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Whole transcriptome analysis reveals an 8-oxoguanine DNA glycosylase-1-driven DNA repair-dependent gene expression linked to essential biological processes

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Abstract

Reactive oxygen species inflict oxidative modifications on various biological molecules, including DNA. One of the most abundant DNA base lesions 8-oxo-7,8-dihydroguanine (8-oxoG) is repaired by 8-oxoguanine DNA glycosylase-1 (OGG1) during DNA base excision repair (OGG1-BER). 8-OxoG accumulation in DNA has been associated with various pathological and aging processes, while its role is unclear. The lack of OGG1-BER in *Ogg1*$^{-/-}$ mice resulted in decreased inflammatory responses, increased susceptibility to infections and metabolic disorders. Therefore, we proposed that OGG1 and/or 8-oxoG base may have a role in immune and homeostatic processes. To test our hypothesis, we challenged mouse lungs with OGG1-BER product 8-oxoG base and changes in gene expression were determined by RNA sequencing and data were analyzed by gene ontology and statistical tools. RNA-Seq analysis identified 1592 differentially expressed (≥ 3-fold change) transcripts. The upregulated mRNAs were related to biological processes,

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including homeostatic, immune-system, macrophage activation, regulation of liquid-surface tension, and response to stimulus. These processes were mediated by chemokines, cytokines, gonadotropin-releasing hormone receptor, integrin and interleukin signaling pathways. Taken together, these findings points to a new paradigm showing that OGG1-BER plays a role in various biological processes that may benefit host, but when is in excess could be implicated in disease and/or aging processes.

Keywords

OGG1-BER; 8-oxoguanine; gene expression; biological processes

Introduction

Reactive oxygen species (ROS) are produced as a by-product of cellular metabolism and/or as a consequence of environmental insults. Due to their reactivity, ROS modify biological molecules, including DNA. The accumulation of ROS-induced base and strand modifications, as well as their defective or decreased repair, has been associated with various diseases and aging processes [1, 2]. Due to guanine's highest susceptibility to reactive species among DNA bases [3, 4], 8-oxoG is one of the most abundant. 8-OxoG(syn) mispairs with adenine during DNA replication, resulting in a G:C to T:A transversion mutation. The mutagenic burden imposed by 8-oxoG is prevented primarily by OGG1-initiated DNA base excision repair pathway (OGG1-BER), which is a multi-step process involving recognition and excision of the damaged base, followed by cleavage of the DNA backbone. The resulting apurinic/apyrimidinic site is then processed by the apurinic/apyrimidinic endonuclease 1, and the DNA is repaired in sequential steps of BER [1, 5, 6]. Due to OGG1-BER, 8-oxoG levels in body fluids such as urine are one of the best biomarkers of environmental exposures and inflammatory processes [7, 8].

To study the consequences of 8-oxoG accumulation in DNA and role of OGG1 in patho-physiological processes, OGG1 knock out (*Ogg*1$^{-/-}$) mice were developed [9, 10]. Intriguingly, the resulting supraphysiological levels of genomic 8-oxoG did not affect embryonic development or life span. Moreover, increased levels of 8-oxoG in the mitochondrial DNA did not affect mitochondrial respiratory parameters or ROS production [11]. Under conditions of chronic oxidative stress, genomic 8-oxoG levels can be increased up to 250-fold in *Ogg*1$^{-/-}$ mice, without apparent consequences, including precancerous lesions or tumors in various organs [12]. More surprisingly, *Ogg1*$^{-/-}$ mice have shown a decreased inflammatory response to various stimuli, including lipopolysaccharide (LPS) [13], consequently increased susceptibility to bacterial infection [14]. A lack of 8-oxoG repair by OGG1 in airway epithelium decreased allergic airway inflammation in mouse models of asthma [15, 16]. Intriguing studies also documented that, following administration of a high-fat diet, *Ogg1*$^{-/-}$ mice have increased plasma insulin levels, impaired glucose tolerance, enhanced adiposity and hepatic steatosis when compared to wild-type animals [17]. These data together suggest that OGG1 and/or its repair product 8-oxoG base may play an important role in signaling various cellular physiological and pathophysiological processes.

Recent studies have shown that OGG1 binds its repair product, 8-oxoG base, at sites other than its catalytic center, and forms an OGG1•8-oxoG complex. This complex interacts with and activates canonical small GTPases and induces guanine nucleotide exchange (GDP→GTP) *in vitro* and *in cellulo* [18-21]. Moreover, studies showed that activation of OGG1-BER was associated with an increase in RAS-GTP levels as well as phosphorylation of downstream RAS targets, including v-*raf-1* murine leukemia viral oncogene homolog 1 (RAF1), mitogen-activated protein kinase (MAPK) kinase (MEK1/2), extracellular signal-regulated kinase (ERK1/2), phosphoinositide 3-kinase [18, 19], and an innate immune response [22].

The goal of the present study was to define the role of OGG1-BER in overall gene expression and identify its involvement in biological processes. To mimic OGG1-BER, mouse lungs were challenged with 8-oxoG base via the intranasal route, and changes in whole transcriptome were assessed by RNA-Seq analysis. Results showed that out of 23,337 identified transcripts, 8-oxoG challenge induced upregulation of 983 and downregulation of 1,398. Gene ontology analysis of RNA-Seq data by using the PANTHER classification system showed that the upregulated transcripts were related to various biological processes, among which homeostatic and immune system processes were the most represented. Thus, we document for the first time that, in addition to maintaining genome fidelity, OGG1-BER plays a role in a range of cellular and biological processes that are essential and could either benefit a host or be implicated, when in excess, in diseases or aging processes.

Materials and Methods

Animals and treatment

Animal experiments were performed according to the NIH Guide for Care and Use of Experimental Animals and approved by the University of Texas Medical Branch (UTMB) Animal Care and Use Committee (approval no. 0807044A). Eight-week-old female BALB/c mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used for these studies. Mice (n = 5 per group) were challenged intranasally (i.n.) with 60 μL of pH-balanced 8-oxoG solution (pH: 7.4; 0.005 μg per gram), or saline under mild anesthesia [18]. LPS was below detectable levels in all reagents. Animals were sacrificed at various time points to isolate lung protein/extracts, RNA, and bronchoalveolar lavage fluid (BALF).

RNA isolation

After intranasal challenges, mouse lungs were excised and homogenized with a TissueMiser® (Fisher). RNA was extracted using an RNeasy kit (Qiagen, Valencia, CA) per the manufacturer's instructions. Briefly, lung tissue homogenate was loaded onto an RNeasy column and subjected to washes with RW1 and RPE buffers. RNA was eluted by using the RNase-free water included in the kit. Eluted RNA was digested with RNase-free DNase as previously described [23]. The RNA concentration was determined spectrophotometrically on an Epoch Take-3™ system (Biotek, Winooski, VT) and by using the software Gen5 v2.01. Equal amounts of RNA from each mouse lung within an experimental group (n = 5) were pooled and analyzed in triplicate. Quality of the total RNA was confirmed spectrophotometrically by using the 260/280 nm ratio, which varied from 1.9 to 2.0. RNA integrity was also evaluated by agarose gel electrophoresis.

Library construction and next-generation RNA sequencing

Library construction and deep sequencing analysis were performed in UTMB's Next-Generation Sequencing (NGS) Core Facility (Director: Dr. Thomas G. Wood). RNA sequencing analysis was carried out on an Illumina HiSeq 1000 sequencing system (Illumina Inc., San Diego, CA, USA). Poly-A+ RNA was selected from total RNA (1 μg) using poly-T oligo-attached magnetic beads. Bound RNA was fragmented by incubation at 94°C for 8 minutes in 19.5 μl of fragmentation buffer (Illumina, Part # 15016648). First- and second-strand synthesis, adapter ligation, and amplification of the library were performed using the Illumina TruSeq RNA Sample Preparation kit per the manufacturer's instructions (Illumina). Samples were tracked through the “index tags” incorporated into the adapters. Library quality was evaluated utilizing an Agilent DNA-1000 chip on an Agilent 2100 Bioanalyzer. Library DNA templates were quantitated by qPCR and a known-size reference standard.

Cluster formation of the library DNA templates was performed using the TruSeq PE Cluster Kit v3 (Illumina) and the Illumina cBot workstation under the conditions recommended by the manufacturer. The template input was adjusted to obtain a cluster density of 700-1000 K/mm^2. Paired-end, 50-base sequencing by synthesis was performed with a TruSeq SBS kit v3 (Illumina) on an Illumina HiSeq 1000 by protocols defined by the manufacturer. Base call conversion to sequence reads was performed using CASAVA-1.8.2. Sequence data were analyzed with the Bowtie2, Tophat and Cufflinks programs using NCBI's mouse (*Mus musculus*) genome build reference mm10. RNA-Seq data have been deposited in the NCBI's Gene Expression Omnibus (GEO) and are accessible through GEO Series accession number (GSE61095). Reads per kilobase transcript per million (RPKM) were normalized for each experimental group to its corresponding control [24].

Gene expression analysis

Heat maps and hierarchical clusters from whole transcriptomes were constructed with GENE-E software from Broad Institute (http://www.broadinstitute.org/cancer/software/GENE-E/). Venn diagrams were constructed by using online Venny software (http://bioinfogp.cnb.csic.es/tools/venny/). Gene ontologies (GO) and signaling pathways were analyzed by the PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System (http://www.pantherdb.org/) version 9.0. The lists of differentially expressed genes (fold change ≥3) were processed via GO annotations in this database (22160 genes, for Mus musculus). PANTHER's overrepresentation test uses the binomial method with Bonferroni correction for multiple comparisons to annotate classification categories for a list of genes [25, 26]. Significance was considered at *P* values < 0.05. The overrepresentation test was used to identify functional classes from the submitted gene lists according to PANTHER's reference lists. This test assumes that under the null hypothesis, genes in the uploaded list are sampled from the same general population as are genes from the reference set, i.e., the probability p(C) of observing a gene from a particular category C in the uploaded list is the same as in the database reference list [25].

Down-regulation of Ogg1

Mouse airways were depleted of OGG1 by RNAi as previously described [15]. We introduced Stealth™ RNAi (Invitrogen Life Technologies, Carlsbad, CA, USA. Cat # MSS237431) i.n. in transfection reagent (Polyplus-transfection SA, New York, NY, Cat# 201-10G) to target *Ogg1*. Control mice were treated with negative control RNAi (Invitrogen, Cat# 4404020). Oligos were of *in vivo* purity as described by the manufacturer. Mouse lungs were pre-treated with RNAi at 0 and 25 h and challenged with 8-oxoG or saline 48 h later.

Cytokine Bio-Plex assay

BALF samples were collected after 2h of exposure to 8-oxoG or saline and centrifuged (800 × g for 5 min at 4°C), and the resulting supernatants were stored at -80°C for further analysis of chemokines and cytokines [23, 27]. We used the Bio-Plex Pro™ Mouse Cytokine Assay (Bio-Rad, Hercules, CA), a bead-based, multiplex protein immunoassay (Bio-Rad, Cat # M60-009RDPD) [28], and processed BALF samples (n=3) and cytokine standards (in triplicate) per the manufacturer's instructions. Readings were performed on a Bioplex® 200™ system (Bio-Rad). Data analysis was performed using Bio-Plex Manager™ Software Version 6.0 Built 617 (Bio-Rad).

Assessment of RAS-GTP levels

RAS-GTP levels were quantified with the Active RAS pull-down assay kits (Pierce, Thermo Scientific, Inc) per the manufacturer's instructions, with slight modifications. Freshly isolated mouse lungs were homogenized in 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 60 mM $MgCl_2$, 1% Nonidet P-40, and 5% glycerol, and 500 μg of lung protein homogenate was incubated with the RAS-binding domain of Raf1 immobilized to glutathione resin, as we previously described [18]. After washing with binding buffer, GTP-bound RAS was eluted with Laemmli buffer (0.125 m Tris-HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, pH 6.8) and subjected to Western blotting. Total RAS was evaluated by Western blotting by using 20 μg of protein from the same lung lysates provided for pull-down assays.

qRT-PCR

Total RNA (1 μg) was reverse-transcribed using the SuperScript® III First-Strand Synthesis System (Invitrogen) per the manufacturer's instructions. To evaluate transcript levels for selected genes, we used the ΔΔCt method, as previously described [23]. The commercial (IDT) validated primers were: *Cxcl1*, Cat # Mm.PT.58.42076891; *Cxcl2*, Cat # Mm.PT.530.16380094; *Ccl3*, Cat # Mm.PT.58.29283216; *Tnf*, Cat # Mm.PT.S6a.12S7S861; *Il1a*, Cat # Mm.PT.58.32778767 and *Il1b*, Cat # Mm.PT.58.41616450. Housekeeping gene primers were: *Hprt*, Cat # Mm.PT.S30.32092191. qRT-PCR was performed on an ABI7000 thermal cycler. The amplification thermal profile was: 2 min 50 °C, 10 min 95 °C, and 15 sec 95 °C followed by 1 min 60 °C (40 cycles). To confirm the presence of a single product after amplification, a dissociation stage was carried out: 15 sec, 95 °C ; 20 sec, 60 °C ; and 15 sec, 95 °C.

Statistical analysis

Statistical analysis was performed using Student's *t*-test or ANOVA, followed by post hoc tests: Bonferroni's and Dunnett's T3 with SPSS 14.0 software. The data are presented as the means ± the standard error of the mean. Differences were considered statistically significant at $p < 0.05$.

Results

The OGG1-BER product 8-oxoG induced changes in global transcriptome

Mouse lungs were challenged via the i.n. route with the 8-oxoG base product of OGG1-BER, and then BALFs, total RNA, and protein extracts were isolated. Preliminary characterizations by qRT-PCR showed that mRNA levels of tumor necrosis factor (*Tnf*), interleukin 1 alpha (*Il1a*) and chemokine (C-X-C motif) ligand 1 (*Cxcl1*) were increased from 30 min on, reaching a maximum at 60 min. Multiplex protein analysis identified *de novo*-synthesized soluble mediators (e.g., CXCL1, TNF-α) from 120 min on, which was similar to results in our previous studies [22, 29]. Therefore to define the primary effects of OGG1-BER (8-oxoG challenge) on global gene expression by RNA-Seq, we limited the exposure time to 0, 30, 60, and 120 min.

RNAs were pooled from challenged lungs (n = 5), RNA sequencing was carried out twice, and the average of the two consequent analyses is presented. A total of 23,337 transcripts were identified (GEO Series accession number GSE61095). To identify groups of transcripts with similar expression patterns, we performed an unsupervised clustering and generated a heat map from whole-transcriptome data using GENE-E online software (Broad Institute). Hierarchical clustering of whole-genome data from 30, 60 and 120 min rendered a dendogram including three major clusters of transcripts (Fig. 1A). In general, cluster 1 contained 5678 mostly upregulated transcripts at all time points. Transcripts (10106) in cluster 2 show either an immediate increase or decrease, and those that were not altered. Cluster 3 included transcripts (3090) without changes in their expression or that increased only at 120 min post exposure. Red represents expression values greater than 1-fold, and green shows expression less than 1-fold.

The number of upregulated and downregulated transcripts (≥ 3-fold, mRNAs, non-coding transcripts and miRNAs) in each cluster at every time point is depicted in Fig. 1B. Venn diagrams show the unique and overlapping differentially expressed transcripts at each time point after challenge with 8-oxoG (Fig. 1C) corresponding to transcript described in Fig. 1B. Whole transcriptome data showed upregulation (≥ 3-fold) of 158 common transcripts and 96, 118 and 251 unique transcripts at 30, 60 and 120 min, respectively. Besides the unique and common transcripts to all time points, only 360 upregulated transcripts were shared between any two time points (Fig. 1C, upper panel). The total of upregulated transcripts (≥ 3-fold) at any time point was 983, of which 570 corresponded to 60 min. Among the 1398 downregulated transcripts, 99 were common, while 110, 663, and 232 were unique at 30, 60, and 120 min, respectively. A total of 294 transcripts were shared between any two time points only (Fig. 1C, lower panel). Together, cumulatively 2,381 transcripts were differentially expressed (≥ 3-fold change) after 8-oxoG challenge.

Results show a continuous increase in the number of transcripts at all time points (Fig. 1D, upper panel). On the other hand, the numbers of downregulated transcripts were decreased from 60 min (Fig. 1D, lower panel). These results could be interpreted as the effect of *de novo* synthesized, soluble mediators in line with preliminary studies [22, 29]. Therefore, to characterize the impact of 8-oxoG challenge, in our analysis the 60-min time point was examined.

OGG1-BER induces gene expression involved in various biological processes

Next, the list containing 570 upregulated transcripts from RNA-Seq at 60 min post 8-oxoG exposure (Fig. 1C) was searched against the PANTHER classification system [25] which identified 443 mRNAs distributed in various GO categories. The highest overrepresented biological processes were: homeostatic process (P = 9.30E-04), immune system process (P = 5.68E-03), macrophage activation (P = 6.40E-03), regulation of liquid surface tension (P = 7.07E-03), response to stimulus (P = 1.12E-02), and metabolic process (P = 7.30E-01) (Fig. 2A). Overrepresented protein classes were: cytokine (P = 2.01E-07), chemokine (P = 6.37E-05), interleukin superfamily (P = 1.23E-03), surfactant (P = 7.28E-03), microtubule family cytoskeletal (P = 1.11E-02) and signaling molecule (P =1.41E-02) (Fig. 2B). The most overrepresented cellular component was the microtubule (P = 3.33E-02) (Fig. 2C). Among molecular functions, cytokine (P = 4.77E-07), chemokine (P = 5.59E-05) and receptor binding (P = 1.53E-02) were significantly overrepresented (Fig. 2D).

The homeostatic process is defined as any biological process involved in the maintenance of an internal steady state according to the Gene Ontology Consortium (http://geneontology.org/). The upregulated (≥ 3-fold) genes involved in homeostatic processes (Fig. 2A, Table 1) were: anterior gradient 2 (*Agr2*); anterior gradient 3 (*Agr3*); ATPase, H^+/K^+ exchanging, gastric, and alpha polypeptide (*Atp4a*); ATPase, class I, type 8B, member 5 (*Atp8b5*); chemokine CC motif ligand 9 (*Ccl9*); collagen type II alpha 1 (*Col2a1*); collagen type IX alpha 1 (*Col9a1*); alpha 3 (*Col9a3*); collagen type X alpha 1 (*Col10a1*); collagen type XI alpha 1 (*Col11a1*); calcitonin/calcitonin-related polypeptide alpha (*Calca*); forkhead box A3 (*Foxa3*), and G protein-coupled receptor 37 (*Gpr37*).

The immune system process was represented by C-X-C chemokines (e.g., *Cxcl1*, *Cxcl2*, *Cxcl3*) C-C motif chemokines (e.g., *Ccl3*, *Ccl4*, *Ccl9*, *Ccl20* and *Ccl28*); interleukins *Il1a*, *Il1b*, *Il6*, *Il10*, *Il17b*, *Il17c*. *Tnf* and the cytokine receptor *Il23r* were also upregulated by 8-oxoG challenge. The Gene Ontology Consortium (http://geneontology.org/) considers collagen genes (*Col2a1*, *Col9a1*, *Col9a2*, *Col9a3*, *Col10a1*, *Col11a1* and *Col19a1*) part of the immune system and homeostasic process. Moreover, the 8-oxoG challenge (OGG1-BER mimic) upregulated the kallikrein family of serine proteases kallikrein B, plasma 1 (*Klkb1*), kallikrein 1-related peptidases *Klk1b3*, *Klk1b9*, *Klk1b21*, *Klk1b24*, and *Klk1b27*. All fold-increase values are shown in Table 1.

The macrophage activation process is defined by the Gene Ontology Consortium as a change in morphology and behavior of macrophages resulting from exposure to a cytokine, chemokine, cellular ligand, or soluble factor. This GO term was represented by 22 genes, which are overlapping with those listed in immune system processes (above and listed in Table 1).

Another, significantly upregulated biological process was regulation of liquid surface tension, which included genes encoding primarily for collagens (*Col2a1*, *Col9a1*, *Col9a2*, *Col9a3*, *Col10a1*, *Col11a1* and *Col19a1*). Genes included in response to stimulus were chemokines, cytokines, interleukins, and collagens (listed above and Table 1). Interestingly, a large number of genes were significantly upregulated in lungs and involved in metabolic processes (lipid, fatty acid, protein, nucleobase metabolic, and primary metabolic process). However, due to the large number of genes included in the PANTHER' metabolic categories, representation of 8-oxoG challenge–induced genes was below significance (P = 7.30E-01).

Signaling pathways induced by OGG1-BER

PANTHER analysis identified and ranked 443 upregulated (≥ 3 fold) genes (induced at 60 min after 8-oxoG challenge) contributing to signaling pathways. Fig. 3 shows the distribution by percentages of genes for various signaling pathways. Among signaling pathways, the most represented one was inflammation mediated by chemokines and cytokines, followed by gonadotropin-releasing hormone receptor (GnRHR), integrin signaling, and interleukin signaling.

The inflammatory signaling pathway included various significantly upregulated genes (≥ 3 fold, Table 1). For example, among C-X-C chemokines, *Cxcl1* (54.45-fold) and *Cxcl2* (192.95-old) are involved in multiple inflammatory processes, such as chemo-attraction of neutrophils [30]. *Cxcl3* (23.78-fold) of which protein product CXCL3 (also known as macrophage inflammatory protein-2-beta, *or* MIP2b) controls migration and adhesion of monocytes has chemotactic activity for neutrophils [31]. Members of the C-C chemokine family represented in this signaling pathway included: *Ccl3* (27.25-fold), which encodes for protein CCL3, a chemoattractant for polymorphonuclear leukocytes, and regulates macrophage function [32], and *Ccl20* (58.87-fold), implicated in the mucosal immunity via chemoattraction of lymphocytes (T-cells and B-cells) and DCs [33].

We also observed increased expression levels of *Ccl4* (5.15-fold), *Ccl9* (3.09-fold) and *Ccl28* (4.80-fold) (Table1). The upregulated interleukins included *Il17c* (12.62-fold); *Il1a* (4.17-fold); *Il1b* (4.15-fold); and *Il6* (4.15-fold); and these have the following attributes: *Il17c* has a protein product with a critical role in innate immunity of the epithelium, that stimulates the release of TNF-α and IL1B from monocytic cells, and maintains epithelial homeostasis during inflammation [34]. *Il1a* has a protein product released by damaged airway epithelial cells acting as an alarmin and triggering inflammatory responses in lung fibroblasts [35]. *Il1b* has a protein product that is a mediator of the inflammatory response and also involved in various cellular processes, including cell proliferation, differentiation, and apoptosis [36]. *Il6* encodes a protein that contributes to acute and chronic inflammation and plays an active role, e.g., in the pathogenesis of asthma and COPD [37]. *Tnf* (16.65-fold) encodes for TNF-α a pleiotropic cytokine involved in various immune responses, inflammatory processes, hematopoiesis, cell proliferation, differentiation, and apoptosis [38]. The highly expressed *Bpifa1* encodes for palate, lung, and nasal epithelium, and its protein is reported to be involved in innate immune responses in the upper airways in addition to other biological processes [39].

The gonadotropin-releasing, hormone receptor-signaling pathway was represented by: nuclear receptor subfamily 4, group A (*Nr4a1*; 8.36-fold); dual specificity phosphatase 1 (*Dusp1*; 4.37-fold); orthodenticle homolog 1 (*Otx1*; 27.16-fold); period circadian clock 1 (*Per1*; 3.53-fold), activating transcription factor 3 (*Atf3*; 5.03-fold), Finkel–Biskis–Jinkins oncogene (*Fos*; 3.71-fold); and Jun B proto-oncogene (*Junb*; 3.28-fold). *Nr4a1's* protein product is a nuclear receptor that regulates gene transcription and has an anti-inflammatory effect [40]. *Dusp1* encodes for a dual specificity phosphatase 1, which dephosphorylates the oxidative-stress stimulated MAPKs p38, c-Jun N-terminal kinase and ERK [41]. *Otx1* plays a role in cell differentiation of the mammalian cortex [42], and *Per1* encodes for the protein PER1, which regulates the expression of genes involved in maintaining homeostasis of blood pressure [43]. In addition, a significant increase in immediate early genes, *Atf3*, *Fos*, and *Junb*, was observed.

The integrin signaling pathway was represented by members of the collagen family: *Col2a1* (29.07-fold); *Col9a2* (13.53-fold); *Col11a1* (7.56-fold); *Col9a1* (50.12-fold); *Col9a3* (6.13-fold); *Col10a1* (45.05-fold); and integrin beta 2-like (*Itgb2l*). The interleukin signaling pathway was represented by: *Il1a*, *Il6*, and *Il10* (5.84-fold); *Il17c* (12.62-fold); *Il17b* (3.9-fold); interleukin-23 receptor (*Il23r*, 3.9-fold); *Il23a* (alpha subunit, 3.2-fold); *Fos* (3.71-fold); and *Mapk15* (3.28-fold). One of the highly expressed genes in the *Il* pathway was *Il10*, which encodes for a pleiotropic cytokine having multiple functions, including inhibition of pro-inflammatory cytokine production in macrophages and other immune cells regulating mucosal immune homeostasis [44]. The expression of *Mapk15*, also known as *Erk8*, and the activity of its protein product are regulated by DNA damage, which does not appear to require an upstream activating kinase [45].

Validation of RNA-Seq by qRT-PCR

To confirm the results of RNA-Seq analysis, we verified the expression levels of selected highly (*Ccl20*, *Ccl3*, *Cxcl1*, *Cxcl2*) and moderately expressed (*Il1a*, *Il1b*, and *Tnf*) pro-inflammatory chemokines and cytokines by qRT-PCR by using the same RNAs as those utilized for RNA-Seq analysis isolated from 8-oxoG-challenged mouse lungs. Fig. 4 shows the comparison of fold changes in RPKM by RNA-Seq analysis (Fig. 4 A) and mRNA levels as determined by qRT-PCR (Fig. 4B). Results from these studies demonstrated that fold changes in RPKMs from RNA-Seq and in mRNA levels determined by qRT-PCR are consistent. The observed differences in fold changes between transcript and mRNA levels were probably due to differences in sensitivity of the two methods.

RAS signaling-dependent genes induced by 8-oxoG challenge

It has been documented that OGG1-BER, or addition of 8-oxoG to cells, increased the GTP-bound levels of small GTPase (RAS, RHOA, RAC1) family proteins [18-20]. Among small GTPases, RAS is one of the central modulators of signal transduction and gene expression related to cell proliferation and differentiation and inflammation [46-48]. To define whether some of the differentially expressed genes are associated with RAS activation, we first tested whether 8-oxoG challenge altered the expression of genes previously defined to be dependent on RAS signaling. To do so, we utilized a published RAS signature gene list [49], which includes 105 upregulated (Fig. 5A) and 42 downregulated genes (Fig. 5B) and then overlaid the 570 upregulated transcripts from RNA-Seq.

From the 105 genes, 8-oxoG challenge increased the expression levels of 28 genes and downregulated 36 genes (Fig. 5A). The remaining 41 genes were not significantly affected. Among the 42 genes downregulated by RAS, 26 genes were downregulated, while 4 genes showed some increase in expression levels, and 16 remained unaltered (Fig. 5B). Fig. 5C shows a subset of RAS signature genes with significant differential expression (≥3-fold) levels. Notably, the highest upregulated (>20-fold) RAS signaling-dependent genes were *Ccl20*, *Cxcl1*, *Cxcl2* and *Cxcl3* (red bars), all related to immune system and cellular processes and response to stimulus as described above. The most downregulated (>5-fold) genes were the tumor suppressors: serine (or cysteine) peptidase inhibitor, clade B, members 2 (*Serpinb2*) and 5 (*Serpinb5*) and the adapter protein implicated in the regulation of various signaling pathways: stratifin (*Sfn*) as shown in Fig. 5C (green bars).

To validate the role of RAS activation-dependent gene expression, we examined whether there was an increase in RAS-GTP levels in lungs after 8-oxoG challenge. As described (first section of results) in parallel with isolation of RNA for RNA-seq, protein extracts were prepared and subjected to active RAS pull-down assays. Results in Fig. 5D, upper panel, show a time-dependent increase in RAS-GTP levels. These results imply an association between RAS activation and expression of RAS-dependent genes, which are in line with the results of previous studies showing an activation of RAS GTPases by OGG1-BER or 8-oxoG challenge[18, 19, 50].

Next, we examined whether an increase in RAS-GTP levels is OGG1-dependent. To do so, we depleted Ogg1 expression from the airways by RNAi [15], the lungs were challenged with saline containing 8-oxoG base, and protein extracts were prepared. The extent of Ogg1 down regulation in airway epithelium is shown in Fig. 5E. *Ogg1*-RNAi prevented activation of RAS after 8-oxoG challenge (Fig. 5D, lower panel). Next, RNAs isolated (at 60 min) from OGG1-deficient mice (n = 5) and mRNA levels of *Cxcl1* and *Cxcl2* were compared to those of OGG1-proficient ones using qRT-PCR. Fig. 5F shows that *Cxcl1* and *Cxcl2* were increased 117-fold and 592-fold, respectively, in OGG1-proficient airways, while OGG1 depletion nearly prevented their expression (***$p < 0.001$).

Discussion

Oxidative modifications of biomolecules, including DNA, are inevitable consequences of environmental exposures and cellular metabolic processes. DNA base and strand lesions impose a continuous threat to the genomic integrity of all organisms. Among such lesions, one of the most abundant is 8-oxoG, and its accumulation in DNA has been linked to carcinogenesis and to various age-related diseases and aging processes [51]; however, the mechanism is not fully elucidated. Results from the present study show that exposure of airways to the specific product of OGG1-BER, 8-oxoG free base, induced differential gene expression associated with various signaling pathways and biological processes. Although a great amount of work remains to be completed in defining the precise role of OGG1-BER-driven cellular physiological responses, these results suggest that it may not be the genomic accumulation of 8-oxoG that has biological significance, but rather its release from DNA. Elucidation of the significance of each induced pathway identified in this study will be useful for rational design of therapeutics to treat diseases and delay aging processes that previously linked DNA damage/repair.

Upon challenge, *Ogg1*$^{-/-}$ mice exhibit decreased innate and allergic inflammatory responses and increased susceptibility to infections and metabolic disorders [15, 16, 17, 22]. These disorders have been linked to a lack of signaling by 8-oxoG base in some studies [17, 18, 20, 22]. To extend our understanding, we examined the overall impact of 8-oxoG challenge on lungs at the whole-transcriptome level utilizing RNA-Seq and bioinformatics for analysis. The 8-oxoG base is rapidly up taken by cells [20], and, thus, instead of inducing oxidative stress and consequent DNA damage repair in these studies, we challenged animals with 8-oxoG base, to mimic OGG1-initiated BER, to avoid signaling by ROS and generation of oxidatively modified molecules. RNA sequencing identified a total of 23,337 transcripts, out of which cumulatively (at 30, 60 and 120 min) 2,381 were differentially expressed (≥ 3-fold change) after 8-oxoG challenge. Changes in transcript levels were continuously increased from 30 min and peaked at 60 min. Preliminary characterization of bronchoalveolar fluids derived from challenged lungs showed that from 120 min on *de novo* synthesized soluble mediators (e.g., CXCL1, TNF-α) were present in high titers. These data were similar to those in our previous studies [22, 29]. Therefore, to define the primary effects of OGG1-BER (8-oxoG challenge) on global gene expression, RNA-Seq data derived from the 60-min-time point were analyzed in detail. At 60 min post-challenge, 570 transcripts were up-regulated, while 1022 were down-regulated. The down-regulated genes did not represent any biological processes based on the PANTHER data base.

The PANTHER classification system [25], identified 443 (out of 570) mRNAs at 60 min post 8-oxoG exposure distributed in various GO categories. In addition, there were 71 microRNAs and 55 non-coding, full-length RNAs identified according to Mouse Genome Informatics. The functions of these transcripts will be examined in future studies. The encoded proteins from mRNAs are significant contributors to various biological processes (e.g., homeostasis, immune system processes, regulation of liquid surface tension, metabolic), and signaling pathways (e.g., driven by chemokines/cytokines, gonadotropin-releasing hormone receptor, integrins and interleukins). The most overrepresented protein classes were cytokines, chemokines, interleukins, integrins, and the microtubule cytoskeletal family. Experimental validation of these biological processes and signaling pathways will be undertaken in future studies. These results points to the role of OGG1-BER in wide-range of cellular responses, which primarily associated with immune and homeostatic processes. One may also suggest that OGG1-BER can trigger immune defense upon environmental challenge.

Biological processes such as liquid surface tension and homeostatic/metabolic processes are in a broad sense, associated with the regulation of cellular homeostasis. It is well-documented that exposure to various environmental chemicals, and physical and biological agents dysregulate cellular/tissue physiological states (redox, osmolarity, energy demand), induces oxidative DNA damage, and activates repair pathways including OGG1-BER. We speculate that OGG1-BER and consequent activation of signaling pathways are links between environmental exposures and cellular responses, including reestablishment of homeostasis. Indeed, genes upregulated by 8-oxoG challenge (e.g., ATPases participating in H^+/K^+ exchange, GTPase alpha polypeptide, class 1 type 8B ATPases, C-C chemokines, collagen types II, IX, X, XI or G protein-coupled receptors) were previously associated with homeostatic states (PANTHER). Another, significantly overrepresented biological process was that of immune system processes, mediated by chemokines and cytokines. This finding leads to the likely scenario that 8-oxoG challenge may not be inducing homeostasis per se, but rather inducing inflammatory responses. One may argue that this only seems to be contradictory, in that an immune response is an attempt to eliminate environmental insult and thus re-establish homeostatic pre-exposure conditions. By the same token, our data also imply that an 8-oxoG base may be classified as an “alarmin,” an endogenous molecule released by OGG1-BER upon oxidative challenge of DNA. In general, alarmins play a role in the various tissue homeostatic processes and benefit a host. However, there is evidence that excessive release of alarmin(s) contributes to dysregulated processes such as inflammation. Indeed, challenge with the 8-oxoG base (but not with guanine, 8-oxodeoxyguanosine, or 2,6-diamino-4-hydroxy-5-formamidopyrimidine, a ring-opened derivative of 8-oxoG) or when the base was released from DNA by OGG1-BER induced activation of NF-κB pathway, expression of chemokines, cytokines and recruitment of neutrophils to the airways as previously documented [22].

Intriguing studies have documented changes in whole-body energy homeostasis and susceptibility to obesity of OGG1-BER-deficient (*Ogg1*$^{-/-}$) mice [17]. Specifically, when *Ogg1*$^{-/-}$ mice were kept on a high-fat diet, they showed increased plasma insulin levels, impaired glucose tolerance, enhanced adiposity and hepatic steatosis [17]. In addition to other explanations for these observations authors proposed that increased susceptibility to obesity of *Ogg1*$^{-/-}$ mice could be due to a lack of 8-oxoG base released from DNA by OGG1-BER. Therefore, we examined whether challenge with 8-oxoG base modulated expression of genes participating in metabolic processes in lungs. Indeed, we identified genes involved in lipid, fatty acid, carbohydrate, protein, and nucleobase metabolism; however, due to the large number of genes cataloged in each subcategory in the PANTHER data base, number of upregulated genes in our data sets did not reach statistical significance. Future studies are warranted to sort out the roles of 8-oxoG (OGG1•8-oxoG complex) in regulating gene expression involved in metabolic processes. In our present studies, we did not utilize *Ogg1*$^{-/-}$ mice, as we have documented both OGG1 and 8-oxoG base are required for activation of small GTPases and down-stream signaling [18, 20, 22].

Studies undertaken previously show that OGG1 binds the repair product, 8-oxoG base (but not guanine or 8-oxodesoxyguanosine), with high affinity ($Kd = 0.56$ nM) and that the complex physically interacts with and activates small GTPases *in vitro* and in cultured cells [18, 20, 21]. Therefore, critically addressing the means by which 8-oxoG challenge of OGG1-expressing lungs could induce biological responses, we asked whether it could be linked to small GTPases. Small GTPases play an important role in various biological processes by regulating the expression of multiple genes [47]. To examine the role of the most relevant GTPase in signaling 8-oxoG-induced gene expression, we matched our RNA-Seq datasets to a published list of RAS signaling-dependent genes [49]. As described in the

“Results” section, among the 147 genes for which the expression has been reported to be dependent on RAS signaling, only 28 (out of 105) were up-regulated and 26 (out of 42) down-regulated by 8-oxoG challenge. In theory, all RAS-dependent genes should be modulated, as 8-oxoG challenge induces a robust RAS activation; however, this was not the case. Although we cannot fully explain these observations, these data suggest that gene activation was dependent on cell types and organ and whether cells were normal or had an oncogenic phenotype. Indeed, the RAS signature set of genes was developed based on cell lines mostly isolated from tumors [49]. It is also possible that the OGG1•8-oxoG complex induced a subset of RAS-dependent genes that mediate signaling cascades and biological processes primarily serving cellular/host immune homeostasis.

Challenging airways with 8-oxoG increased the levels of GTP-bound RAS in an OGG1-dependent fashion similar to that in previous studies [18, 20, 22]. Activation of RAS was observed as early as 15 min, and mRNA levels were increased from 30 min on, so we propose that these events are not only overlapping, but are also etiologically related. In support, Sparmann and coworkers showed that activated RAS led to upregulation of C-X-C chemokines [53]. Another study documented that introduction of *Kras* into the bronchiolar epithelium is associated with robust pro-inflammatory gene expression (e.g., MIP-2, KC, MCP-1 and LIX chemokines) [54]. These data are consistent with an increase in RAS-GTP and *Cxcl1* and *Cxcl2* mRNA levels after 8-oxoG challenge. Moreover, both the activation of RAS and an increase in mRNA levels of *Cxcl1* and *Cxcl2* were significantly prevented in OGG1-depleted airways [22].

For RNA-sequencing the entire mouse lung was used to isolate RNA by raising the possibility that fold changes in mRNA and transcript levels could be different (actually be higher) in the airway epithelium which constitutes ~25% of total lung cells [52]. At this point we cannot exclude the contribution of other cells types (e.g., mast and dendritic cells, which represent <1% of all cells in the lung epithelium). Resident macrophages are affected by 8-oxoG challenge (unpublished data); however, they were removed before RNA extraction by lavage of airways with ice-cold PBS. Therefore, we speculate that the observed changes in mRNA levels primarily represent the response of airway epithelium to 8-oxoG challenge. In support, a) 8-oxoG is rapidly taken up by cells [20], primarily by the epithelium in our model; b) RAS GTPases were activated from 15 min on; and c) from 30 min on (60-min maximum) robust changes in gene expression were observed. This hypothesis is supported by the overrepresentation of immune response processes and is in line with the innate immune defense functions of airway epithelium [52].

In summary, we document for the first time that challenge of lungs with 8-oxoG base induced the expression of large numbers of transcripts, contributing to the activation of various signaling pathways and biological processes, primarily triggering cellular homeostatic and immune responses. Although future studies are required, these data are in accord with decreased innate and allergic immune responses, and the susceptibility of *Ogg1* null mice to obesity. We may speculate that 8-oxoG serves as an endogenously generated alarmin that benefits the host, while its excessive release contributes to dysregulated processes. Finally, we raise the possibility that OGG1-BER and consequent activation of signaling pathways could be a link between environmental exposures and cellular responses.

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Abbreviations

8-oxoG	7,8-dihydro-8-oxoguanine
BALF	bronchoalveolar lavage fluid
BER	base-excision repair
CASAVA	Consensus Assessment of Sequence and Variation
COPD	chronic obstructive pulmonary disease
C-X-C	C-X-C motif chemokines
C-C	C-C motif chemokines
i.n.	intranasal
GENE-E	is a matrix visualization and analysis platform
GEO	Gene Expression Omnibus
GO	gene ontology
Kras	Kirsten rat sarcoma viral oncogene homolog
LPS	lipopolysaccharide
miRNA	micro RNA(s)
OGG1	8-oxoguanine DNA glycosylase-1
OGG1-BER	OGG1-initiated DNA base excision repair
PANTHER	Protein ANalysis THrough Evolutionary Relationships
RAC1	Ras-related C3 botulinum toxin substrate 1
RHOA	Ras homolog gene family, member A
RPKM	reads per kb transcript per million
RNA-Seq	RNA sequencing
Ras	rat viral sarcoma oncogene homolog
RNAi	RNA interference
ROS	reactive oxygen species
TNF-α	tumor necrosis factor alpha

Research Highlights

- 8-Oxoguanine DNA glycosylase1 (OGG1) prevents accumulation of genomic 8-oxoguanine
- The released 8-oxoG base, in complex with OGG1, induces signaling via small GTPases
- OGG1-BER signaling increases levels of 2381 transcripts, out of which 443 are mRNAs
- These genes regulate essential biological processes and signaling pathways
- OGG1-BER signaling may be a link between DNA damage-repair and cellular responses

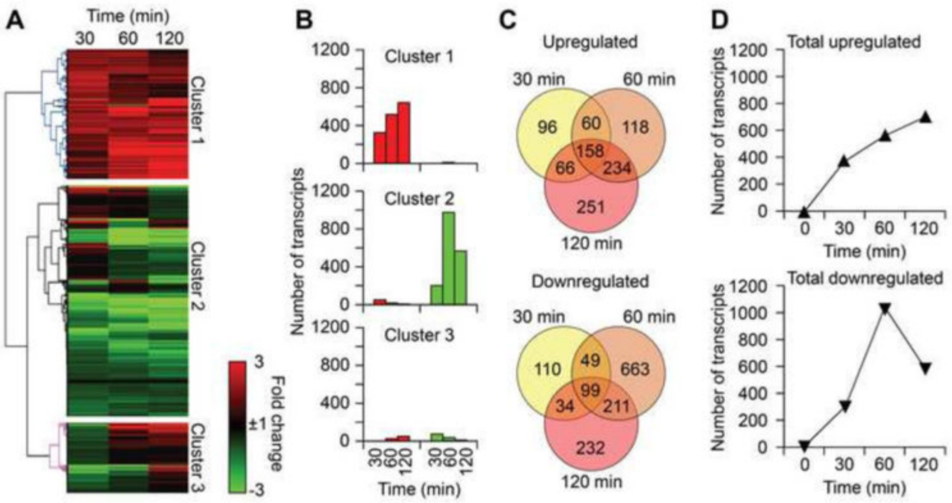

Fig. 1.

Whole-transcriptome profile induced by 8-oxoG challenge. (A) Graphical depiction of results from hierarchical clustering showing whole-transcriptome profile. Total RNA isolated from challenged lungs at 30, 60 and 120 min were subjected to RNA-Seq, heat maps were generated using GENE-E (http://www.broadinstitute.org/cancer/software/GENE-E/), and transcripts were clustered according to their expression patterns. Transcript levels (RPKM) were normalized to control (time 0 min). Rows represent identified transcripts and columns at time points after 8-oxoG challenge. The intensity of the red or green colors shows the degree of upregulation or downregulation, respectively. (B) The total number of upregulated (≥3-fold) transcripts (red bars) and downregulated (≤3-fold) transcripts (green bars) in each cluster. (C) Number of unique and shared transcripts altered by 8-oxoG challenge. Venn diagrams were constructed by using online software Venny (Materials and Methods). (D) Kinetic changes in transcript levels induced by 8-oxoG challenge.

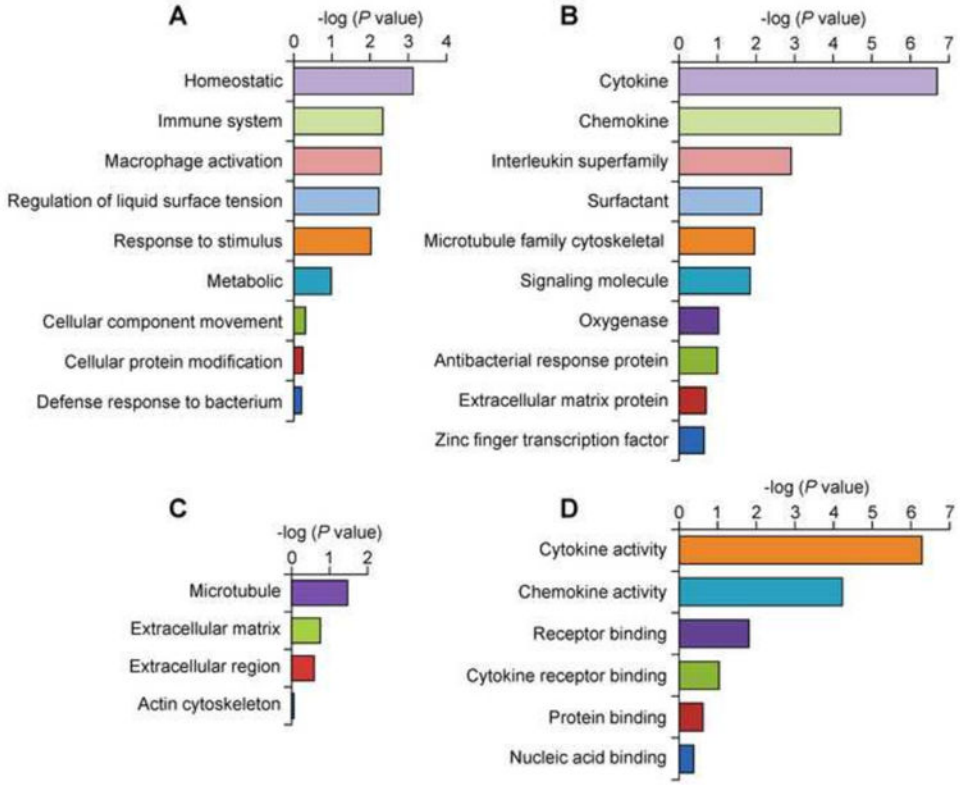

Fig. 2.

Gene ontology categories represented by the upregulated genes induced after introduction of the OGG1-BER product 8-oxoG into mouse lungs. Overrepresentation test of the genes upregulated (≥ 3 fold) at 60 min was performed by using PANTHER (http://www.pantherdb.org). (A) Biological process. (B) Protein class. (**C**) Cellular component. (**D**) Molecular function. X axis represent −log(*P* value).

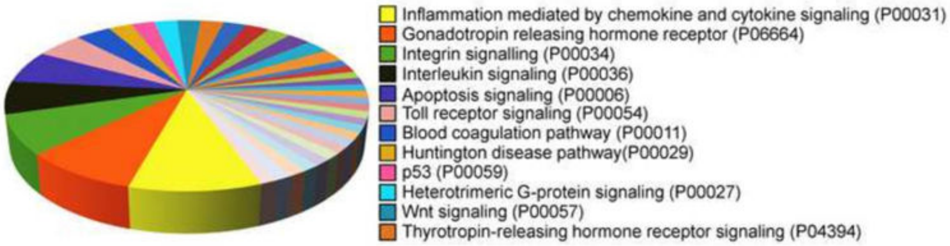

Fig. 3.

Signaling pathways induced by OGG1-BER product, 8-oxoG in mouse lungs. Pie chart was generated by using PANTHER (Materials and Methods). Pathway analysis included only those genes with expression levels ≥ 3-fold at 60 min.

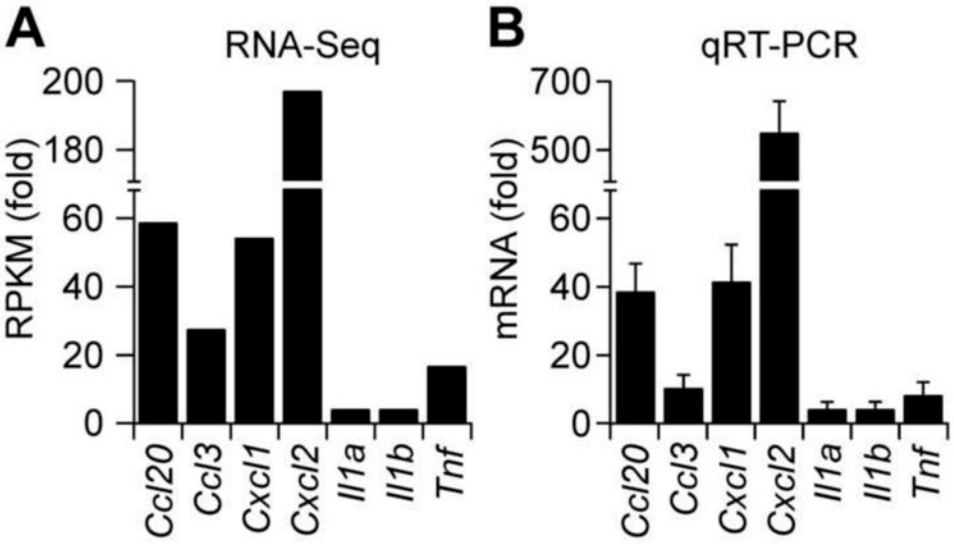

Fig. 4.

Validation of RNA-Seq data. (A) Fold changes in RPKM of selected genes as determined by RNA-Seq analysis. RNA was isolated from lungs at 60 min post challenge and pooled (n = 5) or kept separately. One µg of pooled RNA was subjected to RNA-Seq as in Materials and Methods. (B) qRT-PCR confirmation of RNA-Seq data. One µg RNA isolated from individual control and challenged mice (n = 5) was reverse transcribed, and cDNAs were amplified by using specific primer pairs as described in Materials and Methods.

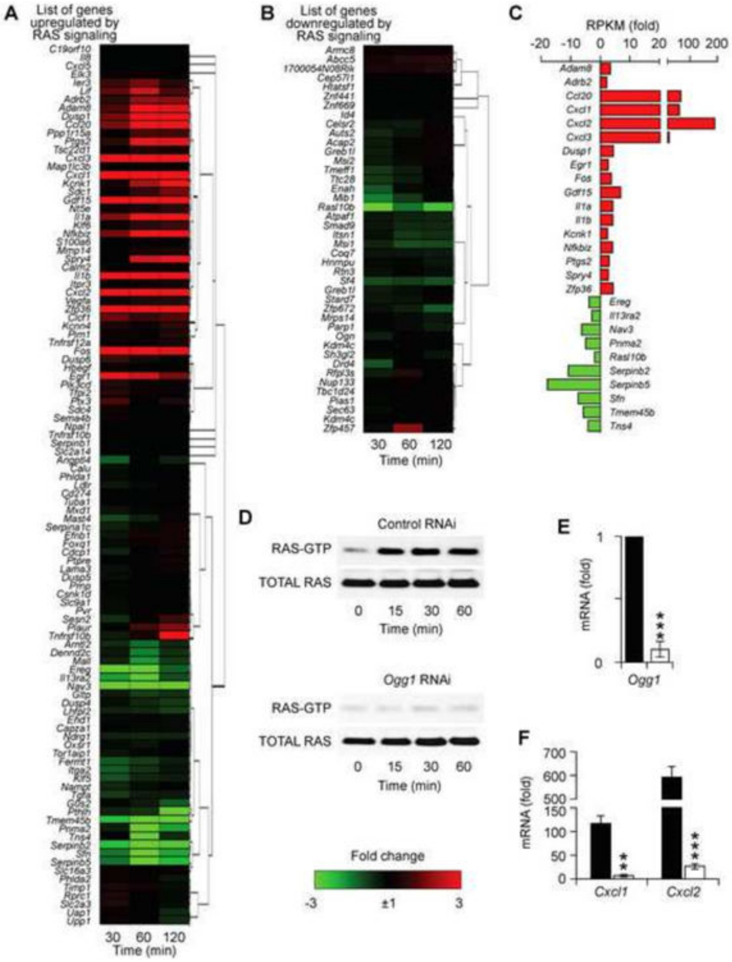

Fig. 5.

OGG1-BER alters expression of RAS signature genes. Effect of 8-oxoG challenge on the expression of genes reported to be up-regulated (A) or down regulated (B) by RAS signaling. (**C**) Differentially expressed RAS signature genes with ≥ 3-fold expression levels. Upregulated genes are shown in red bars and down regulated ones in green bars. (D) Activation of RAS-GTPase by 8-oxoG challenge in lungs (upper panel) and dependency on OGG1 expression in 8-oxoG-exposed lungs. (E) Down-regulation of *Ogg1* in mouse airway epithelium by RNAi. (F) Decreased expression of *Cxcl1* and *Cxcl2 in Ogg1-depleted airways*. $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 1

List of genes up-regulated by OGG1-BER involved in the most represented biological processes and signaling pathways

Symbol	RefSeq ID	Name	Biological processes					Signaling pathways				Fold change
			H	I	M	S	R	In	Gn	It	Il	
Cxcl2	NM_009140	Chemokine (C-X-C motif) ligand 2		X	X		X					192.95
Bpifa1	NM_011126	BPI[a] fold containing family A, member 1		X	X		X					107.60
Matn1	NM_010769	Matrilin 1					X	X		X		83.06
Ccl20	NM_001159738	Chemokine (C-C motif) ligand 20		X			X	X				58.87
Cxcl1	NM_008176	Chemokine (C-X-C motif) ligand 1		X	X		X					54.45
Col9a1	NM_007740	Collagen, type IX, alpha 1	X	X	X	X	X			X		50.12
Bpifb1	NM_001012392	BPI[a] fold containing family B, member 1		X	X		X					48.76
Col10a1	NM_009925	Collagen, type X, alpha 1	X	X	X	X	X			X		45.05
Capn13	NP_001028616	Calpain 13		X								34.00
Col2a1	NM_031163	Collagen, type II, alpha 1	X	X	X	X	X			X		29.07
Ccl3	NM_011337	Chemokine (C-C motif) ligand 3		X			X	X				27.25
Otx1	NM_011023	Orthodenticle homolog 1							X			27.16
Cxcl3	NM_008176	Chemokine (C-X-C motif) ligand 3		X	X		X					23.78
Agr2	NM_011783	Anterior gradient 2	X			X						22.72
Matn3	NM_010770	Matrilin 3					X					20.33
Hsf5	NP_001038992	Heat shock transcription factor 5		X								19.63
Tnf	NM_013693	Tumor necrosis factor		X			X					16.65
Klk1b3	NM_008693	Kallikrein 1-related peptidase b3		X			X					15.42
Col9a2	NM_007741	Collagen, type IX, alpha 2		X	X		X			X		13.53
Il17c	NM_145834	Interleukin 17C		X								12.62
Rgs1	NM_015811	Regulator of G-protein signaling 1						X				10.54
Clec4e	NM_019948	C-type lectin domain family 4, member e		X	X		X					10.45
Il6	NM_031168	Interleukin 6		X							X	10.44
Lypd2	NP_080947	Ly6/Plaur domain containing 2		X								10.23
Klk1b21	NM_010642	Kallikrein 1-related peptidase b21		X			X					10.16

Symbol	RefSeq ID	Name	Biological processes					Signaling pathways				Fold change
			H	I	M	S	R	In	Gn	It	Il	
Pgc	NM_025973	Progastricsin (pepsinogen C)					X					9.81
Lyzl4	NP_081191	Lysozyme-like 4		X								9.81
Nr4a1	NM_010444	Nuclear receptor subfamily 4, group A		X			X		X			8.36
Tacr1	NM_009313	Tachykinin receptor 1		X			X					7.84
Vmn1r3	NM_001167535	Vomeronasal 1 receptor 3					X					7.71
Col11a1	NM_007729	Collagen, type XI, alpha 1	X	X	X	X	X			X		7.56
Ctla4	NM_009843	Cytotoxic T-lymphocyte-associated protein 4		X			X					7.08
Calca	NM_007587	Calcitonin/calcitonin-related polypeptide, alpha	X									6.63
Wfikkn1	NP_001093924	WAP[b], follistatin/kazal immunoglobulin KKN1[c]		X			X					6.53
Col9a3	NM_009936	Collagen, type IX, alpha 3	X	X		X	X	X		X		6.13
Fcrlb	NM_001160215	Fragment crystallizable (Fc) receptor-like B		X	X		X					5.89
Vmn1r2	NM_001167534	Vomeronasal 1 receptor 2					X					5.84
Il10	NM_010548	Interleukin 10		X	X		X				X	5.84
Sik1	NM_010831	Salt inducible kinase 1					X					5.82
Klk1b24	NM_010643	Kallikrein 1-related peptidase b24		X			X					5.61
Ngp	NM_008694	Neutrophilic granule protein		X			X					5.51
Klkb1	NM_008455	Kallikrein B, plasma 1		X			X					5.37
Wfdc8	NM_029325	WAP[b] four-disulfide core domain 8		X			X					5.26
Asgr1	NM_009714	Asialoglycoprotein receptor 1		X	X		X					5.22
Crb1	NM_133239	Crumbs homolog 1		X								5.21
Ccl4	NM_13652	Chemokine (C-C motif) ligand 4		X			X	X				5.17
Atf3	NM_007498	Activating transcription factor 3		X					X			5.03
Alox12e	NM_145684	Arachidonate lipoxygenase, epidermal		X				X				5.02
Camp	NM_009921	Cathelicidin antimicrobial peptide		X			X					4.98
Tbr1	NM_009322	T-box brain gene 1		X								4.91
Ccl28	NM_007930	Chemokine (C-C motif) ligand 28		X	X		X					4.80
Klk1b27	NM_020268	Kallikrein 1-related peptidase b27		X			X					4.74
Nfkbia	NM_010907	NF-LP[d] gene enhancer in B cells inhibitor, alpha		X			X	X				4.73

Symbol	RefSeq ID	Name	Biological processes					Signaling pathways				Fold change
			H	I	M	S	R	In	Gn	It	Il	
Acsm1	NM_054094	Acyl-CoA synthetase medium-chain family member 1		X								4.69
Pigr	NM_011082	Polymeric immunoglobulin receptor		X			X					4.68
Agr3	NP_997414	Anterior gradient 3	X			X						4.39
Cd14	NM_009841	CD14 antigen		X								4.38
Dusp1	NM_013642	Dual specificity phosphatase 1		X					X			4.37
Foxa3	NM_008260	Forkhead box A3	X									4.36
Gpr37	NM_010338	G protein-coupled receptor 37	X	X			X					4.33
Col19a1	NM_007733	Collagen, type XIX, alpha 1		X								4.21
Vmn1r79	NM_001166835	Vomeronasal 1 receptor 79					X					4.21
Gnat3	NM_001081143	Guanine nucleotide binding protein alpha T3[e]					X					4.21
Il1a	NM_010554	Interleukin 1 alpha		X	X		X				X	4.17
Il1b	NM_008361	Interleukin 1 beta		X	X		X					4.15
Nfkbiz	NM_030612	NF-LP[d] gene enhancer biz		X			X					4.10
Osm	NP_001013383	Oncostatin M		X								3.90
Il23r	NM_144548	Interleukin 23 receptor		X			X				X	3.86
Il17b	NM_019508	Interleukin 17B		X								3.86
Trim30b	NM_175648	Tripartite motif-containing 30B		X			X					3.84
Tlr2	NM_011905	Toll-like receptor 2		X								3.81
Vtcn1	NM_178594	V-set domain containing T cell activation inhibitor 1					X					3.75
Fos	NM_010234	Finkel–Biskis–Jinkins oncogene		X					X		X	3.71
Cd69	NP_001028294	CD69 antigen		X			X					3.69
Bdkrb1	NM_007539	Bradykinin receptor, beta 1		X			X					3.67
Lcn4	NM_010695	Lipocalin 4					X					3.61
Nr4a2	NM_013613	Nuclear receptor subfamily 4, group A, member 2		X			X					3.61
Sntn	NM_177624	Sentan, cilia apical structure protein		X	X							3.60
Dnajb13	NM_153527	DnaJ related, subfamily B 13 (Hsp40[f])		X			X					3.58
Per1	NM_011065	Period circadian clock 1							X			3.53
Vmn1r238	NM_001167539	Vomeronasal 1 receptor, 238					X					3.50

Symbol	RefSeq ID	Name	Biological processes					Signaling pathways				Fold change
			H	**I**	**M**	**S**	**R**	**In**	**Gn**	**It**	**Il**	
Ms4a15	NM_001034898	Membrane-spanning 4a15					X					3.50
Klk1b9	NM_010116	Kallikrein 1-related peptidase b9		X			X					3.50
Atp4a	NM_018731	ATPase, H^+/K^+ exchanging, gastric alpha pp[g]	X									3.50
Nlrp3	NM_145827	NOD-like receptor family, pyrin domain containing 3[h]		X			X					3.48
Proc	NM_008934	Protein C					X					3.40
Lrrc26	NM_146117	Leucine rich repeat containing 26		X								3.39
Atp8b5	NM_177195	ATPase, class I, type 8B, member 5	X									3.30
Mapk15	NM_177922	Mitogen-activated protein kinase 15					X	X			X	3.28
Junb	NM_008416	Jun B proto-oncogene						X	X			3.28
Klra5	NM_008463	Killer cell lectin-like receptor A, member 5		X			X					3.27
Mog	NM_010814	Myelin oligodendrocyte glycoprotein					X					3.15
Pkd1l2	NM_029686	Polycystic kidney disease 1 like 2					X					3.15
Zfp352	NM_153102	Zinc finger protein 352		X			X					3.15
Gadd45g	NM_011817	Growth arrest and DNA-damage-inducible 45		X			X					3.13
Ccl9	NM_011338	Chemokine (C-C motif) ligand 9	X	X			X					3.09

Biological processes: H = Homeostatic process (GO:0042592); I = Immune system process (GO:0002376);M = Macrophage activation (GO:0042116); S = Regulation of liquid surface tension (GO: 0050828); R = Response to stimulus (GO:0050896). “X” denotes presence of the genes in each biological process category. **Signaling pathways**: In = Inflammation mediated by chemokine and cytokine signaling (P00031), Gn = Gonadotropin releasing hormone receptor (P06664), It = Integrin signaling, Il = Interleukin signaling (P00036).

[a] Bactericidal/permeability-increasing fold containing family a1;

[b] Whey acidic protein;

[c] Knitz and netrin domain containing 1;

[d] Nuclear factor of kappa light polypeptide;

[f] Heat shock protein 40 kD;

[e] Guanine nucleotide binding protein alpha transducing 3,

[g] ATPase, H^+/K^+ exchanging, gastric alpha polypeptide,

[h] Nucleotide-binding oligomerization domain-containing protein 1.