Enzymatically inactive OGG1 binds to DNA and steers base excision repair toward gene transcription

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Abstract
8-Oxoguanine DNA glycosylase1 (OGG1)-initiated base excision repair (BER) is the primary pathway to remove the pre-mutagenic 8-oxo-7,8-dihydroguanine (8-oxoG) from DNA. Recent studies documented 8-oxoG serves as an epigenetic-like mark and OGG1 modulates gene expression in oxidatively stressed cells. For this new role of OGG1, two distinct mechanisms have been proposed: one is coupled to base excision, while the other only requires substrate binding of OGG1—both resulting in conformational adjustment in the adjacent DNA sequences providing access for transcription factors to their cis-elements. The present study aimed to examine if BER activity of OGG1 is required for pro-inflammatory gene expression. To this end, Ogg1/OGG1 knockout/depleted cells were transfected with constructs expressing wild-type (wt) and repair-deficient mutants of OGG1. OGG1’s promoter enrichment, oxidative state, and gene expression were examined. Results showed that TNFα exposure increased levels of oxidatively modified cysteine(s) of wt OGG1 without impairing its association with promoter and facilitated gene expression. The excision deficient K249Q mutant was even a more potent activator of gene expression.
INTRODUCTION

Reactive oxygen species (ROS) generated during aerobic respiration or environmental exposures induce modifications to macromolecules including proteins, lipids, and polynucleotides (both DNA and RNA). While oxidatively damaged proteins, lipids, and RNAs are usually deemed to undergo degradation and recycling, DNA base and strand lesions need to be repaired to maintain genomic integrity. In DNA, guanine is a preferential target for oxidative modification due to its lowest ionization potential, its single electron oxidation results in 7,8-dihydro-8-oxoguanine (8-oxoG) or a one electron reduction yields 2-6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG). Both 8-oxoG and FapyG together constitute the most abundant DNA damages and are regarded as cellular biomarkers of oxidative stress (OS). During replication, 8-oxoG can pair not only with C, but also with A resulting in a G:C to T:A mutation, thus, its accumulation in DNA is postulated to be associated with pathophysiological processes including aging-associated diseases and carcinogenesis.

In eukaryotic cells, repair of oxidatively modified guanines (8-oxoG, FapyG) is primarily initiated by 8-oxoguanine DNA glycosylase 1 (OGG1) through the DNA base repair excision (BER) pathway. In addition to its glycosylase activity that attacks the N-glycosyl bond, releasing the damaged base and leaving an abasic site in the DNA, OGG1 also possesses β-lyase activity that cleaves the DNA strand 3’ of the AP site by β-elimination, generating an unsaturated hydroxy-aldehyde to the 3′ end (3′dRP) and a phosphate to the 5′ end. OGG1’s in vivo β-lyase activity is under examination as data suggest that OGG1 is a mono-functional enzyme. Nevertheless, AP endonuclease 1 (APE1) successively takes the place to create a nick at the DNA 5′ of the AP site by β-elimination, generating an unsaturated hydroxy-aldehyde to the 3′ end (3′dRP) and a phosphate to the 5′ end. OGG1’s in vivo β-lyase activity is under examination as data suggest that OGG1 is a mono-functional enzyme. Nevertheless, AP endonuclease 1 (APE1) successively takes the place to create a nick in the DNA at 5′ of the AP site resulting in a priming-ready 3′hydroxyl group. DNA polymerase β (pol β) then incorporates a normal nucleotide into the damage site to fill up the gap. In the case where OGG1 behaves as a mono-functional glycosylase, APE 1 would create a 5′-deoxyribos-phosphate terminus (5′dRP), which could be removed by the intrinsic lyase activity of pol β. Finally, DNA ligase III seals the nick, finishing the last step of OGG1-initiated BER.

Although guanine is vulnerable to ROS and 8-oxoG is mutagenic, evolutionally, genes tend to be embedded in the GC-rich genomic regions, and 72% of human gene promoters are guanine-rich. In addition, the transcriptional activity of genes is positively correlated with the GC content in genome. These facts imply that guanine oxidation may be an approach to modulating gene expression in response to OS. Recently, OGG1’s role in transcription regulation has drawn increasing attention. At least two different mechanisms have been proposed: one needs OGG1’s glycosylase activity; while the other only requires the binding of OGG1 with the substrate. For example, exposure of cells to estrogen results in demethylation of histone by lysine-specific demethylase 1 (LSD1), which produces ROS, generates 8-oxoG, and activate OGG1-initiated DNA BER. BER-introduced strand break is exploited by topoisomerase II for long-range changes in DNA topology needed for assembly of transcription machinery, and thereby estrogen-induced gene expression. Another set of studies documented that G oxidation within a potential G-quadruplex-forming sequence (PQS) in the promoter enables a structural shift from B-DNA to a G-quadruplex fold (G4). When 8-oxoG was located in the coding vs template strand, base excision repair led to an on/off transcriptional switch. For example, a hypoxia-induced transcription activation model showed that expression of vascular endothelial growth factor (VEGF) or endonuclease III-like protein 1 (NTHL1) was enhanced when 8-oxoG was formed in G-quadruplex sequences on the coding strand in promoters. Following OGG1’s removal of base lesion, the yielded AP site enables the melting of the duplex to adopt a G-quadruplex fold that is crucial for gene expression. Likewise, oxidation-induced activation of genes including mammalian homolog of myelocytomatosis viral oncogene (c-MYC), mammalian homolog of Kirsten rat sarcoma virus oncogen (KRAS), KIT proto-oncogene (c-KIT), hypoxia inducible factor 1 subunit alpha (HIF-1 α), platelet derived growth factor subunit A (PDGF-A), and telomerase reverse transcriptase (hTERT) is also regulated by such mechanism.

KEYWORDS

8-oxoG, OGG1, oxidative stress, pro-inflammatory gene expression
On the contrary, our previous studies have shown that mRNA levels of TNF, CXCLs, CCLs, and ILs rapidly and robustly increased in response to pro-inflammatory stimuli, which coincided with the peak level of intracellular ROS, as well as the increased enrichment of OGG1 at the promoter regions. Accumulation of 8-oxoG at the proximal promoters of human TNF, CCL20, and CXCL1, and mouse Tnf, Il-1β, and Cxcl12 genes, indeed, increased in response to the pro-inflammatory stimuli. The kinetics of 8-oxoG accumulation was parallel with the recruitment of OGG1, as well as the enhanced expression of pro-inflammatory cytokines/chemokines. However, under the OS condition, OGG1 binding to substrate was not followed by base excision as OGG1 may temporally lose its enzymatic activity due to cysteine oxidation. Engagement of transiently inactivated OGG1 with its substrate at the promoter region was associated with recruitment of transcription factors (eg, NF-κB and Sp1) and RNA polymerase, thereby the transcriptional activation of pro-inflammatory genes.

It has been well accepted that expression of pro-inflammatory cytokines/chemokines in innate immune response (IIR) is modulated by ROS signaling. Given that BER results in strand gap intermediates, binding of transiently inactivated OGG1 is plausible to preserve the integrity of the DNA template and prioritize a prompt expression of ROS-responding genes for IIR. To further examine that OGG1 modulates inflammatory gene transcription without involving its enzymatic activity and the removal of its substrate, we constructed plasmids expressing wild-type (wt) human OGG1 and its mutants K249Q and C253A. K249Q OGG1 is able to recognize and bind to DNA substrates, but lacks both N-glycosylase and AP-lyase activities. Replacement of cysteine (C) 253 with alanine (A) leads to the perturbed substrate engagement, and thereby the decrease in glycosylase activity of OGG1. The expression constructs were transfected into OGG1-knockdown HEK 293 or Ogg1−/− MEF cells and HEK293 cells were grown in DMEM (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% of fetal bovine serum. HEK 293 were treated with or without 10 mM of NAC, 10 µM of TH5487, 10 µM of O8 for 1 hour, then, exposed to 20 ng/mL TNFα for various length of time.

2.2 Cell culture and treatment

Ogg1−/− MEF cells and HEK293 cells were grown in DMEM (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% of fetal bovine serum. HEK 293 were treated with or without 10 mM of NAC, 10 µM of TH5487, 10 µM of O8 for 1 hour, then, exposed to 20 ng/mL TNFα for various length of time.

2.3 Constructs and transfection

The eukaryotic expression plasmids Flag-OGG1, YFP-OGG1 were constructed by inserting the coding sequence of OGG1 into pcDNA3.1 (+) and pZsYellow1-C1 vectors, respectively. Restriction enzyme sites EcoR I and Hind III were selected for cloning of pcDNA3.1 (+). Hind III and BamH I were chosen for pZsYellow1-C1. Flag-OGG1 K249Q, Flag-OGG1 C253A, YFP-OGG1 K249Q, and YFP-OGG1 C253A were created using the Fast Mutagenesis System based on Flag-OGG1 or YFP-OGG1, respectively. Cxcl2-Luc plasmid including mouse Cxcl2 promoter (~571 to +81) was cloned from the MLE 12 genome and insert into the reporter vector pGL4.2 using restriction enzyme sites Kpn I and Bgl II.
The plasmid pRL-SV40 encoding Renilla luciferase driven by an SV40 promoter (Promega) was used as an internal control as described previously. All the plasmids were transfected using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Cells were treated with or without TNFα 36 hours post transfection. The prokaryotic expression plasmid GST-OGG1 was produced by inserting the OGG1 coding sequence into pGEX-4T-2 using restriction enzyme sites EcoRI and XhoI. The mutation plasmids GST-OGG1 K249Q, GST-OGG1 C253A were generated as mentioned above.

### 2.4 GST-fused protein purification

GST and GST-fused proteins were induced in BL21 of *Escherichia coli*. The overexpression was achieved by adding 1 mM of isopropyl-b-D-thiogalactopyranoside and 40 µM of ZnSO4 to an OD 1.0 culture at 16°C overnight. Whole bacteria lysates were applied to glutathione Sepharose 4B (GE Healthcare Life Science, Uppsala, Sweden) after sonication (48 mL of *E.coli* culture, 20% of powder, 6s on, 9s off, 32 times) and GST-tagged proteins were purified according to the manufacturer’s instructions. Proteins were purified and eluted in 150 µL of buffer (50 mM of Tris-HCl (pH 8.3), 100 mM of KCl, and 40 mM of glutathione). These proteins were used for EMSA and cleavage assay experiments in vitro.

### 2.5 Electrophoretic mobility shift assay

The oligonucleotides were synthesized by Sangon Biotech (Shanghai, China), and the site-specific guanine residue within each of the oligonucleotides was modified with a single 8-oxoG (Table 1). To perform the EMSA experiment, we utilized LightShift Chemiluminescent EMSA Kit (Cat # 20148, Thermo Scientific). To examine the interaction of wild-type OGG1 or OGG1 mutants with DNA, 10 fmol Bio-labeled or 100 fmol Cy5-labeled duplex oligo was mixed with 2 µL of GST-fused protein (20, 10, or 5 nM) in a total volume of 10 µL including 10 mM of Tris-HCl (pH 7.5), 10 mM of NaCl, 1 mM of EDTA, and 1 mg/mL BSA. The binding assay was performed for 10 minutes at 4°C. The reaction mixtures were separated at 100 V for 90 minutes in a 6% of polyacrylamide gel (0.5 x TBE) at 4°C.

### 2.6 Oligonucleotide incision assay

To examine the lesion incision abilities of wt OGG1 and OGG1 mutants, a 37-mer oligonucleotide containing an 8-oxoG at position 16 and labeled at the 3’ end with Cy5 was used (Table 1). For the incision assay, 100 fmol Cy5-labeled probe was incubated with 2 µL of GST-fused protein (20, 10, or 5 nM) in a total volume of 10 µL comprising 10 mM of Tris-HCl (pH 7.5), 10 mM of NaCl, 1 mM of EDTA, 1 mg/mL BSA, and 1 mM of DTT. After incubation for 10 minutes at room temperature, the reaction was halted by adding 10 µL loading buffer (containing 8 µL of formamide, 10 mM of NaOH) and heating for 5 minutes at 95°C. The reaction mixtures were separated in a 20% of polyacrylamide gel containing 8 M of urea in Tris-borate-EDTA buffer (pH 8.4). Fluorescence detection of the DNA bands was visualized via a LI-COR Odyssey CLx system.

### 2.7 Western blotting

HEK293 cells or *Ogg1−/−* MEF cells that were transfected with various OGG1 mutation plasmids were lysed. The obtained whole cell extraction was mixed with 2 x SDS sample buffer, separated by 10% of SDS-PAGE, and transferred onto...
nitrocellulose membranes. Membranes were blocked with 5% of skim milk (in 0.1% of TBST) for 1 hour and incubated with primary Abs overnight at 4°C. The dilution of monoclonal antibody against FLAG, GFP, and β-Actin were 1:8000 and that of polyclonal antibody against biotin was 1:2000. After three washes with TBST, membranes were incubated with secondary Abs and detected by ECL Plus western blot detection reagents.

2.8 | Reverse transcription and real-time PCR

Total RNA was isolated from cultured cells using TRIzol reagent (CWBio, China). One microgram purified RNA from each sample was reverse-transcribed to the complementary DNA using PrimeScript RT reagent Kit (TaKaRa, Japan). The cDNA was used as template for RT-qPCR. RT-qPCR was accomplished by using the SYBR Green qPCR Master Mix (Cat# 638320, TaKaRa). The target genes Cxcl2, Tnfα, and Il-1β expression was calculated by ΔΔ Ct method. The relative levels of each sample were normalized to the Gapdh housekeeping gene. Primer sequences are shown in Table 2.

2.9 | Luciferase assay

Ogg1−/− MEF cells were seeded in 12-well plates overnight in the absence of antibiotics. The cells were transfected with 1µg of PcdNA3.1, or Flag-OGG1 or Flag-OGG1 K249Q or Flag-OGG1 C253A plasmids, respectively, together with 100 ng pRL-SV40 Renilla luciferase and 500 ng firefly luciferase plasmids (PGL4.2-vector or Cxcl2-promoter-luc). To determine the prompt activation of the Cxcl2 promoter, the cells were exposed to 20 ng/mL TNFα for 30, 60, and 120 minutes after transient transfection for 36 hours. The transcriptional activity of the Cxcl2 promoter was represented by the mRNA level of firefly luciferase normalized to that of Renilla. Expression was calculated by the ΔΔ Ct method. Primer sequences are shown in Table 2.

2.10 | RNA fluorescence in situ hybridization

Ogg1−/− MEF cells grown on a glass coverslip were transfected with YFP, YFP-OGG1, YFP-OGG1 K249Q, or YFP-OGG1 C253A plasmids. After TNFα treatment for various lengths of time, cells were fixed with 4% paraformaldehyde (Dingguo, China) for 10 minutes at room temperature, permeabilized with 0.5% Triton X-100 for 30 minutes, and then, washed twice with 40% formamide for 10 minutes at room temperature. Thereafter, cells were pre-incubated with 1 mg/mL Salmon sperm DNA solution (10% of Dextran sulfate sodium salt, 50% of formamide, 2 × SSC, 0.02% of BSA, 2 mM of VRC) at 42°C for 1 hour. After that, the cells were incubated with 80 ng/mL Cxcl2-Cy3 probe overnight at 42°C. The nuclei of the cells were stained with 4,6-diamidino-2-phenylindole (DAPI) for 5 minutes. Cells were visualized using a confocal microscope (Nikon, Tokyo, Japan) equipped with a 60x oil-immersion objective lens. The probe used in this experiment was synthesized at Sangon Biotech (Shanghai, China) and labeled with Cy3 at the 3′ end. The sequence of the probe was 5’-TTGACCGCCCTTGAGAGTGGCTATGACTTCTGTCTGGGCGCAGTGGGGTCCTGGGGCGG-3′-Cy3. Cells transfected with YFP.

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TABLE 2 qRT-PCR primers used in this study

| Gene name       | Sequence                      |
|-----------------|-------------------------------|
| Gapdh           | F: 5′-AAT GGT GAA GGT CGG TGT G-3′<br>R: 5′-GTG GAG TCA TAC TGG AAC ATG TAG-3′ |
| Cxcl2           | F: 5′-CAG AAG TCA TAG CCA CTC TCA AGA-3′<br>R: 5′-CTT TCC AGG TCA GTT AGC CTT-3′ |
| Tnfα            | F: 5′-AGA CCC TCA CAC TCA GAT CA-3′<br>R: 5′-TCT TTG AGA TCC ATG CCG TTG-3′ |
| Il-1β           | F: 5′-ACTGTGTTCATATGCGCTCGG-3′<br>R: 5′-ATGGTGTTCATATGCGCTCGG-3′ |
| CXCL1           | F: 5′-TCTCTCTTCTCCGTGGCAGTG-3′<br>R: 5′-CATCCCCTAGTTAAGAAAATCATC-3′ |
| Renilla luciferase | F: 5′-AGC CAG TAG CGC GGT GTA TT-3′<br>R: 5′-TCA AGT ACG CTA TAA GAA CCA TTA CCA GAT T-3′ |
| Firefly luciferase | F: 5′-CTG AAC AGC ATG GGC ATC A-3′<br>R: 5′-AAA TGG GAA GTC ACG AAG GT-3′ |
YFP-OGG1, YFP-OGG1 K249Q, or YFP-OGG1 C253A were chosen to measure and compare the fluorescence intensity (red color) which signified Cxcl2 expression level.

### 2.11 Chromatin immunoprecipitation assay

Parallel 10-cm² dish cultures of HEK 293 cells were transfected with Flag-OGG1, Flag-OGG1 K249Q, or Flag-OGG1 C253A plasmid for 36 hours, and then, exposed to 20 ng/mL TNFα for 30 minutes. DNA-protein complexes were cross-linked with 1% of paraformaldehyde for 10 minutes at room temperature and sheared by sonication with 30-sec pulses (average 300 bp fragment) six times on high power (Bioruptor® Plus, Diagenode). DNA-protein complexes (near 25 µg of genomic DNA fragment per sample) were immunoprecipitated using 2 µg of Flag Ab overnight at 4°C, then, incubated with 30 µL of magnetic beads for 3 hours. The precipitates were washed three times, de-cross-linked, and subjected to qPCR following the manufacturer's instructions for the Simple ChIP Enzymatic Chromatin IP Kit (cell signal-technology, US). Primers for PCR amplification (CXCL1 promoter) are F: 5′-GGAGTTACTCTGAAGGGCGAG-3′; R: 5′-AAAGGGTTGCAGATCTCTC-3′. The size of the PCR product is ~200 bp. The fold enrichment of wtOGG1 or its mutants on the specific region of genomic DNA was calculated as described previously. 33,34

### 2.12 Assessment of cysteine-modified OGG1

Cells expressing YFP or YFP-OGG1 were pretreated with or without 10 nM of NAC for 60 minutes then exposed to TNFα for 60 minutes. Whole cell lysates were made in an ice-cold de-oxygenized buffer containing 50 mM of Tris-HCl (pH 7.5), 50 mM of NaCl, 1 mM of EDTA, 1 mM of EGTA, 1% of Nonidet P-40, 2.5 mM of sodium pyrophosphate, protease inhibitor mixture (Sigma). 100 µM of diethylenetriaminepentaacetic acid, 5 µM of iodoacetamide, 200 units/mL catalase, and 0.1 mM of 3-(2,4-dioxocyclohexyl)propyl 5-((3aR,6S,6aS)-hexahydro-2-oxo-1H-thieno[3,4-d]imidazol-6-yl) pentaanoate (DCP-Bio1; KeraFAST, Inc, Boston) as documented previously. 25,33 The supernatants of whole cell extracts were incubated with 30 µL of GFP beads at 4°C for 3 hours. The immunoprecipitates were resolved by SDS-PAGE, and OGG1-DCP-Bio1 was detected by biotin antibody.

### 2.13 Statistical analysis

All experiments were performed at least three times. Statistical analyses were performed using Student's t test or two-way ANOVA SPSS to analyze changes at the mRNA levels. The data are presented as the mean ± the standard error. The level of significance was accepted at *P < .05, **P < .01, and ***P < .001.

### 3 RESULTS

#### 3.1 Modulation of pro-inflammatory gene expression is independent of OGG1' enzymatic activity

To examine whether OGG1's enzymatic activity is required for modulation of gene expression, we constructed a series of prokaryotic and eukaryotic expression plasmids according to previous structural analyses 15,40,43 We purified GST-tagged OGG1 recombinant proteins (GST-wt OGG1, GST-OGG1 K249Q, and GST-OGG1 C253A), and their substrate binding as well as base excision activities were determined by EMSA and cleavage assays, respectively, using 8-oxoG-containing Cy5- and/or biotin-labeled DNA probes. EMSA assays showed GST-wt OGG1 but not GST formed a DNA-protein complex (Figure 1A; Figure S1A). Moreover, compared with wt OGG1 the level of OGG1 K249Q mutant bound to 8-oxoG-containing DNA was significantly higher. For example, at 20 and 10 nM, the level of probe-associated OGG1 K249Q was 1.4 ± 0.14-fold and 2.7 ± 0.1-fold higher, respectively (Figure 1B, lane 5-7 vs 2-4; Figure S1B, lane 8-10 vs 5-7), most likely due to its slow dissociation from DNA substrate. 40-41,44,45 Whereas, OGG1 C253A bound poorly to 8-oxoG-containing DNA duplex since Cys253 is expected to stabilize the interaction of 8-oxoG with the active site. 40,41,44 The amounts of DNA-protein complexes decreased to ~ 0.3 ± 0.05 and 0.16 ± 0.04, respectively, when 20 nM and 10 nM of OGG1 C253A were applied (Figure 1B, lanes 8-10 vs 2-4; Figure S1B, lanes 11-13 vs 5-7). Next, cleavage assays were performed. Compared with wt OGG1, the C253A mutation caused more than 70% decrease in OGG1's excision activity (Figure 1C, lane 8-10 vs lane 2-4), whereas the K249Q mutant showed no catalytic activity (Figure 1C, lane 5-7 vs lane 2-4).

To examine whether OGG1's enzymatic activity is required for modulation of gene expression, eukaryotic plasmids expressing FLAG- and YFP-tagged wt OGG1 and the mutants were transfected in Oggl−/− mouse embryonic fibroblast (MEF) cells. Cells were exposed to TNFα (20 ng/mL) for various lengths of time, and expression of Cxcl2, Tnf, and Il-1β was determined using qRT-PCR. Cells transfected with PcDNA3.1 (Figure 2A; Figure S2) or YFP (Figure 2B) plasmids had a slight increase in mRNA levels of Cxcl2 (Figure 2A, upper panels), Tnf and Il-1β (Figure S2) after TNFα exposure and served as a control. While wt OGG1 augmented the expression of Cxcl2,
OGG1 K249Q increased gene expression to a higher level. For example, in FLAG-tagged wt OGG1-transfected and TNF-exposed cells, Cxcl2 expression increased 6 ± 2.3-fold at 60 minutes and 5.8 ± 2-fold at 120 minutes, while OGG1 K249Q increased the expression of this gene 9.7 ± 3.8-fold and 8.6 ± 3.3-fold, respectively (Figure 2A). In YFP-tagged OGG1-transfected cells, wt OGG1 increased gene expression 16 ± 2.9-fold at 60 minutes and 9.7 ± 3.2-fold at 120 minutes, whereas, OGG1 K249Q increased gene expression 18 ± 3.8-fold and 14 ± 4.5-fold, respectively (Figure 2B). On the contrary, the C253A mutant, which has decreased abilities to bind with the substrate and remove the lesion, failed to show significant changes in gene expression (Figure 2). The lower panels of Figure 2A, B show the equal transfection efficiency. Two-way ANOVA statistical analysis revealed that wt OGG1 and K249Q mutant, but not the C253A mutant, significantly increased Cxcl2 gene expression. Similar results were also obtained with Tnf and Il-1β expression (Figure S2). Taken together, these data suggest that OGG1 binding to its DNA substrate in the gene regulatory regions, but not its base excision activity is associated with modulation of pro-inflammatory gene expression.

3.2 | Cells expressing OGG1 proteins proficient in DNA binding are the primary source of pro-inflammatory mediators

Data from qRT-PCR (Figure 2A,B; Figure S2) clearly showed a positive association between the binding ability of OGG1 and the mRNA levels of Cxcl2, Tnf, and Il-1β at the whole cell population level. To further attribute the up-regulation of pro-inflammatory mediators to those cells expressing particular OGG1 mutants, we performed RNA fluorescence in situ hybridization (FISH) assays. Parallel cover-slip cultures of wt OGG1- or OGG1 mutant-expressing Ogg1−/− MEF cells were treated with TNFα (20 ng/mL) for various lengths of time, fixed, processed, and hybridized with Cxcl2-specific Cys3-labeled probes. The FISH signals were determined by confocal microscopy to assess Cxcl2 mRNA levels. Figure 3A shows that TNF exposure induced only background levels of FISH signals in Ogg1−/− MEF cells with or

**FIGURE 1** Binding and enzymatic activity of OGG1 mutant's. A and B, Binding of wt and mutant OGG1's to 8-oxoG-containing probes. Purified recombinant proteins (20, 10, and 5 nM) were incubated with 100 fmol 8-oxoG-containing Cy5-labeled DNA oligos (total volume is 10 µL) for 10 minutes on ice. Retardation of protein/DNA complex was analyzed by EMSA. The right panels show quantification of the corresponding experiments. C, Changes in amino acids crucial for enzymatic function decreases/prevents base excision activity of OGG1. Purified recombinant proteins as described above were mixed with 100 fmol 8-oxoG-containing Cy5-labeled DNA oligos for 10 minutes at room temperature. The cleaved fragments were separated from the intact strand in a 20% of polyacrylamide gel. DNA bands were visualized by using a LI-COR Odyssey CLx system. The right panels show quantification of the corresponding experiments. D, Equal concentrations and titrations of OGG1 proteins were used in the above Figures. All experiments were performed three times. Quantification analyses were performed using Image J and the data are presented as the means ± the standard error. A two-way analysis of variance (ANOVA) was applied to determine the significance of the difference. ***P < .001, compared with GST in (A), and with wt OGG1 in (B) and (C)
without YFP expression. In the cells expressing wt OGG1, significant increases in \(\text{Cxcl2} \) mRNA at 30 and 60 minutes, and a slight decrease at 120 minutes were observed (Figure 3B). A similar intensity of FISH signal was observed in cells expressing OGG1 K249Q (Figure 3C). However, the FISH signal in OGG1 C253A mutant-transfected cells was significantly weaker compared to the wt OGG1- or OGG1 K249Q-transfected cells after TNFα stimulation (Figure 3D). The results of the FISH assays suggested that only the cells expressing binding-proficient OGG1 showed increased expression of inflammatory chemokines at the mRNA level.

### 3.3 | OGG1 promoter interaction but not base excision accounts for pro-inflammatory gene expression

To confirm that pro-inflammatory gene expression is dependent on the OGG1-DNA interaction but not the excision of substrate, we tested the stimuli-dependent binding of wt OGG1 and the mutants to genomic DNA by chromatin immune-precipitation (ChIP) assay. HEK 293 cells were overexpressed with FLAG-tagged wt OGG1 and the mutants, and then, exposed to TNFα for 60 minutes. ChIP was performed and the enrichment of OGG1 at promoter sequences was determined using quantitative PCR (qPCR) as described previously.\(^{33,34}\) The right panel of Figure 4A shows that wt OGG1 and the mutants were expressed equally in transfected HEK 293 cells. In response to TNFα exposure, wt OGG1 enrichment showed a 2.9 ± 0.15-fold increase (Figure 4A, left panel). OGG1 K249Q enrichment was 4 ± 2.1-fold, significantly higher than that of wt OGG1; whereas, enrichment of OGG1 C253A was not significant on the same genomic region (Figure 4A, left panel). The enrichment of wt OGG1 and the mutants on the specific promoter region was correlated with their magnitude of interaction with 8-oxoG containing DNA (Figure 1B; Figure S1B).

**FIGURE 2** Binding to the DNA substrate but not base excision activity is required for OGG1 to modulate pro-inflammatory gene expression. Ogg1\(^{−/−}\) MEF cells were transfected with plasmids expressing Flag-tagged (A) or YFP-tagged (B) wt OGG1 or site-specific mutants, and then, exposed to 20 ng/mL TNFα for 0, 30, 60, and 120 minutes. \(\text{Cxcl2} \) gene expression was determined by real-time PCR. The lower panels of A and B show the transfection efficiencies of OGG1 and mutants. Changes in gene expression in cells transfected with control plasmids PcDNA 3.1 (+) (A) and YFP (B) were taken as control. All experiments were performed three times. The data are presented as the means ± the standard error. A two-way analysis of variance (ANOVA) was applied to determine the significance of the difference. ***\(P < .001\), ns: no significance, compared with PcDNA3.1 in (A) and with YFP in (B).
with the enrichment of wt OGG1 in the CXCL1 promoter (Figure 4A), suggesting oxidatively modified OGG1 maintains its ability to bind substrate. Pretreatment of cells with NAC decreased OGG1-associated cysteine sulfenic acid to background levels (Figure 4B, right panel) and prevented TNF-induced enrichment of wt OGG1 on the CXCL1 promoter (Figure 4C). Of note, decreased OGG1 enrichment may also result from the absence of 8-oxoG in the promoter sequences.

We further tested the effects of OGG1 inhibitors on the recruitment of wt OGG1 on chromatin. TH5487, which blocks OGG1 substrate binding48 prevented TNFα-induced enrichment of OGG1 on the CXCL1 promoter (Figure 4C). Another potent OGG1 inhibitor O8, which inhibits catalytic imine formation in OGG1 without blocking its substrate binding,49 allowed recruitment of wt OGG1 to the CXCL1 promoter (Figure 4C). As expected, TH5487 inhibited gene expression, while O8 slightly increased it (Figure 4D). Taken together, these data strongly suggest that OGG1 facilitates gene expression when bound to DNA at its substrate.

3.4 | Substrate-binding is required for OGG1-driven promoter activation

Next, we questioned whether the enzymatic activity of OGG1 is required for a rapid activation of pro-inflammatory gene promoters. A Cxcl2 promoter-based (−571 to +81
nt, containing Sp1 and NF-κB binding sites) dual reporter assay (Firefly/Renilla luciferases) was performed utilizing Ogg1/MEF cells co-expressing wt OGG1 or the mutants. In controls, vector pGL4.2 was used (Figure S3). Thirty-six hours after transfection, cells were exposed to TNFα for 0, 30, 60, and 120 minutes and promoter activation was determined. Results show that firefly luciferase mRNA levels in TNFα-exposed wt OGG1-expressing cells increased by 3 ± 1.4-, 13 ± 2.7- and 3.9 ± 1.1-fold at 30, 60, and 120 minutes, respectively, whereas in cells expressing the K249Q mutant, firefly luciferase mRNA levels increased 4 ± 1.6-, 17 ± 1.9-, and 6.6 ± 0.6-fold. The OGG1 C253A-expressing cells only showed a moderate increase in firefly mRNA levels (4 ± 1.3-fold) at 60 minutes (Figure 4E). Statistical analysis by two-way ANOVA showed that wt and K249Q OGG1 significantly contributed to activation of the Cxcl2 promoter upon TNFα stimulation, whereas the C253A mutant had poor effect.

4 | DISCUSSION

8-OxoG is one of the predominant lesions in genomic DNA caused by OS and has been associated with various
pathophysiological processes, genomic instability and mutagenesis. Recently, increasing amounts of data are suggesting that OGG1-mediated repair of 8-oxoG, at different stages (base recognition/excision) can be steered toward expression regulation of various ROS-responding genes. Indeed, a rapid up-regulation of pro-inflammatory cytokines and chemokines correlated well with an increase in both levels of 8-oxoG and OGG1 enrichment on the TSS adjacent regulatory sequences, and intriguingly, OGG1 depletion impeded gene expression. In-depth studies revealed that OGG1’s interaction with its DNA substrate facilitated DNA occupancy of sequence-specific transcription factors on their motifs. It has been proposed that to prioritize a prompt expression of ROS-responding genes, OGG1 interacts with DNA without removal of its substrate, which, in turn, facilitates the recruitment of transcription factors and assembly of the transcriptional machinery. Here, we utilized a set of constructs expressing wt OGG1 and mutants as tools to further examine the mechanism by which OGG1 modulates expression of pro-inflammatory genes. Results showed that oxidative inactivation or inactivating enzymatic activity through mutation of OGG1 augmented gene expression, suggesting that the pre-excision binding with the substrate and the consequent conformation change of the regulatory sequences is the primary mode by which OGG1 modulates inflammatory gene expression in oxidatively stressed cells.

The HhH-GPD domain of DNA glycosylases is responsible for the recognition and removal of damaged bases from DNA. OGG1 active sites in this domain include Phe319, Gln315, Gly42, and Cys253 for “capture” of 8-oxoG; His270, Asn150, Val250, Gln24, and Gly245 for binding with the phosphate backbone; and Asn149, Tyr203, Arg204, and Arg154 for interaction with the opposite cytosine; which all together form the “8-oxoG-specificity pocket”. Within the HhH-GPD domain, the lysine 249 residue of OGG1 is crucial due to its function as the catalytic nucleophile. From a large number of mutational and functional analyses of OGG1 variants, we noticed that the OGG1 249Q mutants were repair-inactive but substrate recognition-competent, and the replacement of Cys 253 to Ala (C253A) decreased the interaction of OGG1 with substrate-containing DNA, as well as its base excision activity although Cys253 is not crucial for 8-oxoG recognition. Remarkably, in line with the properties of the mutants, our ChIP data showed that while TNFα stimulation induced an increase in chromatin enrichment of wt OGG1, the K249Q mutation resulted in an even higher level of interaction of OGG1 with the promoters of pro-inflammatory genes; whereas OGG1 C253A was not significantly enriched. Accordingly, promoter activation was enhanced, and mRNA levels of the pro-inflammatory cytokines/chemokines were elevated in wt and K249Q OGG1-expressing cells, but not C253A OGG1-expressing ones.

In wt OGG1-expressing cells, pro-inflammatory gene upregulation is proposed to be attributed to the oxidative inactivation of OGG1. OGG1 oxidative modification at cysteine residues is a reversible modification that can be regained after redox balance is restored. Inactivation of OGG1 via cysteine oxidation does not interfere with genomic substrate binding, but halts its glycosylase activity. The vast numbers of OGG1 K249Q analyses help us to comprehend the mechanism of how oxidatively inactivated OGG1 modulates gene expression. Cysteine-oxidized OGG1 persists its ability to recognize the substrate, bend DNA and extrude the damaged base into the active pocket; however, the conformation of the enzyme/DNA complex does not accommodate for base excision, probably due to the disfavor of the lysine 249 to attack C1′ of the deoxyribose moiety using its e-amino group. The biological significance of OGG1 oxidation was proclaimed by studies suggesting that the pre-excision
steps and the associated adjustment of the adjacent DNA by oxidized OGG1 facilitate DNA occupancy of TFD II, Sp1, and NF-κB, and consequently enhance expression of pro-inflammatory genes (TNF, CXCLs, and IIs) under OS conditions. Upon normalization of the cellular redox state, genome fidelity was accomplished via DNA-BER. Taken together, these data imply that this timely compromise of lesion repair is a part of an elaborate orchestration of the ROS-responding cellular processes, which ensures the prioritized transcription of rapidly responsive genes, such as those for the IIR.

In order to further understand the importance of DNA binding for OGG1 in modulation of gene expression, the present study also examined the effects of OGG1 inhibitors TH5487 and O8. The small-molecule TH5487 binds OGG1’s active site and inhibits its searching for and binding to 8-oxoG in the genome. The addition of TH5487 to wt OGG1 and OGG1 K249Q-expressing cells prevented OGG1 enrichment on CXCL1 regulatory sequences and decreased TNFα-induced gene expression. O8, which does not block OGG1 binding to DNA but appeared to interfere with both glycosylase and β-lyase activities, increased the enrichment of OGG1 on the CXCL1 promoter and gene expression. In this study, the OGG1 K249Q mutant reproduced the action of inhibitor O8; whereas, the OGG1 Cys253A mutant mimicked the effect of inhibitor TH5487. Collectively, these data suggest that the substrate binding ability but not the enzymatic activity is essential for OGG1’s role in transcription regulation.

Other than cysteine-based oxidation, functional regulation of OGG1 in transcription activation may also implicate post-translational modifications and protein-protein interactions, some of which has been documented to result in a decrease in OGG1 BER activity. For example, hyperglycemia is conventionally associated with the over-production of ROS. OGG1 was found highly O-GlcNAcylated in diabetic mice compared with controls, and O-GlcNAcylation inhibited OGG1 activity. In light of the results of the present study, we may propose that O-GlcNAcylated OGG1 affects the expression of those genes implicated in diabetic pathogenesis despite the impaired enzymatic activity. Another study documented a direct interaction between OGG1 and poly(ADP-ribose) polymerase 1 (PARP1). The interaction with PARP1 and poly(ADP-ribosylation) (PARylation) by PARP1 were enhanced by OS, which decreased the BER activity of OGG1. Of note, PARP1’s involvement in promoter activation of pro-inflammatory genes has been well documented, which raises a question as to whether OGG1’s oxidation and PARylation cooperate in transcriptional activation of pro-inflammatory genes. The results of our studies and others’ urge us to reassess the pathophysiological significance of hampered OGG1 BER activity in transcription modulation and other cellular processes.

Studies by others have also depicted mechanisms by which OGG1 plays key roles in transcriptional modulation of gene expression, where OGG1 functions as a bona fide repair enzyme, and transcription is achieved upon the formation of the strand break or AP site. These observations may suit the regulation of genes downstream of different signaling pathways, or in other types of cells. Since OGG1 requires reducing cellular conditions for base excision, the pathophysiological contexts of these studies may reflect a post-ROS-assault cellular status. As far as the G-rich promoters of the pro-inflammatory cytokines/chemokines are concerned, potential PQS has also been suggested by a study utilizing a computational approach. Formation of G4 on the

**FIGURE 5** Substrate binding is required for OGG1-dependent pro-inflammatory gene expression. ROS generate 8-oxoG in the promoter region. A, Oxidative inactivation, posttranslational modifications or mutations that halt enzymatic activity but allow binding and pre-excision steps by OGG1 are exploited to promote gene expression. B, Mutation(s) or inhibitors of OGG1 that blocks substrate engagement inhibits OGG1 function in gene expression.
coding strand or template strand leads to either a boost or block of transcription.\textsuperscript{25} Thus, the regulatory role of the G4, if it forms, may not be for a prompt transcriptional activation of genes.

The lack of phenotype and the absence of a significant elevation in the mutation rate in \textit{Ogg1}\textsuperscript{−/−} mice\textsuperscript{62-64} implied that 8-oxoG may not readily mis-pair with A during DNA replication or transcription. The experimental evidence shown by our group and others’ studies suggests that 8-oxoG functions as an OS-generated epigenetic-like marker, which, through engagement with its cognate protein OGG1, regulates the ROS-responding transcriptome. Immune defects in the \textit{Ogg1}\textsuperscript{−/−} mice\textsuperscript{65-67} and the established involvement of OGG1 in the modulation of NF-κB-dependent transcriptional activation of cytokines/chemokines suggest a translational significance for targeting OGG1 inhibition. Moreover, the results generated based on the OGG1 mutants suggest that a positive association between some single nucleotide polymorphisms of OGG1 (eg, OGG1 Ser326Cys) in certain populations with aging-related diseases (eg, malignancies)\textsuperscript{68,69} may be due to the deviations/variations from the coordination between the OGG1-mediated transcriptional regulation and the OGG1-initiated repair process, rather than the insufficient removal of 8-oxoG.

5 | CONCLUSIONS

The transcriptional regulation of pro-inflammatory genes is an important component of innate immune responses, which are associated with changes in cellular redox homeostasis.\textsuperscript{23,25,70} Paradoxically, guanine in gene regulatory regions and the repair enzyme OGG1 are targets of ROS. Oxidative modification of OGG1 halts DNA repair, raising the possibility that oxidatively modified OGG1 (possible with other posttranslational modifications) has a fundamentally different function. The data of the present study provide evidence that enzymatically inactivated OGG1 binds with but does not cleave its substrate, which steers DNA BER toward modulation of gene expression (Figure 5). With regard to the therapeutic aspect, we affirm that the pharmacological inhibition of OGG1 binding to DNA is a promising strategy for treatment of inflammation-related diseases of humans. Finally, our studies revealed that a tight and elaborate spatiotemporal control has evolved in aerobic cells to meet their need for timely gene transcription and the preservation of the integrity of the DNA template.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

W. Hao, I. Boldogh, and X. Ba designed research; W. Hao, J. Wang, Y. Zhang, C. Wang, L. Xia, W. Zhang, M. Zafar, J. Kang, R. Wang, A.A Bohio, and L. Pan performed research; W. Hao, J. Wang, and C. Wang analyzed data; W. Zhang and L. Xia validate the results; W. Hao wrote the paper; W. Hao, X. Zeng, M. Wei, I. Boldogh, and X. Ba review and editing the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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