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Original Contribution

Whole transcriptome analysis reveals a role for OGG1-initiated DNA repair signaling in airway remodeling

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ABSTRACT

Reactive oxygen species (ROS) generated by environmental exposures, and endogenously as by-products of respiration, oxidatively modify biomolecules including DNA. Accumulation of ROS-induced DNA damage has been implicated in various diseases that involve inflammatory processes, and efficient DNA repair is considered critical in preventing such diseases. One of the most abundant DNA base lesions is 7,8-dihydro-8-oxoguanine (8-oxoG), which is repaired by the 8-oxoguanine DNA glycosylase 1 (OGG1)-initiated base-excision repair (OGG1-BER) pathway. Recent studies have shown that the OGG1-BER by-product 8-oxoG base forms a complex with cytosolic OGG1, activating small GTPases and downstream cell signaling in cultured cells and lungs. This implies that persistent OGG1-BER could result in signaling leading to histological changes in airways. To test this, we mimicked OGG1-BER by repeatedly challenging airways with its repair product 8-oxoG base. Gene expression was analyzed by RNA sequencing (RNA-Seq) and qRT-PCR, and datasets were evaluated by gene ontology and statistical tools. RNA-Seq analysis identified 3252 differentially expressed transcripts (2435 up- and 817 downregulated, $\geq$3-fold change). Among the upregulated transcripts, 2080 mRNAs were identified whose encoded protein products were involved in modulation of the actin family cytoskeleton, extracellular matrix, cell adhesion, cadherin, and cell junctions, affecting biological processes such as tissue development, cell-to-cell adhesion, cell communication, and the immune system. These data are supported by histological observations showing epithelial alterations, subepithelial fibrosis, and collagen deposits in the lungs. These data imply that continuous challenge by the environment and consequent OGG1-BER-driven signaling trigger gene expression consistent with airway remodeling.

Airway remodeling is characterized by subepithelial fibrosis, myofibroblast hyperplasia, thickening of the lamina reticularis, and collagen deposition. These histological changes, a decline in lung function, and a poor response to various therapies are common in chronic lung diseases such as asthma and COPD [1, 2]. The underlying molecular mechanisms of tissue remodeling are not fully understood, but in most cases, they are linked to chronic inflammation continuously generating mediators and reactive oxygen

Abbreviations: 8-oxoG, 7,8-dihydro-8-oxoguanine; BER, base-excision repair; CASAVA, consensus assessment of sequence and variation; COPD, chronic obstructive pulmonary disease; ECM, extracellular matrix; GENE-E, a matrix visualization and analysis platform; GEO, Gene Expression Omnibus; GO, gene ontology; GSEA, Gene Set Enrichment Analysis; OGG1, 8-oxoguanine DNA glycosylase 1; OGG1-BER, OGG1-initiated DNA base-excision repair; PANTHER, protein analysis through evolutionary relationships; RPKM, reads per kilobase of transcript per million; RNA-Seq, RNA sequencing; ROS, reactive oxygen species

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species (ROS), which are also considered signaling entities and indiscriminately cause damage to various biomolecules, including DNA [3]. The primary target of ROS in the DNA is guanine, because of its lowest oxidation potential among the DNA bases [4, 5]. Among guanine's oxidation products, 7,8-dihydro-8-oxoguanine (8-oxoG) is the most abundant in both DNA and RNA, and its accumulation in DNA is considered one of the best biomarkers of oxidative stress [6, 7]. 8-OxoG does not induce changes in DNA structure and is not an obstacle to DNA or RNA polymerases [8, 9]. However, 8-oxoG is capable of pairing with adenine in its "*syn*" conformation and is considered one of the most mutagenic base lesions [10]. 8-OxoG must thus be removed to maintain genomic integrity; this occurs primarily via the 8-oxoguanine DNA glycosylase 1 (OGG1)-initiated DNA base-excision repair (OGG1-BER) pathway [11, 12].

Our recent studies documented the association of OGG1-BER with activation of rat viral sarcoma oncogene homolog (Ras) family small GTPases [13]. The pathway's repair product, free 8-oxoG base, forms a complex with cytosolic OGG1. The resulting conformational change allows its interaction with small GTPases, including Kirstein (K)-Ras, which activates them [13–16]. The end result includes the activation of v-Raf-leukemia viral oncogene 1 and phosphatidylinositol, mitogen, stress-activated, and IκB kinases as well as nuclear factor-κB, resulting in robust innate inflammation in the mouse airways [14, 17]. Additional studies showed that OGG1-BER activates Rac1 GTPase, which is involved in cell redox balance [15], and RhoA GTPase, which induces α-smooth muscle actin polymerization into stress fibers [16]. Studies have also shown that after administration of a high-fat diet, *Ogg1*$^{-/-}$ mice have increased plasma insulin levels, impaired glucose tolerance, enhanced adiposity, and increased hepatic steatosis compared to similarly fed wild-type animals [18]. Another study documented an essential role for OGG1 in embryonic development, because its absence resulted in abnormalities such as severe brain defects [19]. It has recently been shown that OGG1-BER mimicked by a single 8-oxoG challenge increased gene expression involved in homeostatic, immune system, and macrophage activation processes mediated by chemokines, cytokines, integrin, and interleukin signaling pathways [20].

In the present study, we postulated that continuous repair of oxidatively damaged DNA by OGG1-BER, and consequent downstream signaling via small GTPases, induces gene expression, which results in functional and structural changes in lungs. To test this hypothesis, we repeatedly challenged mouse lungs with 8-oxoG to mimic ongoing OGG1-BER after environmental exposures and/or during chronic inflammation. RNA was isolated and analyzed by RNA-Seq. Gene ontology analysis showed that the OGG1-BER product 8-oxoG induced gene expression and signaling pathways consistent with histological changes, including epithelial alterations, increased smooth muscle mass, and collagen deposits in the airways.

1. Materials and methods

1.1. 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.) on day 0 (single challenge) or days 0, 2, and 4 (multiple challenge) with 60 μl of pH-balanced 8-oxoG (Cayman Chemicals, Ann Arbor, MI, USA) solution (pH 7.4; 0.0005 mg/kg) or saline under mild anesthesia [13]. In controls, we used identical concentrations of 7,8-dihydro-8-oxoadenine (BioLog Life Science Institute, Axxora, San Diego, CA, USA), 8-oxodeoxyguanosine (Sigma–Aldrich (St. Louis, MO, USA), and guanine (Sigma–Aldrich. The lipopolysaccharide concentration was below detectable levels in all reagents. Animals were sacrificed at various time points (0, 30, 60, and 120 min) after the single or multiple challenges to isolate lung RNA.

1.2. RNA isolation

After intranasal challenge, mouse lungs were excised and homogenized in lysis buffer (Qiagen, Valencia, CA, USA) with a TissueMiser (Fisher, Pittsburgh, PA, USA). RNA was extracted using an RNeasy kit (Qiagen) 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 with the RNase-free water included in the kit. Eluted RNA was digested with RNase-free DNase as previously described [21]. The RNA concentration was determined spectrophotometrically on an Epoch Take-3 system (Biotek, Winooski, VT, USA) using Gen5 version 2.01 software. Equal amounts of RNA from each mouse lung within an experimental group ($n=5$) were pooled and analyzed in triplicate. The quality of the total RNA was confirmed spectrophotometrically via the 260/280 nm ratio, which varied from 1.9 to 2.0. RNA integrity was also evaluated by agarose gel electrophoresis.

1.3. Next-generation RNA sequencing

Library construction and deep sequencing analysis were performed in UTMB's Next-Generation Sequencing Core Facility (Dr. Thomas G. Wood, Director) on an Illumina HiSeq 1000 sequencing system (Illumina, San Diego, CA, USA). Poly(A)$^+$ RNA was selected from total RNA (1 μg) with poly(T) oligo-attached magnetic beads. Bound RNA was fragmented by incubation at 94 °C for 8 min 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. Samples were tracked through the "index tags" incorporated into the adapters. Library quality was evaluated using 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 version 3 (Illumina) and the Illumina cBot workstation under the conditions recommended by the manufacturer. 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 version 3 (Illumina) on an Illumina HiSeq 1000 per the manufacturer's protocols. Base calls were converted to sequence reads using CASAVA-1.8.2. Sequence data were analyzed with the Bowtie2, Tophat, and Cufflinks programs using the National Center for Biotechnology Information's (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 No. GSE65031. Reads per kilobase of transcript per million (RPKM) were normalized to the corresponding control for each experimental group [22].

1.4. Gene ontology analysis

Heat maps and hierarchical clusters from whole transcriptomes were constructed with GENE-E online software (version 3.0.204) from the Broad Institute (http://www.broadinstitute.org/cancer/software/GENE-E/). Venn diagrams were constructed using online