RESEARCH ARTICLE

THE FASEB JOURNAL
The Journal of the Federation of American Societies for Experimental Biology

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

Wenjing Hao[1,2] | Jing Wang[3] | Yuanhang Zhang[1,2] | Chenxin Wang[1,2] | Lan Xia[1,2] | Wenhe Zhang[1,2] | Muhammad Zafar[1,2] | Ju-Yong Kang[1,4] | Ruoxi Wang[1,2,5] | Ameer Ali Bohio[1,2] | Lang Pan[2,6] | Xianlu Zeng[1,2] | Min Wei[1,2] | Istvan Boldogh[6] | Xueqing Ba[1,2]

[1]Key Laboratory of Molecular Epigenetics of Ministry of Education, Northeast Normal University, Changchun, China

[2]School of Life Science, Northeast Normal University, Changchun, China

[3]Department of Respiratory Medicine, China-Japan Union Hospital of Jilin University, Changchun, China

[4]Faculty of Life Science, Kim Il Sung University, Pyongyang, DPRK

[5]Key Laboratory of Animal Resistance Biology of Shandong Province, Institute of Biomedical Sciences, College of Life Sciences, Shandong Normal University, Jinan, China

[6]Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, Galveston, TX, USA

Correspondence
Xueqing Ba, Key Laboratory of Molecular Epigenetics of Ministry of Education, School of Life Sciences, Northeast Normal University, 5268 Renmin Street, Changchun, Jilin 130024, China.
Email: baxq755@nenu.edu.cn

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;

whereas, mutant OGG1 with impaired substrate recognition/binding was not. These data suggested the interaction of OGG1 with its substrate at regulatory regions followed by conformational adjustment in the adjacent DNA is the primary mode to modulate inflammatory gene expression.

KEYWORDS

8-oxoG, OGG1, oxidative stress, pro-inflammatory gene expression

1 | 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).[1,2] 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.[1,3,4] In DNA, guanine is a preferential target for oxidative modification due to its lowest ionization potential,[5-7] 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).[8] Both 8-oxoG and FapyG together constitute the most abundant DNA damages and are regarded as cellular biomarkers of oxidative stress (OS).[9] 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 pathophysiologic processes including aging-associated diseases and carcinogenesis.[10-12]

In eukaryotic cells, repair of oxidatively modified guanines (8-oxoG, FapyG) is primarily initiated by 8-oxoguanine DNA glycosylase1 (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.[13] OGG1's in vivo β-lyase activity is under examination as data suggest that OGG1 is a mono-functional enzyme.[14,15] 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′-deoxyribose-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.[16-18]

Although guanine is vulnerable to ROS and 8-oxoG is mutagenic, evolutionarily, genes tend to be embedded in the GC-rich genomic regions,[19-21] and 72% of human gene promoters are guanine-rich.[22] In addition, the transcriptional activity of genes is positively correlated with the GC content in genome.[19] 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.[3,14,19,23-29] 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.[24,27] 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.[28] 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.[19,28-31] 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.[32]

On the contrary, our previous studies have shown that mRNA levels of *TNF*, *CXCL*s, *CCL*s, and *IL*s 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.[25,33,34] These pro-inflammatory cytokine/chemokine genes usually possess G-clusters in their promoter regions that have been proposed to trap positive charge at 5' of runs of guanine due to the long-distance migration of charge along DNA duplex matrix, thereby to protect gene bodies from ROS attacks.[35] Accumulation of 8-oxoG at the proximal promoters of human *TNF*, *CCL20*, and *CXCL1*, and mouse *Tnf*, *Il-1β*, and *Cxcl2* genes, indeed, increased in response to the pro-inflammatory stimuli.[25,33] 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.[33,36-38] 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.[25,33,39]

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.[40,41] Replacement of cysteine (C) 253 with alanine (A) leads to the perturbed substrate engagement, and thereby the decrease in glycosylase activity of OGG1.[15] The expression constructs were transfected into OGG1-knockdown HEK 293 or *Ogg1*$^{-/-}$ MEF cells. Chromatin recruitment of wt OGG1 and the mutants, as well as pro-inflammatory gene expression were determined using various molecular approaches. The results showed that under OS wt and the OGG1 mutants having the ability to bind with 8-oxoG but lacking the enzymatic activity, are potent modulators of inflammatory gene transcription in TNF-exposed cells. These data support the hypothesis that binding of OGG1 with the substrate without lesion excision is key to regulating OS-induced pro-inflammatory gene expression.

2 | MATERIALS AND METHODS

2.1 | Antibodies and reagents

The monoclonal antibody (Ab) against FLAG (Cat # F1804) was purchased from Sigma (Saint Louis, MO, USA). The monoclonal antibody against GFP (Cat # HT801) and β-Actin (Cat # HT201) were purchased from TRANS (Beijing, China). The polyclonal antibody (Ab) against Biotin (Cat # ab53494) was purchased from Abcam (San Francisco, CA). Vanadyl ribonucleoside complexes (VRC) solution (Cat # 94742) was purchased from Sigma (Saint Louis, MO, USA). Salmon sperm DNA solution (Cat # H1060) was purchased from Solarbio Life Science (Beijing, China). Formamide (Cat # A100314) was purchased from Sangon Biotech (Shanghai, China). OGG1 inhibitor 4-(4-Bromo-2-oxo-3H-benzimidazol-1-yl)-N-(4-iodophenyl) piperidine-1-carboxamide (TH5487, Cat # PC-35806) was purchased from ProbeChem (Shanghai, China). About 3,4-Dichloro-benzo[b]thiophene-2-carboxylic acid hydrazide (O8, Cat # SML1697) was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Dextran sulfate sodium salt powder (DE136) was purchased from Spectrum Chemical MFG Corp (Solon, Ohia, USA).N-acetyl-$_L$-cysteine (NAC, Cat # A7250) was from Sigma-Aldrich (St Louis, MO, USA). GFP beads (Cat # CH10001) were purchased from the Chenghe Inc (Jilin, China).

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 hours, 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.[25,39]

The plasmid pRL-SV40 encoding Renilla luciferase driven by an SV40 promoter (Promega) was used as an internal control as described previously.[25] 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 *EcoR* I and *Xho* I. 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 × 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.[42]

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 × SDS sample buffer, separated by 10% of SDS-PAGE, and transferred onto

TABLE 1 Oligos used in EMSA and/or cleavage assay

Probe name	Sequence
WT probe	Sense: 5′-TTCCCTGGTCCCCGGGCTTTTCCAGACATCG-3′
	Anti-sense: 5′-biotin-CGATGTCTGGAAAAGCCCGGGGACCAGGGAA-3′
G2 probe	Sense: 5′-TTCCCTGGTCCCCGGGCTTTTCCAGACATCG-3′
	Anti-sense: 5′-biotin-CGATGTCTGGAAAAGCCCGGGGACCA(G*)GGAA-3′
Cy5 probe	Sense: 5′-AGAGAAGAAGAAGAA(G*)AGATGGGTTATTCGAACTAGC-Cy5Sp/3′
	Antisense: 3′-TCTCTTCTTCTTCTTCTCTACCCAATAAGCTTGATCG-5′

Note: WT probe and G2 probe are derived from mouse *Cxcl2* promoter and were used in previous study. WT refers to no 8-oxoG-modified oligoes, whereas G2 probe contains a single 8-oxoG in antisense strand.[34] Cy5 probe is a 40-mer oligonucleotide containing an 8-oxoG at position 19 and labeled at the 3′ end with Cy5.[42] (G*) signifies the inclusion of 8-oxoG.

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 $\Delta\Delta$ 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 *Cxcl*2-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 *Cxcl*2 promoter was represented by the mRNA level of firefly luciferase normalized to that of Renilla. Expression was calculated by the $\Delta\Delta$ 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% of paraformaldehyde (Dingguo, China) for 10 minutes at room temperature, permeabilized with 0.5% (v/v) of Triton X-100 for 30 minutes, and then, washed twice with 40% of formamide for 10 minutes at room temperature. Thereafter, cells were preincubated 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 60× 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′-TTGACCGCCCTT GAGAGTGGCTATGACTTCTGTCTGGGCGCAGTGG GGTCCTGGGGGCG-3′-Cy3. Cells transfected with YFP,

TABLE 2 qRT-PCR primers used in this study

Gene name	Sequence
Gapdh	F: 5′-AAT GGT GAA GGT CGG TGT G-3′
	R: 5′-GTG GAG TCA TAC TGG AAC ATG TAG-3′
Cxcl2	F: 5′-CAG AAG TCA TAG CCA CTC TCA AG-3′
	R: 5′-CTT TCC AGG TCA GTT AGC CTT-3′
Tnfα	F: 5′-AGA CCC TCA CAC TCA GAT CA-3′
	R: 5′-TCT TTG AGA TCC ATG CCG TTG-3′
Il-1β	F: 5′-ACTGTTTCTAATGCCTTCCC-3′
	R: 5′-ATGGTTTCTTGTGACCCTGA-3′
CXCL1	F: 5′-TCTCTCTTTCCTCTTCTGTTCCTA-3′
	R: 5′-CATCCCCCATAGTTAAGAAAATCATC-3′
Renilla luciferase	F: 5′-AGC CAG TAG CGC GGT GTA TT-3′
	R: 5′-TCA AGT AAC CTA TAA GAA CCA TTA CCA GAT T-3′
Firefly luciferase	F: 5′-CTG AAC AGC ATG GGC ATC A-3′
	R: 5′-AAA TGG AAG TC 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-cm^2 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 signaling technology, US). Primers for PCR amplification (CXCL1 promoter) are F: 5′-GGAGTTACTCTGAAGGGCGAG-3′; R: 5′-AAAGGGGTTCGCGGATCTC-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-OGG1were pretreated with or without 10 mM 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 mM 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) pentanoate (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 $\pm$ 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 $\pm$ 0.14-fold and 2.7 $\pm$ 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.[15,41,44] The amounts of DNA-protein complexes decreased to ~ 0.3 $\pm$ 0.05 and 0.16 $\pm$ 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 $Ogg1^{-/-}$ 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,B, upper panels), *Tnf* and *Il-1β* (Figure S2) after TNFα exposure and served as a control. While wt OGG1 augmented the expression of *Cxcl2*,

Tnf, and *Il-1β* as observed in our previous studies,[25,33-34,39] 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
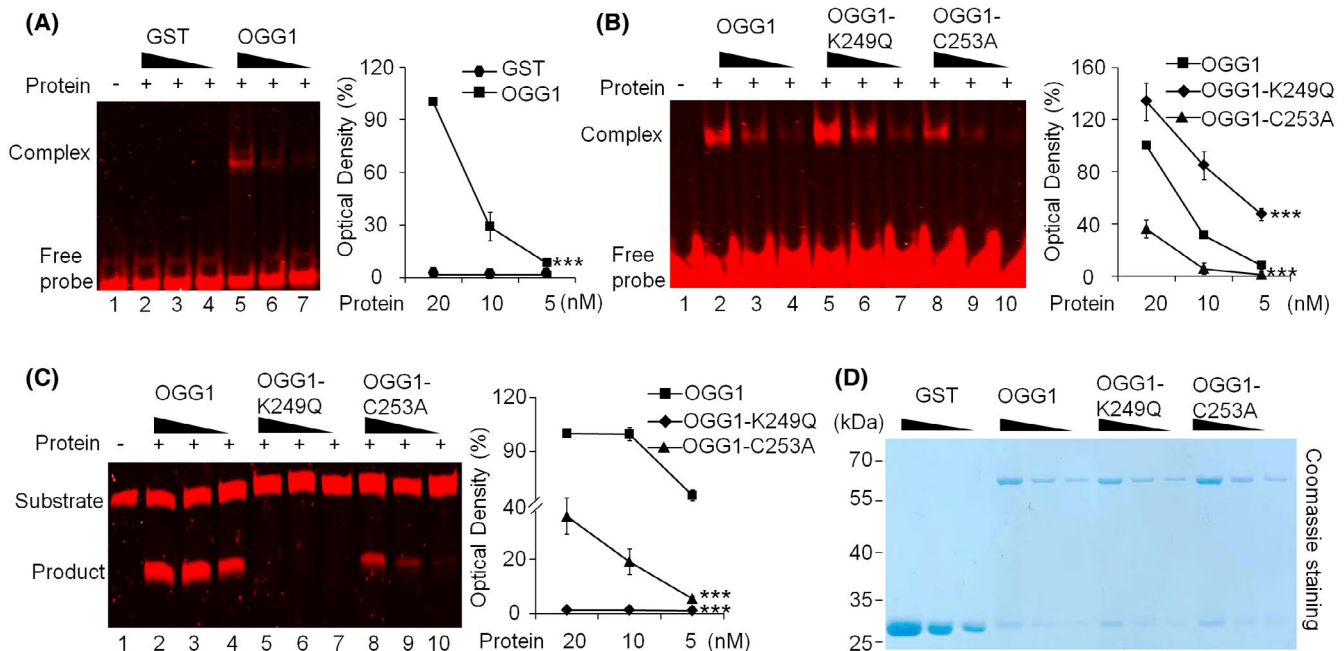

FIGURE 1 Binding and enzymatic activity of OGG1 mutant's. A and B, Binding of wt and mutant OGG1's to 8-oxoG-containing probes. Purified recombinant proteins (20, 10, and 5 nM) were incubated with 100 fmol 8-oxoG-containing Cy5-labeled DNA oligos (total volume is 10 μL) for 10 minutes on ice. Retardation 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 *Cxcl*2 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 over-expressed 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).

TNFα binding to its receptor leads to an increase in cellular ROS levels.[25,46,47] Recent studies have also shown that oxidation of cysteine residues, of which eight are present in OGG1, could be responsible for the decreased activity to excise 8-oxoG.[3,33,36,37] To test whether TNF-ROS induce oxidative modification to OGG1 at cysteine(s), we utilized cysteine sulfenic acid-reacting agent DCP-Bio1.[33] YFP-wt OGG1-expressing cells were exposed to TNFα for 60 minutes, cell extracts were made in the presence of DCP-Bio1, and immunoprecipitates were subjected to Western blotting. The result showed an increase in OGG1-associated cysteine sulfenic acid levels, a strong indication of OGG1 oxidation (Figure 4B), which was temporally correlated
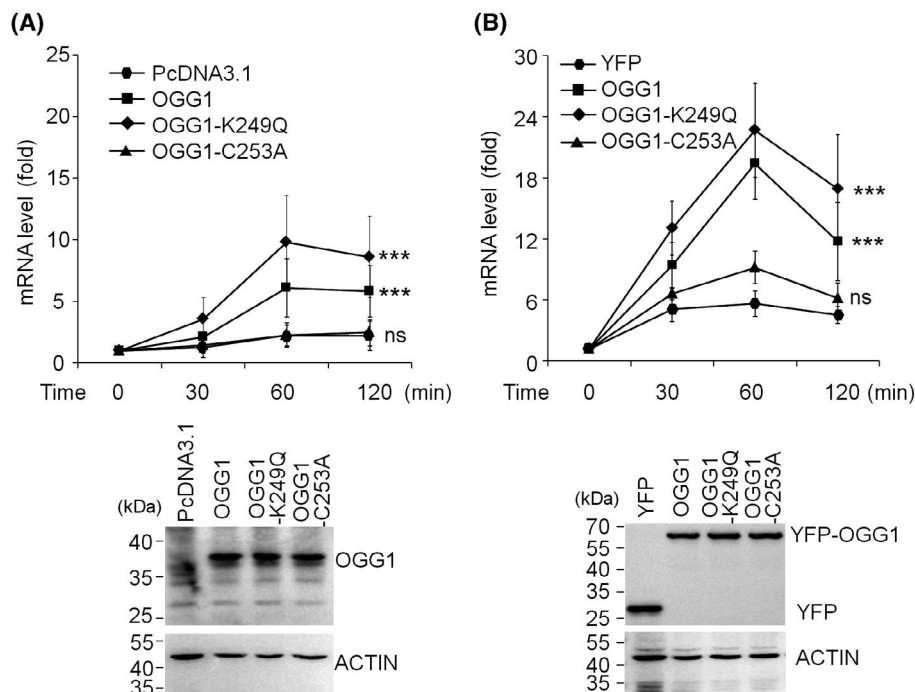

FIGURE 2 Binding to the DNA substrate but not base excision activity is required for OGG1 to modulate pro-inflammatory gene expression. *Ogg1*^{−/−} MEF cells were transfected with plasmids expressing Flag-tagged (A) or YFP-tagged (B) wt OGG1 or site-specific mutants, and then, exposed to 20 ng/mL TNFα for 0, 30, 60, and 120 minutes. *Cxcl*2 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)
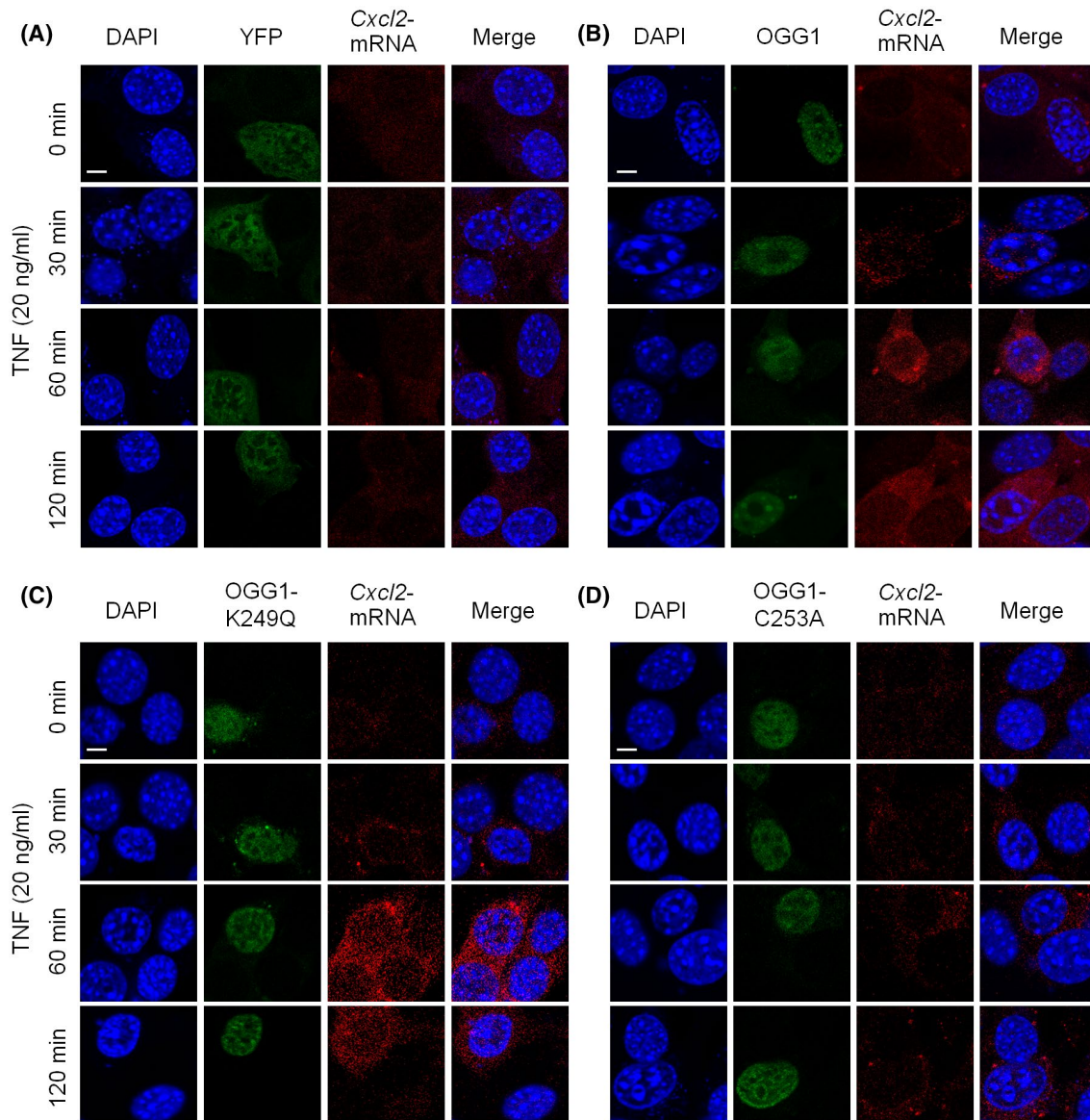
FIGURE 3 Expression of *Cxcl2* in wt and OGG1 variant-expressing cells as shown by FISH *Ogg1*⁻/⁻ MEF cells were transfected with plasmids expressing A, YFP, B, wt OGG1, C, OGG1 K249Q, D) OGG1 C253A, and then, exposed to 20 ng/mL TNFα for 0, 30, 60, and 120 minutes. The levels of *Cxcl2* mRNA were determined by fluorescence in situ hybridization (red). The nuclei of cells were counter stained with DAPI (red: *Cxcl2* mRNA; blue: DNA; green: OGG1). Bar, 10 µm. A representative result of three experiments is shown

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 binding[48] prevented TNFα-induced enrichment of OGG1 to 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
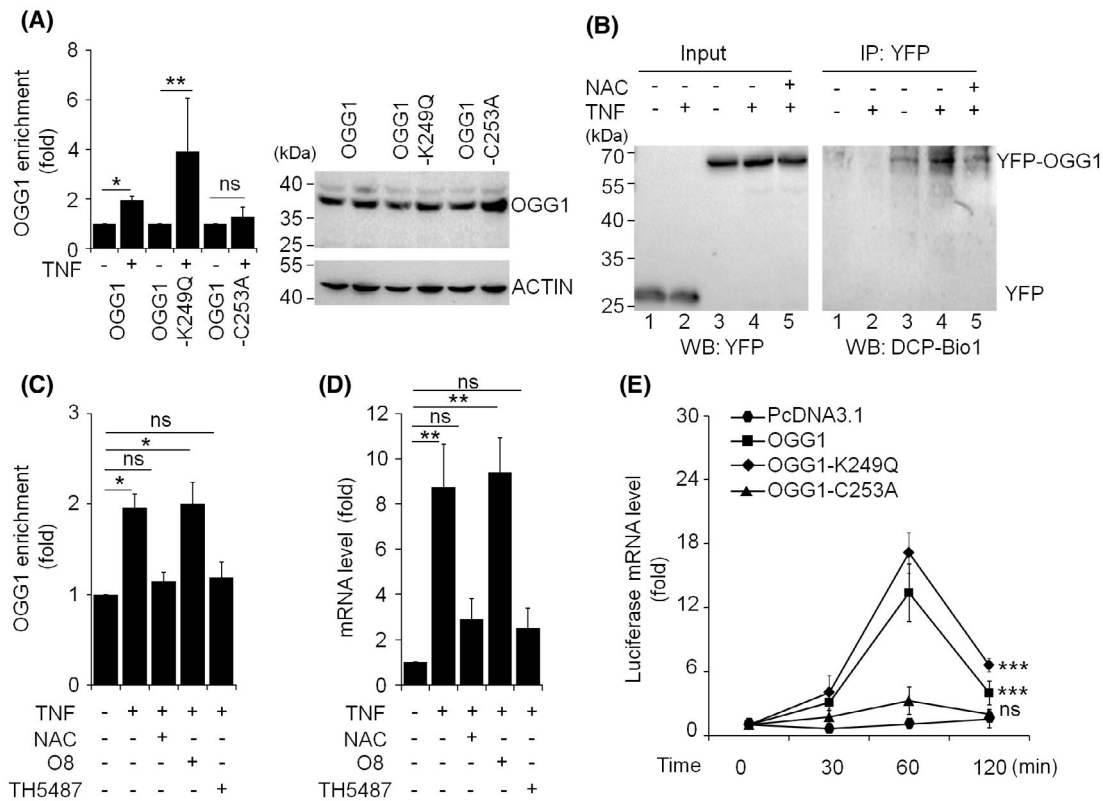
FIGURE 4 Recruitment of enzymatically inactive OGG1 to the promoter facilitates transcriptional activation of pro-inflammatory genes. A, Enrichment of wt OGG1 and the mutants on the *CXCL1* promoter in response to TNFα exposure. HEK 293 cells were transfected with Flag-tagged wt OGG1 or OGG1 mutants, and then, mock treated or TNF-exposed for 60 minutes. Enrichment of wt OGG1 and mutants on the *CXCL1* promoter was determined by ChIP-coupled qPCR (left panel). The right panel shows equal expression of each OGG1 in transfected cells. B, TNFα exposure induces oxidative modification of OGG1 at cysteine residues. YFP or YFP-wt OGG1-expressing cells were mock-treated or NAC (10 mM)-treated for 60 minutes, and then, exposed to TNFα for 60 minutes. Whole cell extracts were made in the presence of DCP-Bio1 (a cysteine sulfenic acid-reacting agent). Immunoprecipitation with YFP beads was conducted and levels of DCP-Bio1-tagged YFP-wt OGG1 were determined by western blot. C, The effects of OS and inhibitors on OGG1 DNA interactions. HEK 293 cells were transfected with Flag-tagged wt OGG1, and then, exposed to TNFα for 60 minutes with or without pretreatment of NAC (10 mM), TH5487 (10 μM), or O8 (10 μM). ChIP was performed using antibody to Flag-tag. PCR amplification of the TSS adjacent *CXCL1* promoter region was performed to determine the enrichment of OGG1. D, The effects of oxidative inactivation or inhibitors on *CXCL1* gene expression. HEK 293 cells were exposed to TNFα for 60 minutes with or without pretreatment of NAC, TH5487, or O8. *CXCL1* gene expression was measured by real-time qPCR. E, The roles of wt OGG1 and the mutants in transcription activation were analyzed using a dual reporter assay. $Ogg1^{-/-}$ MEF cells expressing wt OGG1 or the mutants were co-transfected with a luciferase dual reporter system. Firefly luciferase mRNA expression is driven by the *Cxcl2* promoter. Cells were mock-treated or exposed to TNFα for 0, 30, 60, and 120 minutes. The mRNA levels of firefly luciferase were assessed by real-time qPCR. All experiments were performed three times. The data are presented as the means ± the standard error. A two-way analysis of variance indicated the significance of the difference. *$P < .05$ and ***$P < .001$, ns: no significance, compared with PcDNA3.1

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.[25] In-depth studies revealed that OGG1's interaction with its DNA substrate facilitated DNA occupancy of sequence-specific transcription factors on their motifs.[33,39,48] 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 "oxoG-specificity pocket".[41,50] Within the HhH-GPD domain, the lysine 249 residue of OGG1 is crucial due to its function as the catalytic nucleophile.[15,16] 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,[16] and the replacement of Cys 253 to Ala decreases OGG1 substrate binding and its base excision activity.[15]

The first structure of OGG1 with 8-oxoG-containing DNA was obtained by using an OGG1 K249Q mutant,[51] which revealed a sharp bend in the bound DNA. By utilizing enzyme mutants and real-time approaches, recent studies measured the dynamic changes in protein/DNA interaction and the consequent conformational alteration of DNA.[52] The decrease in intrinsic tryptophan fluorescence reveals OGG1/substrate interaction; the alteration of the fluorescence emitted from 2-aPu that is introduced in DNA probe reflects the eversion of 8-oxoG; and the increase in FRET signal from Cy3 to Cy5 that are labeled at the 5′ ends of annealed 8-oxoG-containing

DNA duplex aims to detect the bend of DNA. The results indicated that OGG1 K249Q interacts with 8-oxoG, bends the DNA and flips out the damaged base into the active pocket. Importantly, wt OGG1 exhibited a similar altered pattern of 2-aPu fluorescence, and a more dramatic change in FRET signal compared with OGG1 K249Q.[51] While OGG1 K249Q has been intensively studied, the effect of mutating the 253 cysteine to alanine has not. C253A mutant bound abasic DNA with similar affinity as the wt enzyme, but weaker for the 8-oxoG-containing DNA, thus, exhibiting impaired catalytic kinetics.[15] Cys 253 and Phe 319 interact with opposite π-faces of 8-oxoG, sandwiching the damaged base,[51] which may contribute to the interaction of OGG1 with its substrate.

Based on these structural and functional analyses, we constructed plasmids carrying various mutated OGG1s to examine the function of OGG1 in gene expression modulation. DNA binding and excision analyses in the present study revealed that OGG1 K249Q has no excision activity despite an increased engagement with substrate-containing DNA compared to wt OGG1, in line with reports showing a slower dissociation of OGG1 K249Q from substrate-containing DNA.[40,51,53] On the contrary, the replacement of Cys253 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.[15] 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.[25,33,36,37] Inactivation of OGG1 via cysteine oxidation does not interfere with genomic substrate binding, but halts its glycosylase activity.[3,23,25,33-34,39] 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 ε-amino group.[51] 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 *ILs*) under OS conditions.[3,25,33-34,39,48] Upon normalization of the cellular redox state, genome fidelity was accomplished via DNA-BER.[33,36,37] 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.[48,49] The small-molecule TH5487 binds OGG1's active site and inhibits its searching for and binding to 8-oxoG in the genome.[48] 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,[49] increased the enrichment of OGG1 on the *CXCL1* promoter and gene expression.[48] 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.[54] 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).[55] The interaction with PARP1 and poly(ADP-ribosy)lation (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,[56-59] 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.[19,23-24,60] 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.[61] Formation of G4 on the
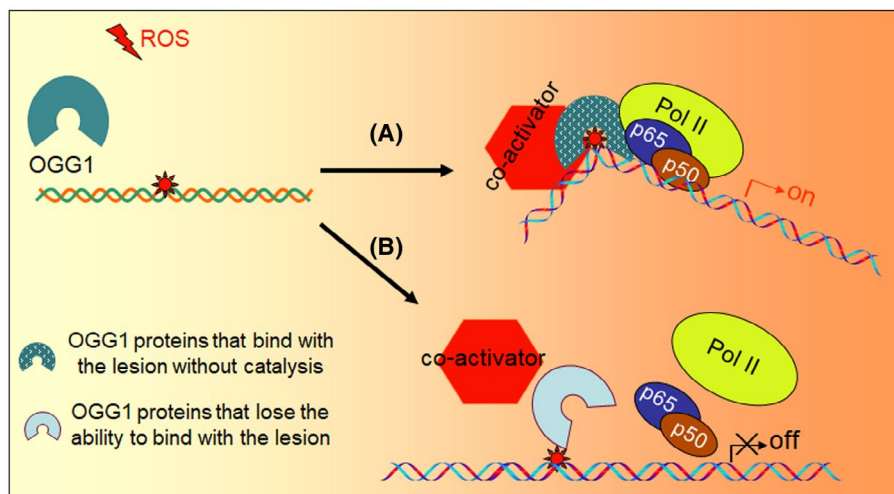

FIGURE 5 Substrate binding is required for OGG1-dependent pro-inflammatory gene expression. ROS generate 8-oxoG in the promoter region. A, Oxidative inactivation, posttranslational modifications or mutations that halt enzymatic activity but allow binding and pre-excision steps by OGG1 are exploited to 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.[28] 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 $Ogg1^{-/-}$ mice[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 $Ogg1^{-/-}$ mice[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)[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.[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.

ACKNOWLEDGMENTS

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.

REFERENCES
1. Radak Z, Boldogh I. 8-Oxo-7,8-dihydroguanine: links to gene expression, aging, and defense against oxidative stress. *Free Radic Biol Med*. 2010;49:587-596.
2. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. *Curr Biol*. 2014;24:R453-R462.
3. Ba X, Boldogh I. 8-Oxoguanine DNA glycosylase 1: beyond repair of the oxidatively modified base lesions. *Redox Biol*. 2018;14:669-678.
4. Friedberg EC. DNA damage and repair. *Nature*. 2003;421:436-440.
5. Neeley WL, Essigmann JM. Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. *Chem Res Toxicol*. 2006;19:491-505.
6. Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis*. 1986;7:1849-1851.
7. Saito I, Takayama M, Nakamura T, Sugiyama H, Komeda Y, Iwasaki M. The most electron-donating sites in duplex DNA: guanine-guanine stacking rule. *Nucleic Acids Symp Ser*. 1995;34:191-192.
8. Boiteux S, Coste F, Castaing B. Repair of 8-oxo-7,8-dihydroguanine in prokaryotic and eukaryotic cells: properties and biological roles of the Fpg and OGG1 DNA N-glycosylases. *Free Radic Biol Med*. 2017;107:179-201.
9. Cadet J, Douki T, Ravanat JL. Oxidatively generated base damage to cellular DNA. *Free Radic Biol Med*. 2010;49:9-21.
10. Dherin C, Radicella JP, Dizdaroglu M, Boiteux S. Excision of oxidatively damaged DNA bases by the human alpha-hOgg1 protein and the polymorphic alpha-hOgg1(Ser326Cys) protein which is frequently found in human populations. *Nucleic Acids Res*. 1999;27:4001-4007.
11. Hart RW, Setlow RB. Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammalian species. *Proc Natl Acad Sci U S A*. 1974;71:2169-2173.

12. Audebert M, Radicella JP, Dizdaroglu M. Effect of single mutations in the OGG1 gene found in human tumors on the substrate specificity of the Ogg1 protein. *Nucleic Acids Res*. 2000;28:2672-2678.

13. Svilar D, Goellner EM, Almeida KH, Sobol RW. Base excision repair and lesion-dependent subpathways for repair of oxidative DNA damage. *Antioxid Redox Signal*. 2011;14:2491-2507.

14. Allgayer J, Kitsera N, Bartelt S, Epe B, Khobta A. Widespread transcriptional gene inactivation initiated by a repair intermediate of 8-oxoguanine. *Nucleic Acids Res*. 2016;44:7267-7280.

15. Dalhus B, Forsbring M, Helle IH, et al. Separation-of-function mutants unravel the dual-reaction mode of human 8-oxoguanine DNA glycosylase. *Structure*. 2011;19:117-127.

16. Bjoras M, Luna L, Johnsen B, et al. Opposite base-dependent reactions of a human base excision repair enzyme on DNA containing 7,8-dihydro-8-oxoguanine and abasic sites. *EMBO J*. 1997;16:6314-6322.

17. Wallace SS. Base excision repair: a critical player in many games. *DNA Repair*. 2014;19:14-26.

18. Izumi T, Wiederhold LR, Roy G, et al. Mammalian DNA base excision repair proteins: their interactions and role in repair of oxidative DNA damage. *Toxicology*. 2003;193:43-65.

19. Fleming AM, Ding Y, Burrows CJ. Oxidative DNA damage is epigenetic by regulating gene transcription via base excision repair. *Proc Natl Acad Sci U S A*. 2017;114:2604-2609.

20. D'Onofrio G, Jabbari K, Musto H, Alvarez-Valin F, Cruveiller S, Bernardi G. Evolutionary genomics of vertebrates and its implications. *Ann N Y Acad Sci*. 1999;870:81-94.

21. Gautier C. Compositional bias in DNA. *Curr Opin Genet Dev*. 2000;10:656-661.

22. Saxonov S, Berg P, Brutlag DL. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proc Natl Acad Sci U S A*. 2006;103:1412-1417.

23. Wang R, Hao W, Pan L, Boldogh I, Ba X. The roles of base excision repair enzyme OGG1 in gene expression. *Cell Mol Life Sci*. 2018;75:3741-3750.

24. Perillo B, Ombra MN, Bertoni A, et al. DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. *Science*. 2008;319:202-206.

25. Ba X, Bacsi A, Luo J, et al. 8-oxoguanine DNA glycosylase-1 augments proinflammatory gene expression by facilitating the recruitment of site-specific transcription factors. *J Immunol*. 2014;192:2384-2394.

26. Tornaletti S, Maeda LS, Kolodner RD, Hanawalt PC. Effect of 8-oxoguanine on transcription elongation by T7 RNA polymerase and mammalian RNA polymerase II. *DNA Repair*. 2004;3:483-494.

27. Amente S, Bertoni A, Morano A, Lania L, Avvedimento EV, Majello B. LSD1-mediated demethylation of histone H3 lysine 4 triggers Myc-induced transcription. *Oncogene*. 2010;29:3691-3702.

28. Fleming AM, Zhu J, Ding Y, Burrows CJ. 8-Oxo-7,8-dihydroguanine in the context of a gene promoter G-quadruplex is an on-off switch for transcription. *ACS Chem Biol*. 2017;12:2417-2426.

29. Ruchko MV, Gorodnya OM, Pastukh VM, et al. Hypoxia-induced oxidative base modifications in the VEGF hypoxia-response element are associated with transcriptionally active nucleosomes. *Free Radic Biol Med*. 2009;46:352-359.

30. Pastukh V, Roberts JT, Clark DW, et al. An oxidative DNA "damage" and repair mechanism localized in the VEGF promoter is

31. Fleming AM, Zhu J, Ding Y, Visser JA, Zhu J, Burrows CJ. Human DNA repair genes possess potential G-quadruplex sequences in their promoters and 5′-untranslated regions. *Biochemistry*. 2018;57:991-1002.

32. Fleming AM, Zhu J, Ding Y, Burrows CJ. Location dependence of the transcriptional response of a potential G-quadruplex in gene promoters under oxidative stress. *Nucleic Acids Res*. 2019;47:5049-5060.

33. Pan L, Zhu B, Hao W, et al. Oxidized guanine base lesions function in 8-oxoguanine DNA glycosylase-1-mediated epigenetic regulation of nuclear factor kappaB-driven gene expression. *J Biol Chem*. 2016;291:25553-25566.

34. Hao W, Qi T, Pan L, et al. Effects of the stimuli-dependent enrichment of 8-oxoguanine DNA glycosylase1 on chromatinized DNA. *Redox Biol*. 2018;18:43-53.

35. Giese B. Long-distance electron transfer through DNA. *Annu Rev Biochem*. 2002;71:51-70.

36. Bravard A, Vacher M, Gouget B, et al. Redox regulation of human OGG1 activity in response to cellular oxidative stress. *Mol Cell Biol*. 2006;26:7430-7436.

37. Bravard A, Campalans A, Vacher M, et al. Inactivation by oxidation and recruitment into stress granules of hOGG1 but not APE1 in human cells exposed to sub-lethal concentrations of cadmium. *Mutat Res*. 2010;685:61-69.

38. Morreall J, Limpose K, Sheppard C, Kow YW, Werner E, Doetsch PW. Inactivation of a common OGG1 variant by TNF-alpha in mammalian cells. *DNA Repair*. 2015;26:15-22.

39. Pan L, Hao W, Zheng X, et al. OGG1-DNA interactions facilitate NF-kappaB binding to DNA targets. *Sci Rep*. 2017;7:43297.

40. Nash HM, Lu R, Lane WS, Verdine GL. The critical active-site amine of the human 8-oxoguanine DNA glycosylase, hOgg1: direct identification, ablation and chemical reconstitution. *Chem Biol*. 1997;4:693-702.

41. Bruner SD, Norman DP, Verdine GL. Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature*. 2000;403:859-866.

42. German P, Szaniszlo P, Hajas G, et al. Activation of cellular signaling by 8-oxoguanine DNA glycosylase-1-initiated DNA base excision repair. *DNA Repair*. 2013;12:856-863.

43. Bjoras M, Seeberg E, Luna L, Pearl LH, Barrett TE. Reciprocal "flipping" underlies substrate recognition and catalytic activation by the human 8-oxo-guanine DNA glycosylase. *J Mol Biol*. 2002;317:171-177.

44. Banerjee A, Yang W, Karplus M, Verdine GL. Structure of a repair enzyme interrogating undamaged DNA elucidates recognition of damaged DNA. *Nature*. 2005;434:612-618.

45. Bruner SD, Norman DP, Fromme JC, Verdine GL. Structural and mechanistic studies on repair of 8-oxoguanine in mammalian cells. *Cold Spring Harb Symp Quant Biol*. 2000;65:103-111.

46. Yang D, Elner SG, Bian ZM, Till GO, Petty HR, Elner VM. Proinflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells. *Exp Eye Res*. 2007;85:462-472.

47. Tezel G. TNF-alpha signaling in glaucomatous neurodegeneration. *Prog Brain Res*. 2008;173:409-421.

48. Visnes T, Cazares-Korner A, Hao W, et al. Small-molecule inhibitor of OGG1 suppresses proinflammatory gene expression and inflammation. *Science*. 2018;362:834-839.

49. Donley N, Jaruga P, Coskun E, Dizdaroglu M, McCullough AK, Lloyd RS. Small molecule inhibitors of 8-oxoguanine DNA glycosylase-1 (OGG1). *ACS Chem Biol*. 2015;10:2334-2343.

50. van der Kemp PA, Charbonnier JB, Audebert M, Boiteux S. Catalytic and DNA-binding properties of the human Ogg1 DNA N-glycosylase/AP lyase: biochemical exploration of H270, Q315 and F319, three amino acids of the 8-oxoguanine-binding pocket. *Nucleic Acids Res*. 2004;32:570-578.

51. Koval VV, Knorre DG, Fedorova OS. Structural features of the interaction between human 8-oxoguanine DNA glycosylase hOGG1 and DNA. *Acta Naturae*. 2014;6:52-65.

52. Kuznetsova AA, Kuznetsov NA, Ishchenko AA, Saparbaev MK, Fedorova OS. Step-by-step mechanism of DNA damage recognition by human 8-oxoguanine DNA glycosylase. *Biochim Biophys Acta*. 2014;1840:387-395.

53. Tyugashev TE, Vorobjev YN, Kuznetsova AA, Lukina MV, Kuznetsov NA, Fedorova OS. Roles of active-site amino acid residues in specific recognition of DNA lesions by human 8-oxoguanine-DNA glycosylase (OGG1). *J Phys Chem B*. 2019;123:4878-4887.

54. Cividini F, Scott BT, Dai A, et al. O-GlcNAcylation of 8-oxoguanine DNA glycosylase (Ogg1) impairs oxidative mitochondrial DNA lesion repair in diabetic hearts. *J Biol Chem*. 2016;291:26515-26528.

55. Noren Hooten N, Kompaniez K, Barnes J, Lohani A, Evans MK. Poly(ADP-ribose) polymerase 1 (PARP-1) binds to 8-oxoguanine-DNA glycosylase (OGG1). *J Biol Chem*. 2011;286:44679-44690.

56. Ke Y, Wang C, Zhang J, et al. The role of PARPs in inflammation-and metabolic-related diseases: molecular mechanisms and beyond. *Cells*. 2019;8:1047.

57. Ke Y, Zhang J, Lv X, Zeng X, Ba X. Novel insights into PARPs in gene expression: regulation of RNA metabolism. *Cell Mol Life Sci*. 2019;76:3283-3299.

58. Bohio AA, Sattout A, Wang R, et al. c-Abl-mediated tyrosine phosphorylation of PARP1 is crucial for expression of proinflammatory genes. *J Immunol*. 2019;203:1521-1531.

59. Liu L, Ke Y, Jiang X, et al. Lipopolysaccharide activates ERK-PARP-1-RelA pathway and promotes nuclear factor-kappaB transcription in murine macrophages. *Hum Immunol*. 2012;73:439-447.

60. Seifermann M, Ulges A, Bopp T, et al. Role of the DNA repair glycosylase OGG1 in the activation of murine splenocytes. *DNA Repair*. 2017;58:13-20.

61. Kumar P, Yadav VK, Baral A, Kumar P, Saha D, Chowdhury S. Zinc-finger transcription factors are associated with guanine quadruplex motifs in human, chimpanzee, mouse and rat promoters genome-wide. *Nucleic Acids Res*. 2011;39:8005-8016.

62. Klungland A, Rosewell I, Hollenbach S, et al. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc Natl Acad Sci U S A*. 1999;96:13300-13305.

63. Minowa O, Arai T, Hirano M, et al. Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice. *Proc Natl Acad Sci U S A*. 2000;97:4156-4161.

64. Arai T, Kelly VP, Minowa O, Noda T, Nishimura S. The study using wild-type and Ogg1 knockout mice exposed to potassium bromate shows no tumor induction despite an extensive accumulation of 8-hydroxyguanine in kidney DNA. *Toxicology*. 2006;221:179-186.

65. Mabley JG, Pacher P, Deb A, Wallace R, Elder RH, Szabo C. Potential role for 8-oxoguanine DNA glycosylase in regulating inflammation. *FASEB J*. 2005;19:290-292.

66. Li G, Yuan K, Yan C, et al. 8-Oxoguanine-DNA glycosylase 1 deficiency modifies allergic airway inflammation by regulating STAT6 and IL-4 in cells and in mice. *Free Radic Biol Med*. 2012;52:392-401.

67. Touati E, Michel V, Thiberge JM, et al. Deficiency in OGG1 protects against inflammation and mutagenic effects associated with H. pylori infection in mouse. *Helicobacter*. 2006;11:494-505.

68. Karahalil B, Bohr VA, Wilson DM 3rd. Impact of DNA polymorphisms in key DNA base excision repair proteins on cancer risk. *Hum Exp Toxicol*. 2012;31:981-1005.

69. Zhou PT, Li B, Ji J, Wang MM, Gao CF. A systematic review and meta-analysis of the association between OGG1 Ser326Cys polymorphism and cancers. *Med Oncol*. 2015;32:472.

70. Sen CK, Packer L. Antioxidant and redox regulation of gene transcription. *FASEB J*. 1996;10:709-720.

SUPPORTING INFORMATION

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