression vector and cell transfection

The pEGFPC1-FPG expression vector [22] was used for cell transfection. In this vector, the FPG gene is

expressed as a fusion to the C-terminus of the fluorescent protein EGFP (excitation = 488 nm, emission = 507 nm). Cells transfected with the pEGFPC1 plasmid alone (vector only) were used as controls.

Transfection of vectors was performed with Effectene reagent (Qiagen) or Nucleofector® technology (Amaxa, Lonza) according to the manufacturer's instructions with minor modifications. In the Effectene procedure, 200 000 cells were seeded into 60 mm dishes. After 24 h, a mixture of 1 μg linearized vector DNA, 8 μl Enhancer and 25 μl Effectene reagent was added to the dishes (1:25 DNA/Effectene ratio). Cells were allowed to take up vector for 42 h. Various vector DNA/Effectene ratios (1:10, 1:50) and vector amounts (2 μg) were also tested. In some experiments, transfected cells were exposed to a low concentration (50 μg/ml) of G418 for 5 days to enrich in EGFP-FPG-expressing cells. Transfection using a Nucleofector® device (Amaxa, Lonza) was performed by harvesting HTMC from a 90% confluent 25 cm^2 flask. Cells were collected by centrifugation and the pellet was resuspended in 100 μl Nucleofector® solution with supplement and combined with 2 μg linearized vector DNA. The cell/DNA suspension was transferred to a Nucleofector® cuvette and the A-034 and U-001 Nucleofector® programmes applied.

Transgene expression analysis and cell sorting

To identify transfected cells, cell fluorescence was analysed under an inverted fluorescence microscope and images were taken with a Cell-R imaging system (Olympus Biosystems GMBH, Planegg, Germany). For fluorescence cell sorting, cells were harvested by trypsinization, re-suspended (10^6 cells/ml) and sorted using a FACSAria system (BD Biosciences). Data were analysed by the FACS DIVA 5.0 software. Sorted cells were collected by centrifugation, washed twice in phosphate buffer (PBS) and stored at −80°C. Transfected HTMC were analysed at the end of the transfection procedure while transfected T24 cells had been cultured for 90 days prior to fluorescence analysis.

Evaluation of DNA oxidative damage by endonuclease digestion and capillary electrophoresis

OD was evaluated in control (vector only) and transfected (vector + Fpg) HTMC in terms of formation of AP sites. AP sites were evaluated by digestion with Endonuclease IV, an enzyme that specifically catalyses formation of single strand breaks at AP sites. Fragmentation of digested DNA was determined by alkaline capillary electrophoresis. Endonuclease IV treatment was performed by adding a mixture composed of 0.1 U Endonuclease IV (Fermentas International Inc, Burlington, Canada), 1 μl 10 × reaction buffer and sterile molecular grade water in a final volume of 10 μl to HTMC DNA (100 ng), then incubating for 30 min at 37°C. Reactions were terminated by heating for 15 min at 80°C. Digested samples were lyophilized and resuspended in 4 μl 20 mM EDTA, pH 12.25 then incubated for 30 min at room temperature. DNA samples underwent capillary electrophoresis in an Agilent 2100 Bioanalyser (Agilent Technologie, Waldbronn, Germany) using a DNA 12000 chip. The chip was prepared by filling each well with 9 μl gel-dye mix (1 μl Agilent DNA dye concentrate in 60 μl filtered gel matrix), 5 μl of marker. DNA (25 ng in 1 μl) was added to each sample well and ladder (1 μl) was added to a separate well. The chip was then vortexed for 1 min at 2400 rpm and run in the Agilent 2100 bioanalyser. An electric field was applied between each well and the microchannels so that the DNA was electrophoretically driven by a voltage gradient and the molecules were separated by size. The molecular sizes that can be analysed with the DNA 12000 chip range between 100–12 000 bp. The dye intercalated with the DNA was detected by laser-induced fluorescence. Data were translated into gel-like images and electropherograms. The integration function of the Bioanalyser software was not applicable in these experiments because no defined peaks were identified, as expected. The signal was inferred by the analysis of a smear due to a wide distribution of the fragment of DNA obtained from enzymatic digestion. Analyses of the gel-like images (reflecting the electropherogram) as obtained from the Bioanalyser output were thus performed with ImageJ software (Wayne Rasband, National Institute of Health, Bethesda, MD, USA). For each lane, a rectangular area corresponding to an elution time interval of 30–60 s (<700 bp as inferred by comparison with the reference ladder) was analysed and the average intensity signal was recorded. This area includes fragmented DNA resulting from endonuclease digestion, thus corresponding to AP sites.

Detection of 8-oxo-dG by ^{32}P post-labelling

8-oxodG was analysed in HTMC by trifluoracetic acid enrichment, ^{32}P-post-labelling, monodirectional thin layer chromatography and electronic autoradiography as previously described [4,23]. DNA (1–3 μg) is depolymerized to 3'-monophosphate nucleotides by incubation with micrococcal nuclease (0.14 U/μg DNA) and spleen phoshpodiesterase (1 mU/μg DNA) at 37°C for 3.5 h. Unmodified dGp nucleotides are selectively removed by incubation with 80% v/v trifluoracetic acid (30 μl) for 10 min at room temperature and 8-oxo-dG labelled by incubation with T4 plasmid polynucleotide kinase (8 U) in the presence of AT-gamma-^{32}P (64 μCi, specific activity ≥750 Ci/mmol) (ICN, Irvine, CA). ^{32}P labelled 8-oxo-dG is purified from the reaction mixture by a monodirectional thin layer in

1.5 M formic acid and identified by electronic autoradiography (Instant Inmager, Packard, Meriden, CT). Positive reference standards were obtained by incubating calf thymus DNA with 1 mM $CuSO_4$ and 50 mM H_2O_2 or using an authentic 8-oxo-dG reference standard (National Cancer Institute Chemical Carcinogen Reference Standard Repository, Midwest Research Institute, Kansas City, MO). DNA-free samples were used as negative control.

Results

Transfection efficiency in HTMC

Figure 1 shows the expression of the fusion protein EGFP-FPG in the human bladder carcinoma cell line T24 and primary HTMC. This fusion protein retains full FPG DNA repair activity as demonstrated by the significantly increased 8-oxodG and AP site repair activities of a number of human cells lines expressing EGFP-FPG [22,24,25]. The increased 8-oxodG and AP site repair capacities of EGFP-FPG-expressing cells can be reproducibly detected in both intact cells and cell-free extracts [22,24,25]. Both T24 and HTMC displayed no background fluorescence on the inverted microscope (top row). After transfection with pEGFP-C1 vectors, clones with elevated EGFP expression could be isolated in T24 cells (T6, middle row, left), while no such clone could be recovered in HTMC. We then tried to concentrate the few EGFP-expressing cells among HTMC by FACSAria sorting, obtaining a limited but detectable enrichment in fluorescence (Figure 1, HTMC-EGFP, middle row, right). Expression of the fusion protein EGFP-FPG is lower than EGFP in clones isolated from the transformed cell line T24 (TFC vs T6, bottom and middle rows, left). Minimal expression of the fusion protein EGFP-FPG (below the detection limits of fluorescence microscopy) occurred in FACSAria-sorted HTMC (HTMC-EGFP-FPG, bottom row, right).

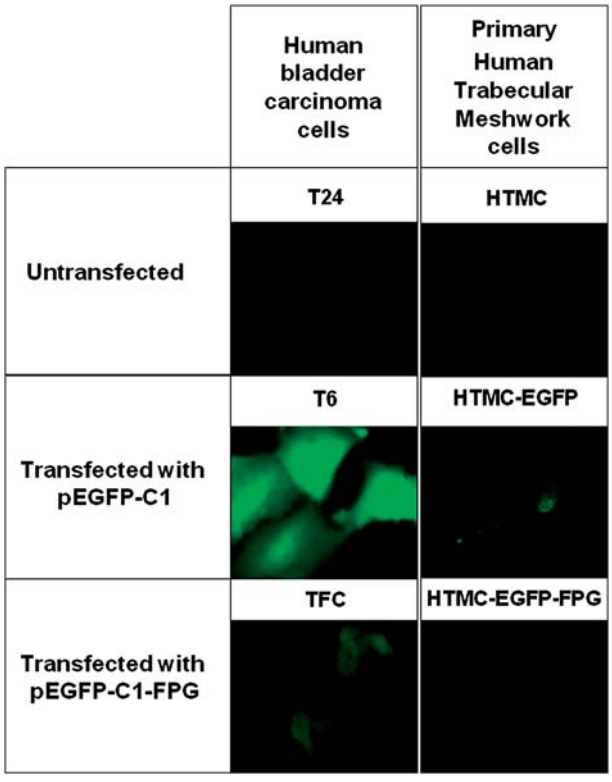

Figure 1. FPG expression in T24 cells and HTMC. T24: human bladder carcinoma cells. T6: T24 cells transfected with the pEGFP-C1 vector (vector only) and expressing the EGFP tag. TFC: T24 cells transfected with the pEGFP-C1-FPG vector and expressing the fusion protein EGFP-FPG. HTMC: primary human TM cells. HTMC-EGFP: HTMC transfected with the pEGFP-C1 vector and expressing the EGFP tag. HTMC-EGFP-FPG: HTMC transfected with the pEGFP-C1-FPG vector and expressing the fusion protein EGFP-FPG.

Figure 2 shows the FACSAria analysis of unsorted HTMC expressing EGFP (left) or EGFP-FPG (right). Only 0.17% HTMC-EGFP cells and 0.09% HTMC-EGFP-FPG cells were fluorescent, with mean fluorescence intensities of 13 270 and 16 353 fluorescence units, respectively (Figure 2, bottom, highlighted values). In control experiments with the transfected T24 cell line, 58.99% T6 cells (T24 cells expressing EGFP) and 1.36% TFC cells (T24 cells expressing EGFP-FPG) were fluorescent, with mean fluorescence intensities of 9993 and 2988, respectively (data not shown).

Influence of Fpg transfection on oxidative DNA damage

Fragmented DNA resulting from endonuclease digestion at AP sites was detectable by alkaline capillary electrophoresis in both control and Fpg-transfected HTMC samples analysed. This fragmented DNA reflects the extent of basal oxidative damage in HTMC under physiological conditions. The fragmented DNA in control is the product of digestion of Endo IV on the AP sites induced by basal endogenous oxidative stress [26,27]. FPG is a bifunctional glycosylase acting both as a glycosylase and a lyase, thus allowing for AP site repair. As a matter of fact a significant difference in fragmented DNA quantity was observed when comparing control HTMC transfected with pEGFP-C1 vector only (Figure 3, lanes 2 and 3) to HTMC effectively transfected by the pEGFP-C1-FPG vector, as selected by FACSAria (Figure 3, lanes 4 and 5). The amount of DNA fragmentation induced by Endo IV at AP sites decreased by 36.2% ($p < 0.05$) in EGFP-FPG-positive cells as compared to control cells. Reporting the amount of fragmented DNA in a colour scale, this protective EGFP-FPG effect results in a dramatic decrease of red colour in the area of damaged DNA (Figure 3).

The levels of oxidative DNA damage in terms of 8-oxodG in HTMC either naïve or after FPG transfection are reported in Table I and Figure 4. The level of 8-oxodG under basal conditions is quite high

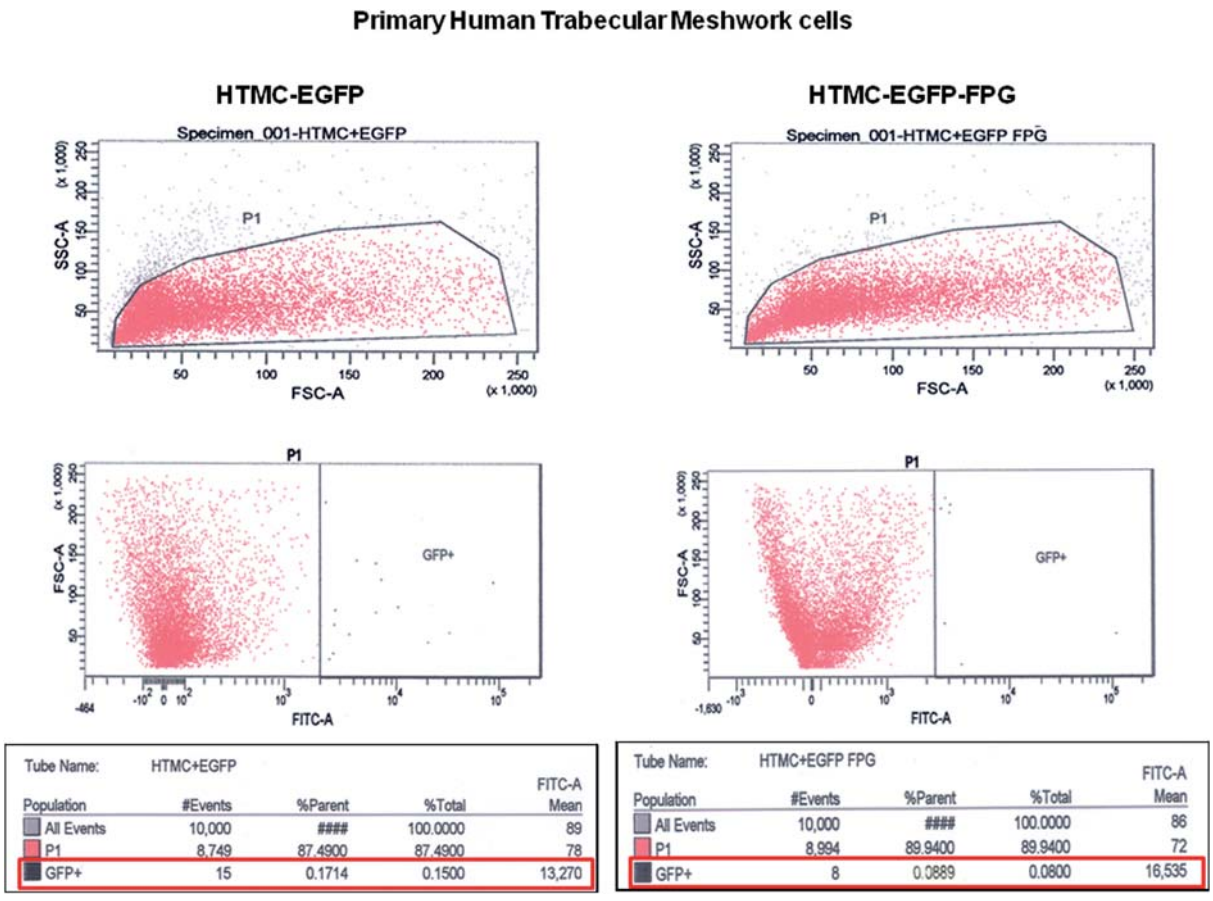

Figure 2. Fluorescence of HTMC transfected with pEGFP-C1 (left) and pEGFP-C1-FPG (right) vectors as determined by FACS. Cells were harvested by trypsinization, resuspended in physiological saline and analysed using a FACSAria cytofluorimeter. Data were analysed by the FACS DIVA 5.0 software. Effectively transfected cells are included in the GFP + square. Relevant data are highlighted by a red frame (bottom row).

(mean ± SD: 3.49 ± 0.33 8-oxodG/10^5 nucleotides; Table I and Figure 4, lanes 1–3), a finding amenable to the high susceptibility of TM to oxidative DNA damage under basal conditions [15]. Following Fpg transfection, 8-oxo-dG was significantly decreased to 2.29 ± 0.58 8-oxodG/10^5 nucleotides (Table I) and a further decrease to 1.16 ± 0.22 8-oxodG/10^5 nucleotides (Table I and Figure 4, lanes 4–6) was observed after enrichment of FPG-expressing cells by exposure to 50 μg/ml G418. Hence, although the overall achievable expression of EGFP-FPG in HTMC was low, it was yet sufficient to significantly decrease the levels of spontaneous oxidatively-damaged DNA.

Discussion

Reports have indicated that HTMC are quite resistant to cell transfection by gene vectors as compared to other cell types. Nevertheless, Fpg transfection is quite efficient in decreasing the basal rate of OD in the small fraction of effectively transfected cells.

Repair of oxidized purines such as 8-oxo-dG is inefficient in human cells in comparison to repair of other major endogenous lesions (e.g. uracil, AP sites or oxidized pyrimidines) [28]. This is due to the poor catalytic properties of hOGG1, the major DNA glycosylase involved in 8-oxodG removal. *E. coli* FPG protein differs from its human counterpart hOGG1 in several aspects. Unlike the well-formed and tight lesion-accomodating pocket of hOGG1, the FPG pocket is flexible and highly dynamic, thus allowing FPG to excise a wide repertoire of damaged bases [20]. For instance, while hOGG1 only excises 8-oxoG and FapyG from DNA, FPG rapidly releases 8-oxoG, FapyG and FapyA with similar excision kinetics [29]. The ability to excise FapyA is thus a major substrate difference between the two enzymes. Furthermore, FPG can remove oxidized pyrimidines (5-OHdC and dihydrouracil) from DNA, although at a lower rate as compared to oxidized purines [20]. Although the N-terminal of FPG is involved in the catalytic excision of oxidized bases from DNA [30], recent work has shown that fusion of the EGFP protein to the FPG N-terminal does not significantly affect the 8-oxodG and AP site repair capacities of the FPG protein, as demonstrated by enhanced DNA repair of 8-oxodG, 5-OHdC and AP sites after expression of EGFP-FPG in human cells [22,24,25]. Consistently, the mutation frequency induced by oxidizing agents at the Na^+-K^+ ATPase locus in human cells

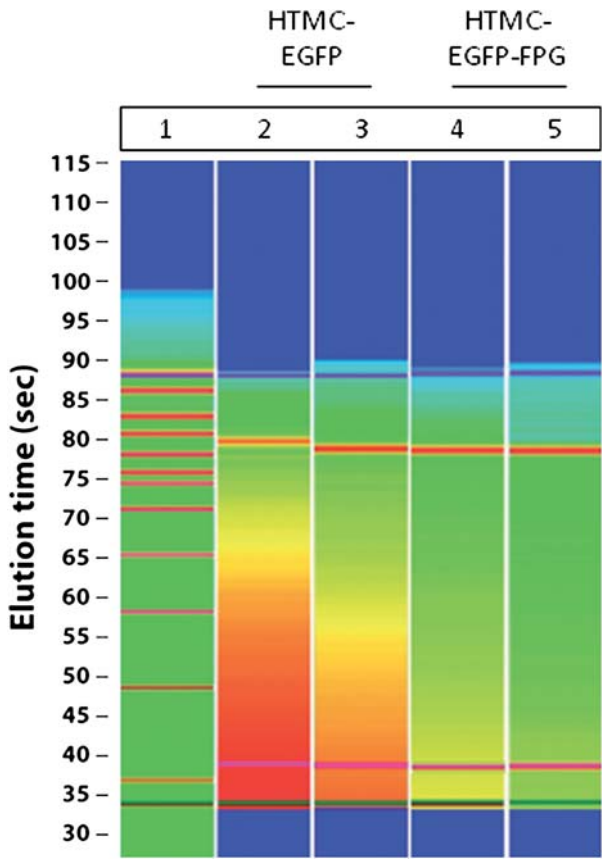

Figure 3. DNA fragmentation as evaluated by detection of AP sites (endoIV digestion) and alkaline capillary electrophoresis. DNA amounts are reported on colour scale (red high, green low). Lane 1, ladder; Lanes 2–3, HTMC transfected with pEGFP-C1 vector and expressing the EGFP tag (HTMC-EGFP); Lanes 4–5, HTMC transfected with pEGFP-C1-FPG vector and expressing the fusion protein EGFP-FPG (HTMC-EGFP-FPG). Numbers in the first column on the left indicate the elution time (s). Fragmented DNA (<700 kB), eluting after 30–60 s, is lower in EGFP-FPG transfected cells (lanes 4–5) than in vector-only transfected cells (lanes 2–3).

expressing EGFP-FPG is reduced by about one order of magnitude in comparison to cells transfected with vector only [22]. Whether FPG expression may similarly protect HTMC from the harmful effects of OD and counteract their degeneration is a worth exploring possibility. Unfortunately, expression of the fusion protein EGFP-FPG in HTMC was inefficient. A limited enrichment of EGFP-FPG expressing cells could be obtained after a 5-day exposure of transfected cells to a low concentration of G418 (50 μg/ml, Table I and Figure 4), but no stable EGFP-FPG-expressing clones could be selected by use of higher concentrations of G418 nor synchronization by 24 h serum starvation prior to transfection improved the recovery of EGFP-FPG expressing clones [31]. Even high throughput procedures such as the Nucleofector® technology (Amaxa-Lonza) did not significantly increase the number of EGFP-FPG-expressing cells. In previous work, EGFP-FPG expression had been achieved at relatively high levels in clones isolated in the human bladder carcinoma cell line T24 (TFC [22]) and the human SV-40-immortalized Cockayne syndrome cell lines CS3BE.S3.G1 and CS1AN.S3.G2 [24,25]. EGFP-FPG expression remained stable in those cells continuously cultured for at least 60 days, after which time a gradual drop in fluorescence was observed. Since TFC cells used as control for FPG expression in this study had been cultured for more than 90 days, their fluorescence values underestimate the FPG expression levels that can be obtained in T24 cells soon after transfection [22]. It is likely that the poor EGFP-FPG expression observed in primary HTMC in the present study is linked to poor transfectability of HTMC, because the vector type and transfection procedures did not differ from those successfully employed with transformed cell lines. The main results of the present paper are that: (1) Fpg transfection significantly increases the DNA repair capacity of HTMC for oxidative stress measured by Endo IV-mediated incision of AP sites and 8-oxodG ^{32}P post-labelling; (2) only <1% of HTMC are effectively transfected by Fpg.

HTMC play a fundamental role in glaucoma pathogenesis. These cells that cover the TM beams possess a wide range of biochemical and structural properties that are pivotal for maintenance of the aqueous outflow pathway. These properties include the growth of trabecular cells as an endothelial monolayer with a non-thrombogenic cell surface, the production of plasminogen activator, avid phagocytosis [32], the ability to synthesize glycosaminoglycans, collagen, fibronectin and other connective tissue elements [33] and the capacity to migrate [34]. The HTMC transfection presented here is proposed as a possible technical tool for pursuing the goal of minimizing oxidative damage and its consequences in glaucoma target tissues in the future. Nevertheless, as demonstrated by the low percentage of transfected

Table I. 8-oxodG levels as detected in HTMC under basal conditions or after EGFP-FPG transfection.

Replicates	HTMC (8-oxo-dG/10^5 nucleotides)	HTMC-EGFP-FPG (8-oxo-dG/10^5 nucleotides)	HTMC-EGFP-FPG + G418 (8-oxo-dG/10^5 nucleotides)
1	3.56	1.56	1.11
2	3.35	2.21	1.18
3	3.91	2.39	0.91
4	3.14	2.98	1.44
Mean ± SD	3.49 ± 0.33*	2.29 ± 0.58	1.16 ± 0.22

*$p < 0.0001$ vs HTMC-EGFP-FPG and HTMC-EGFP-FPG + G418 cells as evaluated by Student's *t*-test for unpaired data.

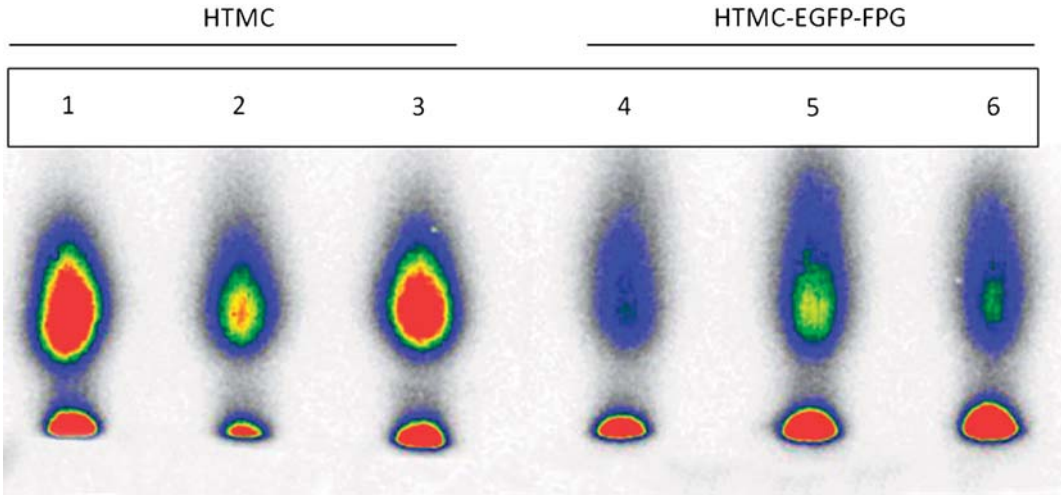

Figure 4. 8-oxodG levels in HTMC under basal conditions (HTMC, lanes 1–3) or after transfection with pEGFP-C1-FPG vector and subsequent exposure to 50 μg/ml G418 for 5 days (HTMC-EGFP-FPG, lanes 4–6). 8-oxodG was detected by trifluoracetic acid enrichment, ^{32}P-post-labelling, monodirectional thin layer chromatography and electronic autoradiography as described in Materials and methods section.

HTMC obtained, it is very difficult to transfect these cells. The precise reason for such a low sensitivity remains obscure. Classical gene transfer by chemical transfection techniques works rather well for a large variety of transformed cells as reported here for T24 bladder carcinoma cells, but is less effective for primary cell types. Further, it is likely that HTMC characteristics, being pivotal elements for aqueous humour clearance, are adapted to be highly resistant to viral infection and intracellular delivery of exogenous genetic material in order to maintain cell survival, function and aqueous humour sterility. It is likely that the poor EGFP-FPG expression observed in primary HTMC in the present study may be overcome in future studies by using appropriate viral vectors for gene transfection, such as adeno-associated viruses [21].

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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