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Activation of cellular signaling by 8-oxoguanine DNA glycosylase-1-initiated DNA base excision repair

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Abstract

Accumulation of 8-oxo-7,8-dihydroguanine (8-oxoG) in the DNA results in genetic instability and mutagenesis, and is believed to contribute to carcinogenesis, aging processes and various aging-related diseases. 8-OxoG is removed from the DNA via DNA base excision repair (BER), initiated by 8-oxoguanine DNA glycosylase-1 (OGG1). Our recent studies have shown that OGG1 binds its repair product 8-oxoG base with high affinity at a site independent from its DNA lesion-recognizing catalytic site and the OGG1•8-oxoG complex physically interacts with canonical Ras family members. Furthermore, exogenously added 8-oxoG base enters the cells and activates Ras GTPases; however, a link has not yet been established between cell signaling and DNA BER, which is the endogenous source of the 8-oxoG base. In this study, we utilized KG-1 cells expressing a temperature-sensitive mutant OGG1, siRNA ablation of gene expression, and a variety of molecular biological assays to define a link between OGG1-BER and cellular signaling. The results show that due to activation of OGG1-BER, 8-oxoG base is released from the genome in sufficient quantities for activation of Ras GTPase and resulting in phosphorylation of the downstream Ras targets Raf1, MEK1,2 and ERK1,2. These results demonstrate a previously unrecognized mechanism for cellular responses to OGG1-initiated DNA BER.

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Keywords

Ogg1; base excision repair; cell signaling

1. Introduction

Reactive oxygen species (ROS), generated by ionizing radiation, physical and chemical agents or particulate matter, cause oxidative damage to guanine residues in DNA. 8-oxo-7,8-dihydroguanine (8-oxoG) is the most abundant among the oxidatively modified guanine lesions. It is highly mutagenic, as it preferentially mispairs with adenine during DNA replication (rev in [1]). Accumulation of 8-oxoG in the DNA is linked to carcinogenesis, aging and aging-associated diseases [2–5]. 8-OxoG is primarily repaired by 8-oxoguanine DNA glycosylase-1 (OGG1), which recognizes the genomic 8-oxoG, flips it into the active site pocket, and excises it as free 8-oxoG base during the base excision repair (BER) pathway [6–8].

Associated with its DNA repair function, OGG1 has a key role in chromatin remodeling and transcriptional initiation of genes. For example, the transcription factor c-Myc and estrogen receptor-directed histone demethylation produces oxygen radicals locally, generates 8-oxoG in proximal regions of the promoters, and recruits OGG1. The nick generated by OGG1 facilitates topoisomerase II binding for chromatin relaxation [9, 10]. Recent observations also proposed DNA repair-independent roles of OGG1 in cellular processes, as it co-localizes with centrioles (microtubule organizing centers), microtubule networks, and mitotic chromosomes [11, 12]. Intriguing studies showed that OGG1 binds its excision product, the 8-oxoG base with high affinity ($K_d = 0.56$ nM), and the resulting complex (OGG1•8-oxoG) interacts with H-, K- and N-Ras proteins nearly stoichiometrically [13]. Importantly, OGG1•8-oxoG increased nucleotide release from Ras-GDP and Ras-GTP and allowed rebinding of either GTP or GDP nucleotides onto Ras *in vitro*. Externally added 8-oxoG base rapidly increased Ras-GTP levels in OGG1-expressing cells but not in OGG1 depleted ones [13], and consequently initiated signal transduction via Raf1-MEK1,2-ERK1,2, leading to the transcriptional activation of genes, as shown by microarray analysis (NCBI, GEO # GSE26813) [13]. Another study has reported that OGG1•8-oxoG physically interacts with guanine nucleotide-free and GDP-bound Rac1 protein. This interaction results in a rapid increase in Rac-1-GTP levels *in vitro*, *in cellulo*, and in an organ environment [14]. Although these data indicate that DNA BER-generated 8-oxoG base•OGG1 can function as a guanine nucleotide exchange factor (GEF), a clear connection between DNA BER and cellular signaling has not yet been rigorously established.

To further test this hypothesis and establish a possible mechanism, we utilized KG-1 cells, which express a thermolabile OGG1 mutant (OGG1^{R229Q}). At physiological temperature (37 °C), cells accumulate 8-oxoG in their genome due to the lack of OGG1^{R229Q}'s enzymatic activity [15]. Shifting KG-1 cell cultures from 37 °C to a lower temperature (e.g., 25 °C) rescues the 8-oxoG excision activity of OGG1^{R229Q}. This system thus provides a unique opportunity to investigate the role of OGG1 and the released 8-oxoG in triggering cellular signaling. Here we show that activation of OGG1-driven BER, and the resulting release of 8-oxoG from the genome, led to the activation of Ras GTPases and phosphorylation of downstream Ras targets. These results strongly suggest a link between the repair of 8-oxoG and cellular signaling, and show an unexpected novel mechanism in the DNA damage response.

2. Experimental Procedures

2.1. Cell culture and treatment

Human diploid fibroblasts (MRC5), and HeLa S3 (human cervical epithelial adenocarcinoma), KG-1 (human myeloid leukemia), and U937 (human monocytic lymphoma), cells were maintained in Earle's minimum essential, Dulbecco's modified Eagle's low glucose, Iscove's modified Dulbecco's medium, and RPMI 1640 medium, respectively. All media were supplemented with 10% Fetal Bovine Serum (FBS), glutamine (2 mM), penicillin (100 U per mL) and streptomycin (100 µg per mL), and the cells were grown at 37 °C in a 5% CO₂ atmosphere. KG-1 cells expressing temperature-sensitive OGG1 [15] were cultured to 1 million cells per ml and assayed at 37 °C or 25 °C. Low oxygen culturing of KG-1 and U937 cells was achieved by using gas-tight tissue culture flasks. Cell cultures were flushed with prepared gas mixtures (5% CO₂; 1% O₂; 94% N₂; Airgas Southwest) to produce a calculated 3% oxygen environment. Gas mixtures were replaced daily [16, 17].

2.2. Oligonucleotide incision assay

OGG1's base excision repair activity in nuclear lysates was determined using a 40-mer oligonucleotide containing an 8-oxoG at position 19 and labeled at the 3' end with indodicarbocyanine 5 AGAGAAGAAGAAGAA(G*)AGATGGGTTATTCGAAC TAGC-Cy5Sp/3 [18, 19]. This oligonucleotide was hybridized to its complementary sequence containing a cytosine opposite the 8-oxoG lesion (G*). In a standard excision reaction, nuclear extracts were added to a 10-µL reaction mixture containing 200 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 bovine serum albumin (BSA), and 5% glycerol. After 15 min at 37 °C, the excision reaction was stopped by adding 4 µL of formamide dye and heating for 5 min at 95 °C. The cleaved product was separated from the intact substrate in a 20% polyacrylamide gel containing 8 M urea in Tris-borate-EDTA buffer, pH 8.4. Fluorescence in the separated DNA bands was visualized using a LI-COR Odyssey CLx system.

2.3. Measurement of activated Ras level

The active form of Ras protein was determined using pull-down affinity purification assays (Active Ras Pull-Down and Detection Kit, Thermo Fisher Scientific) as described previously [13]. Briefly, cells were washed with ice-cold PBS and lysed with 1x Lysis/Binding/Washing buffer [25 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM MgCl₂, 5 mM EDTA, 1% NP-40, 1 mM DTT, 5% glycerol, 20 mM NaF, 1 mM, 1 µg/ml leupeptin and 1 µg/ml aprotinin]. Cell lysates were cleared by centrifugation at 12,000 × g, protein concentrations were determined, and Ras-GTP (active Ras) was captured by the Ras-binding domain of Raf1 (Ras-RBD) [20]. The eluents were fractionated on 4–20% PAGE, and changes in the levels of activated Ras were determined by Western immunoblot analysis using anti-pan-Ras primary antibody (Ab) and a horseradish peroxidase (HRP)-conjugated secondary Ab.

2.4. Assessment of 8-oxoG levels

We assessed 8-oxoG in DNA by determining the levels of OGG1-sensitive sites using an OGG1 FLARE (Fragment Length Analysis by Repair Enzymes) Comet assay (Travigen, Gaithersburg, MD) [21, 22]. The cells were embedded in 0.5% low melting-point agarose and added directly to pre-coated slides (Travigen). The slides were then immersed in lysis buffer [2.5 mol/L NaCl, 100 mmol/L Na₂EDTA, 10 mmol/L Tris-HCl (pH 10), 10% DMSO, 1% Triton X-100] at 4°C for 12h. The slides were washed three times (15 min) in FLARE buffer {250 mM HEPES-KOH (pH 7.4), 2.5 M KCl and 250 mM EDTA} and kept

in 40 mM HEPES (pH 8.0), 0.1 M KCl, 0.5 mM EDTA at room temperature for 1 h. The human OGG1 (6 pmol; Travigen, Gaithersburg, MD) was added to the gel-covered slides in 40 mM HEPES (pH 7.6), 0.1 M KCl, 0.5 mM EDTA containing 0.2 mg/ml bovine serum albumin and incubated for 40 min at 37 °C. The slides were placed on an electrophoresis platform, covered with electrophoresis buffer (1 mmol/L EDTA, 0.3 mol/L NaOH (pH 12.1), and DNA was allowed to unwind for 20 min before electrophoresis (20 min at 0.7 V/cm, 300 mA). After neutralization in 0.4 mol/L Tris-HCl (pH 7.5) for 5 min at room temperature, the DNA was stained with 1 ng per mL SYBER green. Comets were evaluated using the Comet Assay IV v4.2 system (Perceptive Instruments, Suffolk, UK). The tail intensity, defined as the percentage of DNA migrating from the head of the comet into the tail, was measured. A minimum of 200 cells were evaluated for each data point.

2.5. Immunoblotting and antibodies

Cells were lysed in a buffer containing 25 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM MgCl₂, 1 mM EDTA, 1% NP-40, 1 mM DTT, 5% glycerol, 20 mM NaF, 1 mM, 1 µg/ml leupeptin and 1 µg/ml aprotinin. The lysates were clarified by centrifugation at 12,000 × g and the supernatants collected. Protein samples (10 to 40 µg per lane) were separated by 5–20% SDS-PAGE. Proteins were transferred to Hybond-ECL nitrocellulose (Amersham, Biosciences, UK Limited) membrane by electroblotting. The membranes were then blocked with 3% BSA in Tris-buffered saline (TBS) containing 0.1% Tween (TBS-T) for 3 h, and incubated overnight at 4°C with the primary Ab diluted in 3% BSA in TBS-T. Primary Abs were pan-Ras (Millipore Inc), ERK1/2, phospho-ERK1/2, MEK1/2, and phospho-MEK1/2 (Cell Signaling). The blots were then washed four times with TBS-T and incubated for 1 h with HRP-conjugated secondary HRP-conjugated Ab (anti mouse IgG, GE Healthcare, UK Ltd) in 5% non-fat dry milk in TBS-T. After washing, immunoreactive bands on membranes were developed by using an ECL substrate (Amersham Biosciences, UK Limited) and visualized by chemiluminescence.

2.6. Downregulation of gene expression

siRNAs mixed with transfection reagent (INTERFERinTM; Polyplus-transfection Inc., NY) were added to dishes along with 5 × 10⁶ cells in serum-free media for 3 h and incubated in growth media for 72 h. Control siRNA (siGENOME Non-Targeting siRNA) and target-specific siRNAs (siGENOME SMARTpools) to OGG1 (Cat # NM-010957) were obtained from Dharmacon/Thermo Fisher Scientific, Inc. OGG1 was depleted via a repeated siRNA transfection and plating method [13, 21]. The extent of down-regulation was determined by qRT-PCR.

2.7. Quantitative real-time PCR

qRT-PCR was done using an ABI 7000 System equipment and software (Applied Biosystems, Foster City, CA) per the manufacturer's recommended protocol. The thermal profile was: 50°C for 2 min, 95°C for 10 min, and 45 cycles of 95 °C for 15 sec, followed by 60 °C for 1 min. A dissociation stage was added at the end of the run to verify the primers' specificity (95 °C for 15 sec, 60 °C for 20 sec and 90 °C for 15 sec). Expression levels (fold change) were determined by the delta-delta Ct method (ΔΔCt) [23, 24]. Primers were: OGG1, F: 5 -GCATCGTACTCTAGCCTCCA-3, R: 5 -GCTCTTGTCTCCTCGGTACA-3; GAPDH, F: 5 -GAAGGTGAAGGTCGGAGT-3, R: 5 -GAAGATGGTGATGGGATTTC-3 .

2.8. Statistical analysis

Results were analyzed for significant differences using ANOVA procedures and Student's t-tests, and were considered significant at $p < 0.05$. Data are expressed as the mean ± SD.

3. Results and Discussion

The goal of this study was to demonstrate that 8-oxoG base released from the genome during DNA BER processes activates a DNA repair-independent function of OGG1 that links DNA damage repair to cell signaling. To examine the validity of this previously raised hypothesis [13], we used KG-1 cells that express a 8-oxoG repair-deficient OGG1 (OGG1^{R229Q}) that is inactive at physiological temperature (37 °C) both *in vitro* and *in vivo* [15, 25]. Therefore KG-1 cells have supraphysiological 8-oxoG levels in their genome and supposedly mimic normal cells in tissues exposed to oxidative stress generated by endogenous and exogenous mechanisms. Intriguingly, the viability of KG-1 cells is similar to that of cells expressing wt OGG1 (e.g., U937 used in our studies); in fact, they can easily be differentiated into functional dendritic cells [26]. Importantly, the KG-1 cell model allowed us to examine the consequences of 8-oxoG repair without exposing cells to ROS, which operate as intracellular signaling molecules, a function that has been widely documented [27]. Oxidants also function as regulatory molecules for the small GTPases, including the Ras subfamily proteins H-, N-, K-, and E-Ras. These canonical Ras proteins contain redox-sensitive sequences termed NKCD (GXXXXGK(S/T)C) motifs (rev in [28]).

To confirm that the catalytic activity of OGG1 in KG-1 cells is defective under our culture conditions, we prepared nuclear extracts and carried out 8-oxoG excision assays. Fig. 1a shows that there was no detectable 8-oxoG excision activity at 37 °C. The activity of OGG1^{R229Q} was re-established at 25 °C, in line with data published previously [15, 25, 29]. Similar results were observed when mitochondrial extracts of KG-1 were tested at 37 °C and 25 °C (data not shown), in line with data showing a primary role of OGG1 (OGG1a isoform) in the repair of 8-oxoG in mitochondrial DNA [30]. In controls, nuclear extracts of U937 cells expressing wtOGG1 showed nearly identical 8-oxoG excision activity at 25 °C and 37 °C (Fig. 1A). To demonstrate that the low 8-oxoG excision activity of OGG1 in KG-1 cells was not associated with low protein levels, we showed that OGG1^{R229Q} levels in KG-1 cells were higher than wtOGG1 levels in U937 cells (Fig. 1B).

We previously reported that exogenously added 8-oxoG base increases Ras-GTP levels in OGG1-expressing, but not in OGG1 depleted cells [13]. To determine if the release of 8-oxoG from the genome is associated with an increased level of Ras-GTP, cultures of KG-1 cells were transferred from 37 °C to 25 °C for various lengths of time, cell extracts prepared, and GTP-bound Ras levels determined using active Ras pull-down assays (Materials and Methods). Control cells were kept at 37 °C. The results summarized in Fig 2A show that at 25 °C, GTP-bound Ras levels were increased from 15 min on, peaked at 90 min, and returned to baseline by 6 h (Fig. 2A). At 37 °C, there was no change in activated Ras levels (Fig. 2A, lower panels). In contrast, U937 cells (expressing wtOGG1) showed no change in active Ras levels at 25 °C; in fact, Ras-GTP levels were lower at all time points compared to those at 37 °C (Fig. 2B). Incubation of KG-1 and U937 cells at 25 °C resulted in no alteration in total Ras (Fig. 2A,B, lower panels) or OGG1 protein levels (Fig. 2C). These data support the idea that OGG1-initiated BER is associated with activation of Ras GTPase as we proposed previously [13]. Free 8-oxoG base is generated primarily by OGG1, although it may also result from NEIL1- and NEIL2-mediated replication- and transcription-coupled DNA repair, respectively [31, 32]. However, NEIL1 and NEIL2 have a low affinity for 8-oxoG base and do not physically interact with Ras family proteins [13], suggesting a strong specificity between OGG1-BER and activation of the small GTPase Ras.

Small GTPases including Ras family cycle between the GDP-bound, inactive “off state” and the GTP-bound, active “on state,” and so act as molecular switches that regulate multiple signal transduction pathways [33]. Activation involves the replacement of GDP with GTP, a process mediated by GEFs [34]. GEFs first interact with GTPase and dissociate GDP at an

increased rate; the bound GTP then promotes the release of the exchange factor, leaving the GTPase in an active form [34, 35]. The intrinsic GTPase activity of Ras is stimulated by a family of proteins known as GTPase-activating proteins (GAPs) [36]. It has been reported that GEFs' and GAPs' activities were affected by temperature change [37], so they could influence the relative levels of Ras-GTP and Ras-GDP in KG-1 cells. For example, Ras GAP could be inhibited at 25 °C, resulting in the accumulation of Ras-GTP. To exclude this possibility, OGG1 expression in KG-1 cells was downregulated using siRNA [13, 21], and the cells then incubated at 25 °C for 90 min. OGG1-depleted cells showed no increase in Ras-GTP levels (Fig. 3A). In contrast, Ras-GTP levels were increased in cells transfected with control siRNA (Fig. 3A). The extent of OGG1 downregulation is shown in Fig. 3B. These results confirmed that in KG-1 cells, activation of OGG1^{R229Q} and generation of 8-oxoG base is the primary cause of increased Ras-GTP levels, rather than an inhibitory effect of low temperature on Ras GAP. Moreover, incubating U937 cells at 25 °C appeared to decrease Ras-GTP with or without OGG1 depletion (Fig. 3C). Ras-GTP levels in MRC5 cells showed no change, while those in HeLaS3 cells showed some decrease when cells were transferred from 37 °C to 25 °C for 45 and 90 min (Fig. 3D).

To examine KG-1 cells' ability to activate Ras at 25 °C is dependent on OGG1-initiated BER, we ablated OGG1 expression with siRNA [13, 21] and determined Ras-GTP levels after the addition of platelet-derived growth factor (PDGF) [38]. OGG1-deficient KG-1 cells were transferred from 37 °C to 25 °C for an 80 min incubation, and PDGF then added for 10 min. Control cells (at 37 °C) were also exposed to PDGF for 10 min. PDGF addition rapidly increased Ras-GTP levels importantly, this Ras activation was not significantly affected by temperature in either KG-1 or U937 cells (Fig. 3E). Moreover, the lack of OGG1 expression had no effect on PDGF-induced Ras activation in KG-1 and U937 cells. These results also imply that Ras activation occurs via different mechanisms after OGG1 activation and PDGF addition. These data further support the hypothesis that activation of Ras at 25 °C was due to the increased release of 8-oxoG from DNA due to an increase in OGG1^{R229Q} activity in KG-1 cells (Fig. 1A).

To determine if 8-oxoG is indeed repaired and released from the KG-1 genome during incubation of cells at 25 °C, we utilized Flare Comet assays (fCA) [21, 22]. In fCA, agarose-embedded cells are lysed and digested with OGG1 (specifically excising 8-oxoG and the consequent lyase reaction via β -elimination, resulting in cleavage of the DNA phosphate backbone at the abasic site with formation of 3'-phospho-, α,β -unsaturated aldehyde (3,4-hydroxy 2-pentenal) and 5'-phosphate termini [39, 40]), which generates alkaline labile sites [22] and allows accurate assessment of relative 8-oxoG levels. Our data show minor differences in comet tail intensities between KG-1 and U937 cells before OGG1 digestion (images placed in right corners of Fig. 4A). These results are consistent with the view that intrahelical 8-oxoG does not cause a structural modification or make DNA alkaline-sensitive [41].

Significant increases in tail intensities were observed when the DNA of KG-1 and U937 cells was OGG1 digested (Fig. 4A). Quantitation of tail intensities showed that at 37 °C, 8-oxoG levels in genome of KG-1 cells were 3.9-fold higher than those in cells incubated at 25 °C for 6 h (Fig. 4B). When cultured at 37 °C, genomic 8-oxoG levels in KG-1 cells were 4.2-fold higher than those in U937 cells. Genomic 8-oxoG levels in KG-1 cells kept at 25 °C for 60 and 120 min were decreased substantially (Fig. 4B); after 6 h incubation at this temperature, 8-oxoG levels in DNA of KG-1 were nearly identical to those in U937 cells expressing wtOGG1, supporting the activation of OGG1^{R229Q} in KG-1 cells (shown in Fig. 1A). There were slight (statistically not significant) increases in 8-oxoG levels in DNA when U937 cells were incubated at 25 °C for 6 h (Fig. 4B).

As a proof of principle, we generated KG-1 cells without high levels of 8-oxoG in their genome. To do this, KG-1 cells were cultured in a low-O₂ environment, which has been shown to decrease genomic 8-oxoG levels [16, 17]. We hypothesized that in such cells, activation of OGG1-BER at 25 °C would result in insignificant changes in Ras activation due to a low genomic 8-oxoG level. To test this hypothesis, KG-1 cells were cultured in 3% O₂ environment (an optimal O₂ concentration determined in preliminary studies) at 37 °C to lower oxidative genome damage. Indeed, after for 4 weeks in the low-O₂ environment, genomic 8-oxoG levels decreased ~80% compared to those in KG-1 cells maintained at ambient O₂ (Fig. 4C). KG-1 cells maintained at low and ambient oxygen tension were transferred to 25 °C for 45 and 90 min of incubation, and Ras-GTP levels were assessed. The results in Fig. 4D show that KG-1 cells maintained in a 3% O₂ environment had only slightly increased Ras-GTP levels at 25 °C. In contrast, transfer of control KG-1 cells (maintained in ambient O₂) from 37 °C to 25 °C resulted in extensive Ras activation (Fig. 4D). These results imply that OGG1-BER-mediated activation of RasGTPase can occur after exposures to oxidative stress, and that under these conditions 8-oxoG base is released in sufficient quantities to activate cell signaling. These studies also eliminate the possibility of an inherent unexpected characteristic of KG-1 cells in the Ras activation processes.

It has been shown that Ras-GTP binds to the Ras-binding domain of the Raf1 serine/threonine kinase, leading to its phosphorylation [42]. The activated Raf kinase phosphorylates and induces the dual-specificity mitogen-activated protein kinases (MEK1/2), which in turn phosphorylate extracellular signal-regulated kinases (ERK1 and ERK2) [43]. To determine if activation of RasGTPase in KG-1 cells contributes to this downstream signaling, we investigated the levels of phosphorylated (p)-MEK1/2 and extracellular signal-regulated kinase (p-ERK1/2).

When KG-1 cells were transferred to 25 °C for 15, 30, 45 and 60 min, there was an increase in p-MEK1/2 and p-ERK1/2 levels (Fig. 5A) compared to those in KG-1 cells kept at 37 °C. The time course of MAPKs phosphorylation correlates well with activation of Ras (Fig. 2A). To confirm that ERK1/2 phosphorylation is indeed BER-dependent, OGG1 was downregulated by siRNA [13, 21]. Cells were then transferred from 37 °C to 25 °C for 60 min, lysed, and changes in pERK1/2 levels were determined. The results summarized in Fig. 5B show that p-ERK1/2 levels were increased only in control siRNA-transfected cells. PGDF induced ERK1/2 phosphorylation was independent of OGG1^{R229Q} activity.

In conclusion, cells are continuously challenged by exogenous and endogenous oxidative stresses that can ultimately lead to the generation of DNA base lesions, of which 8-oxoG is one of the most abundant. Unrepaired 8-oxoG has been shown to pair with adenine during replication and thus causes GC → TA mutations, and its accumulation in DNA is traditionally associated with various cellular pathophysiological changes and human diseases [3–5, 44]. While the mechanism of OGG1-initiated BER processes such as 8-oxoG recognition, excision and repair re-synthesis have been well characterized, there has been much less consideration given to the possible role of a DNA repair-independent function of OGG1, and to the biological role/fate of the 8-oxoG base released from DNA. 8-oxoG base is considered a potential biomarker for predicting the extent of oxidative exposures and cellular pathology, and for estimating the progression of various human diseases [5, 45].

Our studies here examined the intracellular fate of the 8-oxoG base released from the genome via OGG1-initiated BER. We addressed this important issue by using KG-1 cells expressing the mutated thermolabile OGG1^{R229Q}. Our data show that upon activation of OGG1-BER, 8-oxoG base is released from the genome in sufficient amounts and with adequate kinetics to induce post-repair cellular signaling via activated Ras-GTPase. Because our prior studies showed a high affinity of OGG1 for the 8-oxoG base at an independent site

and not OGG1's DNA lesion-recognition site [13], the present data suggest that it occurs in the intracellular milieu, facilitating a GEF function of OGG1 as a consequence of OGG1-BER in KG-1 and likely in other cell types. Together these observations indicate that OGG1 is a multifunctional protein possessing glycosylase/AP-lyase activity and functioning as a guanine nucleotide exchange factor. In support of this, OGG1 co-localizes with microtubule organizing centers and microtubule networks [11, 12], so the GEF function of OGG1 could be utilized in modification of the cellular architecture, which requires Rho and Rac family GTPases. *Ogg1*^{-/-} mice lack 8-oxoG excision activity, and possibly the GEF function of OGG1, so the results of our studies could also provide a mechanism for the increased resistance of these mice to inflammation [46]. Although the true significance of these novel findings has yet to be uncovered, we speculate that OGG1-BER- dependent Ras GTPase activation could be essential for maintaining the cellular physiological state and for reestablishing pre-exposure conditions after an oxidative insult. It is also attractive to propose that the free 8-oxoG-driven GEF activity of OGG1 and activation of small GTPases are the basis of OGG1's long-suspected involvement in cellular pathologies, aging-associated diseases, and aging processes [3, 4, 44].

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Highlights

- Putative link between OGG1-initiated BER and cell signaling is not yet established
- KG-1 cells expressing a thermolabile OGG1 mutant accumulate 8-oxoG in their genome
- Activation of OGG1-BER by temperature downshift results in Ras GDP → GTP exchange
- Downregulation of OGG1 blocked activation of Ras and downstream targets
- These data document novel properties of OGG1 in modulating cellular signaling

Figure 1. 8-oxoG excision activity of OGG1^{R229Q} at 37 °C vs. 25 °C

A, Nuclear extracts (5 µg per lane) from KG-1 cells were temperature-equilibrated (25 °C and 37 °C) and mixed with an 8-oxoG-containing 3'-Cy5-labeled substrate. Nuclear extracts of U937 cells were used as a control. After a 15 min incubation, the reactions were terminated and then the substrate and product were separated (Materials and Methods). Images were taken using a LI-COR Odyssey CLx system. **B**, OGG1 levels in total extracts (20 µg per lane) of KG-1 and U937 cells. To ensure equal loading, membranes were re-probed with Ab to GAPDH. **S**, substrate; **P**, product; OGG1, recombinant OGG1 (25 fM).

Figure 2. Ras-GTPase activation at OGG1's permissive temperature in KG-1 cells

A, Changes in Ras-GTP levels during incubation of cells at 25 °C. Cells grown at 37 °C were transferred to 25 °C, and GTP-bound Ras levels were determined using an "Active Ras Pull-down Assay" (Materials and Methods) in 250 µg cell extracts at the time points indicated. Extracts prepared from cells kept at 37 °C were used as a control. The lower panels show total Ras levels in cell extracts (25 µg per lane). **B**, Lack of change in Ras-GTP levels in U937 cells at physiological and sub-physiological temperatures. Ras-GTP and total Ras levels were determined as in A. **C**, OGG1 levels was unaltered at 25 °C and 37 °C in KG-1 cells.

Figure 3. OGG1 down-regulation halts Ras activation in KG-1 cells

A, Lack of Ras activation in OGG1-depleted KG-1 cells. OGG1-depleted and control (cont siRNA) cells were transferred to 25 °C for 90 min and Ras-GTP levels determined in cell extracts. **B**, Changes in OGG1 RNA levels after transfection of cells with control and OGG1 siRNA. *** = $p < 0.001$. **C**, Incubation of U937 cells at 25 °C lowers basal Ras-GTP levels. **D**, Lack of changes in Ras-GTP levels in MRC5 and HeLaS3 cells at 25 °C. **E**, PDGF-induced activation of Ras is temperature-independent in KG-1 and U937 cells. In A,C,D and E, Ras-GTP levels were determined using an “Active Ras Pull-down Assay” (Materials and Methods). Upper panels: Ras-GTP levels in 250 µg cell lysate. Lower panels show total Ras levels in 25 µg per lane cell lysate.

Figure 4. Ras activation requires the release of 8-oxoG from genomic DNA

A, Representative images of Comet tail moments of DNA of KG-1 and U937 cells before (insets at the right) and after digestion with OGG1. **B**, Quantitation of Comet tail intensities. KG-1 cells were incubated for 1, 2 and 6h at 25 °C, and DNA embedded in agarose was OGG1-digested (fCA) and electrophoresed under alkaline conditions (pH: 12.1). U937 cells kept at 25 °C for 6h were used as a control. **C**, Decreased levels of 8-oxoG in the genome of KG-1 cells maintained in a low-O₂ environment. Cells were grown in a 3% O₂ environment for 4 weeks and relative levels of 8-oxoG determined using fCA. **B,C**, Graphs show the calculated mean $\pm$ SD of comet tail intensity ($n > 200$). * = $P < 0.05$; *** = $P < 0.01$ (Student's t -test). **D**, Lack of Ras activation in KG-1 cells maintained in a low-oxygen environment. Cells were maintained at 3% O₂ for 4 weeks and then at 25 °C for the times indicated, and Ras-GTP levels were determined in 250 μ g cell lysates (Materials and Methods). Control cells were grown at ambient O₂ tension. Total Ras protein levels were assessed in 25 μ g cell lysates.

Figure 5. Phosphorylation of MEK1/2 and ERK1/2 upon activation of OGG1^{R229Q}

A, MEK1/2 and ERK1/2 phosphorylation in KG-1 cells at 25 °C (upper panels). The lower panels show total MEK1/2 and ERK1/2 levels (as loading controls). **B**, Downregulation of OGG1 in KG-1 cells halts phosphorylation of ERK1, 2. PDGF (10 ng/ml) -induced ERK1, 2 phosphorylation is independent of OGG1-mediated DNA repair. PD098059, an inhibitor of MEK1/2 decreased ERK1/2 phosphorylation induced by OGG1-BER or PDGF. Representative Western blots of three independent experiments are shown.