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8-oxoguanine DNA glycosylase-1 driven DNA base excision repair: role in asthma pathogenesis

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Abstract

Purpose of review—To provide both an overview and evidence of the potential etiology of oxidative DNA base damage and repair-signaling in chronic inflammation and histological changes associated with asthma.

Recent findings—Asthma is initiated/maintained by immunological, genetic/epigenetic and environmental factors. It is a world-wide health problem, as current therapies suppress symptoms rather than prevent/reverse the disease, largely due to gaps in understanding its molecular mechanisms. Inflammation, oxidative stress and DNA damage are inseparable phenomena, but their molecular roles in asthma pathogenesis are unclear. It was found that among oxidatively modified DNA bases, 8-oxoguanine (8-oxoG) is one of the most abundant, and its levels in DNA and body fluids are considered a biomarker of ongoing asthmatic processes. Free 8-oxoG forms a complex with 8-oxoguanine DNA glycosylase-1 (OGG1) and activates RAS-family GTPases that induce gene expression to mobilize innate and adaptive immune systems, along with genes regulating airway hyperplasia, hyper-responsiveness and lung remodeling in atopic and non-atopic asthma.

Summary—DNA's integrity must be maintained to prevent mutation, so its continuous repair and downstream signaling "fuels" chronic inflammatory processes in asthma, and forms the basic mechanism whose elucidation will allow the development of new drug targets for the prevention/reversal of lung diseases.

Keywords

DNA repair; OGG1; 8-oxoG; inflammation; asthma

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Conflict of interest

The authors declare that no conflict of interest exists

INTRODUCTION

Asthma is a complex chronic inflammatory lung disease, thought to result from the interaction between individuals' genetic susceptibility and epigenetic changes induced by environmental factors. It is characterized by heterogeneity in pathogenesis, ventilatory/gas exchange impairment, mediated by multiple inflammatory mediators and oxidative stress induced by reactive oxygen species (ROS) [1,2,3,4].

Environmental agents acting singly or in combination primarily impact the surface of the airway, and when in excess, their interactions with airway epithelium and resident immune cells lead to increased ROS generation and both the onset and worsening of lung diseases, including asthma [1,2]. ROS are signaling molecules, and due to their reactivity, modify proteins, lipids, and DNA [5■]. In the DNA, one of the most common oxidation products is 8-oxo-7,8-dihydroguanine (8-oxoG) [6,7], which is preferentially repaired by the DNA base excision repair (BER) pathway [8], that utilizes glycosylases to excise the lesion by cleaving its N-glycosidic bond, followed by endonucleolytic cleavage and subsequent gap filling [8,9]. Although increases in oxidatively modified DNA bases are common, accumulation of 8-oxoG in the DNA has been linked to various inflammatory disease processes [9,10].

The genetics, development, imunopathogeneses, and pathophysiology of asthma have been elegantly reviewed by leading experts in the field [2,4,11,12,13,14,15,16,17,18]. It mostly agreed that asthma is a highly complex interplay among dysregulated airway epithelial, mast cells, basophils, dendritic cells, B and T cells, neutrophils and eosinophils, and is clinically manifested via a multitude of mediators leading to pathological pulmonary physiology. This review discusses present views the on role of OGG1-initiated repair of oxidatively damaged DNA and provides data to support a novel concept – the association of OGG1-BER with the expression of genes that are implicated in deregulation of the immune system and fundamental to histopathological changes in asthma.

GUANINE OXIDATION IS A MARKER OF ASTHMA

In DNA or RNA, the primary target of ROS is guanine, due to its lowest redox potential among nucleic acid bases [7]. Thus, 8-oxoG is one of the most abundant oxidatively modified DNA base lesions; it is estimated that up to 100,000 8-oxoG DNA lesions could be formed daily per cell [19]. The level of genomic 8-oxoG also correlates well with the dose and length of exposure, chemical composition, and physical nature of inhaled environmental agents [20,21,22,23,24,25,26]. For example, exposure to environmental pollutants and ROS generated by inflammatory cells significantly increased genomic 8-oxoG levels in the lung epithelium, resident macrophages, and peripheral blood monocytes, or of 8-oxoG base or its nucleoside levels in such body fluids as serum, urine, sputum and bronchoalveolar lavage fluid (BALF) [24,25,26,27,28,29,30,31,32].

In asthmatic patients and experimental animal models of asthma, one of the most referenced form of DNA base damage is 8-oxoG and its open-ringed form FapyG (2,6-diamino-4-hydroxy-5-formamidopyrimidine) [33,34,35,36,37,38,39]. Comprehensive studies have also shown that asthmatic patients have elevated levels of 8-oxoG in their sputum, serum, urine and BALF compared to controls [36,40,41]. Increased 8-oxoG levels in the genome and

body fluids are traditionally considered markers of inflammation; however, it remains unknown as to whether these lesions contribute to the development, maintenance and/or progression of inflammatory processes underlying asthma.

PARADOXICAL ROLE OF GENOMIC 8-OXOGUANINE IN INNATE INFLAMMATION

To elucidate the role of genomic 8-oxoG and OGG1 in pathophysiological processes, *Ogg*1^{null} mice were developed [42,43]. Intriguingly, the lack of OGG1 activity and consequent supraphysiological 8-oxoG levels did not affect embryonic development or life span. Despite high levels of 8-oxoG in the mitochondrial DNA (> 20-fold increase vs. wild-type), the mitochondria were functionally normal, with no detectable changes in maximal respiration rates or mitochondrial ROS generation [44]. Under chronic oxidative stress (such as that occurring in asthma), 8-oxoG levels increased by 250-fold in *Ogg*1^{null} mice without apparent consequences, and there was no increased incidence of precancerous lesions or tumors including lungs [45].

Unexpectedly, *Ogg*1^{null} mice show decreased inflammatory responses to bacterial infection [46], and increased resistance to lipopolysaccharide (LPS)-induced inflammation and organ dysfunction [47]. The decreased immune response was associated with significantly lower serum chemokine/cytokine levels and prolonged survival after LPS exposure, despite a marked increase in LPS-induced oxidative stress in the lungs, heart, kidneys, and liver. Moreover, sensitized *Ogg*1^{null} showed significantly decreased contact hypersensitivity to oxazolone when compared to wild-type ones as shown by attenuation of chemokine/cytokine responses including interleukin (IL)-1β, tumor necrosis factor-alpha (TNFα), macrophage inflammatory protein-1 (MIP1-a), IL-4 and lower inflammatory cell accumulation [47]. These data raise the possibility that OGG1 itself and/or OGG1-BER could be the link to immune processes and associated diseases.

DECREASED ALLERGIC IMMUNE RESPONSES IN THE ABSENCE OF 8-OXOG REPAIR BY OGG1

Li and colleagues [48] documented that, compared to wild-type, ovalbumin (OVA) challenge of sensitized *Ogg*1^{null} mice resulted in significantly lower levels of Th1 TNF-α, interferon-gamma (IFNγ), IL-2, IL-12, and T-helper-2 (Th2) cytokines (IL-4, IL-13), and IL-6, IL-17 levels and inflammatory cell infiltration in lung tissues due to decreased NF-κB activation after OVA challenge. In lung epithelial cells, OGG1 downregulation led to both decreased ROS generation and higher IFN-γ production. The authors concluded that OGG1 may influence airway inflammation by regulating the cellular oxidative metabolism [48].

Environmental pollutants primarily affect the epithelium and its constituent cells [2,49,50]. Therefore, Bacsi and colleagues [51] investigated whether OGG1–initiated repair of genomic 8-oxoG in the airway epithelium impacted innate and allergic immune responses. OGG1 was therefore ablated from the airway epithelium of ragweed pollen grain extract (RWPE)-sensitized animals [51] before RWPE challenge, which-induced significantly lower allergic inflammatory responses as determined by the expression of Th2 cytokines, the

number of eosinophils recruited to airways, epithelial metaplasia, and airway hyperresponsiveness (AHR). In contrast, challenging OGG1-proficient lungs with RWPE led to a robust innate and late allergic inflammation [52,53,54].

Besides protein allergens, pollens carry a myriad of molecules with a variety of biological functions, including an NADPH oxido-reductase that generates ROS upon exposure. Indeed, ragweed and 39 other pollens tested induce ROS in the airways, increase genomic 8-oxoG levels, and activate OGG1-BER before innate and allergic inflammation [54]. Ablation of NADPH oxidase activity prevented RWPE challenge-induced recruitment of neutrophils and eosinophils, airway hyperplasia, and AHR [54]. Taken together, these results suggest that oxidative stress, as well as oxidative DNA damage/repair via OGG1 generate activation signals relevant to a robust inflammatory response in sensitized subjects.

ACTIVATION OF SMALL GTPASES BY OGG1-INITIATED DNA REPAIR

The linkage between OGG1-BER and innate/allergic inflammation was not obvious until recent observations showing that OGG1 binds its repair product 8-oxoG base with high affinity, and the resulting complex (OGG1•8-oxoG) physically interacts with small GTPases [55■■, 56]. Importantly, the OGG1•8-oxoG complex caused GDP→GTP exchange in canonical RAS family proteins, and so functions as a guanine nucleotide exchange factor (GEF) in a manner similar to other GEFs [57].

The high sequence homology among the RAS and RHO GTPases [58], provided the rationale to examine whether OGG1-BER and the consequent formation of a OGG1•8-oxoG complex activate the RHO family member RAC1. Hajas and colleagues reported that OGG1•8-oxoG physically interacts with guanine nucleotide-free and GDP-bound RAC1. This interaction resulted in rapid GDP→GTP exchange, indicating that OGG1•8-oxoG functions as a prototypic GEF [55■■,59■]. Luo et al., 2014 provided further insights into the biological consequences of OGG1-initiated release of 8-oxoG from DNA [60■]. These authors demonstrated that only OGG1-expressing cells display increased activation of RHOA-GTPase in oxidatively stressed cells. These observations were intriguing, as many small GTPases are redox-sensitive, and ROS have an effect similar to GEFs in that they modulate guanine nucleotide binding of GTPases [58], which could be observed in OGG1-expressing but not OGG1-deficient cells.

The biological significance of the above observation became evident from studies showing that increasing the cellular 8-oxoG level by adding it to cells, into airways, or activating OGG1-BER *in cellulo*, rapidly increased the GTP-bound levels of RAS, RAC1 and RHOA GTPases. It has been shown that RAS-GTPases activate downstream targets, including mitogen-activated protein kinase kinase, and extracellular signal-regulated kinase, and the latter's nuclear translocation [56,61■]. Activated RAC1 facilitated a spatially controlled increase in cellular ROS levels via a nuclear membrane-associated type 4 NADPH oxidase [59■]. Moreover, RHOA-GTP induced smooth muscle alpha-actin synthesis and its polymerization into stress fibers in cultured cells and lungs [60■]. It was also shown that in the airway epithelium OGG1-BER is a prerequisite for GDP→GTP exchange, KRAS-GTP-driven signaling via mitogen-activated-, phosphoinositide-3-, and mitogen- and stress-

activated protein kinases, leading to activation of the NF-κB pathway by inducing RelA phosphorylation at Serine276 and nuclear translocation [62■■,63■■]. These events are essential for NF-κB-orchestrated activation of the pro-inflammatory innate and adaptive networks, including C-C and C-X-C chemokines and ILs expression leading to mucosal airway inflammation [64,65].

ASTHMA SIGNATURE GENES INDUCED BY 8-OXOG BASE CHALLENGE OF AIRWAYS

The perturbed, primarily NF-κB-driven sub-networks change the sensitivity and response to stimuli of airway epithelial, mast, and dendritic cells, as well as basophils, lymphocytes, neutrophils and eosinophils [64,65]. The multitude of mediators they generate impacts not only the immune system but also airway smooth muscle, vascular endothelium, nerve and other cell types leading to deregulation of cellular interactions and clinical manifestations of asthma [2,13,14,15,16,17,18,66].

In examining the relevance of OGG1-BER to immune deregulation and pulmonary pathophysiology, airways were challenged with the OGG1-BER product 8-oxoG base, and resulting gene expression was determined via RNA sequencing (RNA-Seq). 8-oxoG challenge mimics the impact of OGG1-BER [56,67] and excludes ROS signaling that has been associated with the pathogenesis of asthma [5■,68]. In order to define the primary effect of 8-oxoG challenge, experiments were restricted to early time-points (30, 60, 120 min). From 2 h on, high levels of TNFα, C-C and C-X-C chemokines were present in the lungs. RNA-Seq analyses showed changes in the expression of an unexpectedly large number of transcripts (mRNAs, miRNAs and non-coding RNAs – 18,874). When threshold values of mRNA levels were set to ≥3-fold, a total of 2,381 genes were modulated (983 up- and 1398 downregulated). Gene ontology analysis (PANTHER database) showed that coding transcripts were related to various biological processes, among which immune system processes and inflammation were overrepresented (data not shown).

To elucidate the relevance of 8-oxoG challenge-induced gene expression to immune deregulation and asthma pathophysiology, we inserted our 2,381 genes into a set of 572 genes upregulated (http://www.jci.org/cgi/content/full/111/12/1863/DC1) in *A. fumigatus*- and OVA-induced experimental mouse models of asthma [69,70] and hierarchically clustered them. Fig. 1A shows that 8-oxoG challenge upregulated 344 genes essential in experimental asthma (119 were downregulated, and 109 unchanged; Fig. 1A). These unexpected data were further examined for the relevance of 8-oxoG–induced genes to immune and histopathological changes induced by *A. fumigatus* and OVA. 8-oxoG challenge up-regulated (>3-fold) 85 out of 101 genes associated with immune deregulation by *A. fumigatus* and OVA challenge. These data are visually depicted in Fig. 1B. Examples include C-C motif chemokines [e.g., MIP-1-α,-β, MIP-1-related-protein 6, MIP-1-γ, Th2-attracting C-C motif chemokine ligand-17 and -22, C-X-C motif chemokine ligand-1,-2,-5,-9, their receptors-1,4, and 5, IL-1-α,-1-β, IL-17, IL-6, and IL receptor-2,-3, -*ra2*]. Moreover, 8-oxoG challenge upregulated (by 3- to >50-fold) the expression of genes encoding proteins important in inflammatory cell attachment, migration (e.g. C-X-C motif chemokine ligand-1,-2; transcription factor activating protein 3, chemokine ligand 22,

integrin alpha-M, integrin beta chain beta 2, mannose receptor gene-1, serum soluble E-selectin, thrombospondin-1 protein), T cell development and functions (e.g., plasminogen activator inhibitor-2, TNF-α-induced protein 6 and 9), B-cell responses (e.g. membrane-spanning 4-domain protein), allergen-induced cytokine release (e.g. T-cell surface glycoprotein CD28, granzyme B, monocyte differentiation antigen CD14, secretory leukocyte protease inhibitor protein, Tenascin C, prostaglandin I receptor family) (Fig. 1B).

Given that OGG1•8-oxoG activates RAS, RAC1 and RHOA [55■■,56,59■,60■], it was not entirely unexpected that challenging lungs with 8-oxoG increased the expression of 78 of 92 genes involved in epithelial hyperplasia and mucus secretion (Fig. 1C); 28 of 35 genes linked to airway AHR (Fig. 1D) and 58 of 76 genes important in airway remodeling processes were up-regulated (Fig. 1E). These analyses suggest that OGG1•8-oxoG-induced gene expression is similar to that responsible for immune- and pulmonary pathophysiology in experimental models of allergic asthma induced by *A. fumigatus* or OVA. A detailed gene list is shown in Supplementary Materials (Table 1).

OGG1-BER-DRIVEN GENE EXPRESSION IN HUMAN ASTHMA

Next, it was examined whether 8-oxoG challenge-induced changes in gene expression are similar to those previously associated with immune deregulation and pulmonary pathology in human asthma. To address this, the human equivalent of the mouse gene list was created and compared to a list of human asthma-related genes identified and documented in the GeneCards database (www.genecards.org). GeneCards' database is integrated from the Human Genome Organization, Gene Nomenclature Committee, European Bioinformatics Institute, and National Center for Biotechnology Information and Database of Allergy and Asthma Biomarkers and others. Strikingly, of the 2,381 8-oxoG challenge-regulated genes 1,051 were previously linked to human asthma (731 were upregulated, 169 downregulated and 151 unchanged; Fig. 2A). To better define gene expression resulting from pulmonary challenge with 8-oxoG, genes associated with inflammation, atopic, non-atopic and severe asthma, as well as AHR, epithelial hyperplasia, and remodeling were further analyzed. Six-hundred and fifty-nine genes were identified as immune response-related, of which 454 were up- and 100 downregulated (95 genes were unaltered) by 8-oxoG challenge (Fig. 2B). 758 genes shown to be involved in severe asthma of which 519 were up-, 122 down-regulated and 117 were unaltered (Fig. 2C). Ninety percent of the 320 atopic and 93% of 281 genes up-regulated in non-atopic asthma by 8-oxoG exposure (Fig. 2D,E). Further analysis revealed that >82% of genes previously associated with AHR (158; Fig. 2F), and mucus production/secretion (172 genes; Fig. 2G) were up-regulated by 8-oxoG challenge of airways. GeneCards' database contains 540 genes linked to changes in molecular and cellular (airway smooth muscle, epithelium) composition, and extracellular matrix during airway remodeling [71]. 8-oxoG challenge up- (392 genes), down- (73 genes) regulated and did not change 85 of them (Fig. 2H). These results suggest that OGG1-BER-associated gene expression regulates pulmonary inflammation and cellular/tissue pathology in human asthma. A detailed gene list is in Supplementary Materials (Table 2).

CONCLUSION

A close association between intrahelical 8-oxoG and 8-oxoG base levels in the body fluids of asthmatic subjects has been extensively documented, implying that genomic integrity is continually maintained via OGG1-BER. A review of the literature and data introduced here strongly suggest that OGG1-BER and the consequent OGG1•8-oxoG-driven signaling induces gene expression implicated in innate immune and adaptive immune regulation, AHR, epithelial hyperplasia, mucin production and remodeling (Fig. 3). Together these data imply a role of OGG1-BER signaling as a basic molecular mechanism underlying gene expression in atopic, non-atopic and severe asthma. In support of this, the resistance of OGG1-null mice to deregulation of the innate and adaptive immune systems and decreased pulmonary pathology further underlines the importance of OGG1-BER. Importantly, data provided here point to a novel mechanism – a role of OGG1-BER in the initiation and maintenance of chronic inflammatory disease conditions, not only in the lungs, but other organs. A greater understanding of the molecular mechanisms of OGG1-BER signaling will be essential to the development of better therapeutic modalities to prevent/reverse related disease processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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KEY POINTS

- Asthma is a complex interplay among aberrant pulmonary responses to environmental challenge and intrinsic determinants
- Inflammation in asthma and oxidative damage to DNA are inseparable twins
- Continuous OGG1-driven DNA repair-signaling “fuels” unscheduled gene expression via small GTPases for inflammation and histological changes in asthma
- Elucidating the involved molecular pathways will aid to identify novel targets for prevention of pulmonary inflammation and histological changes

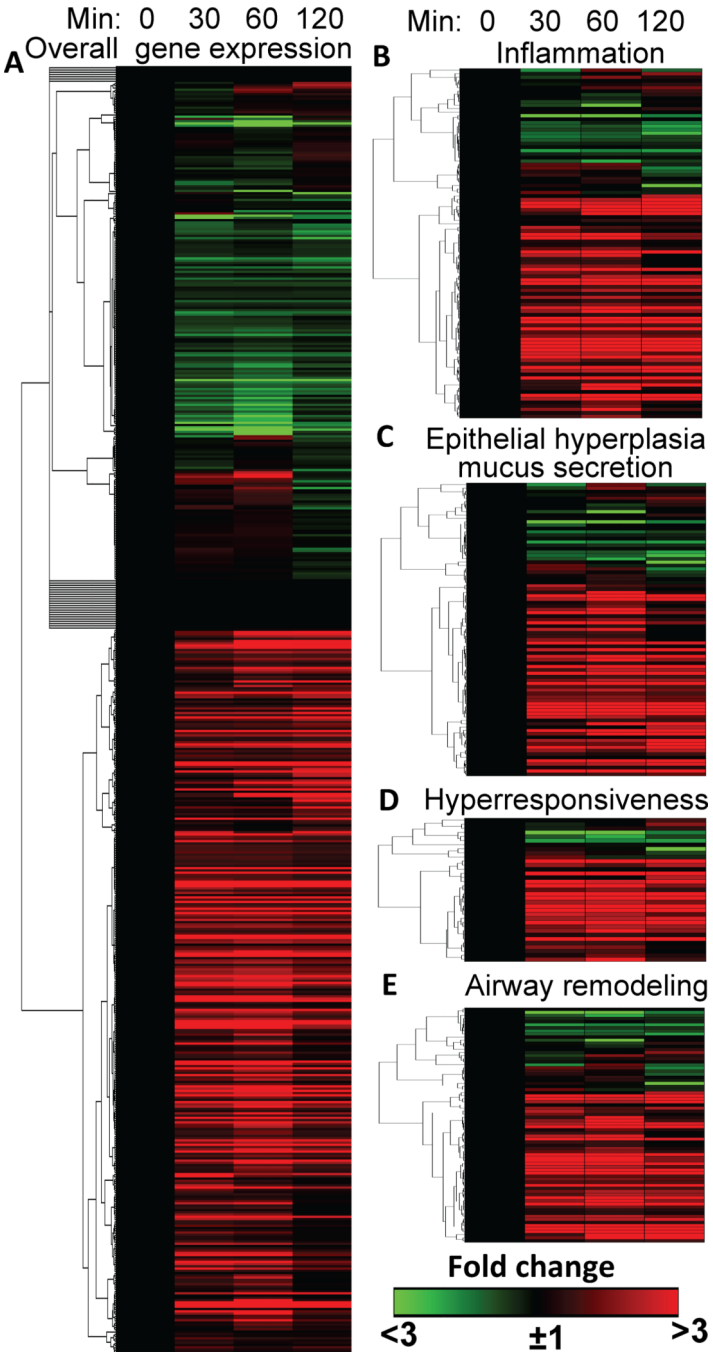

FIGURE 1.

Visual depiction of 8-oxoG-challenge-induced gene expression documented to be signatures of *A. fumigatus*-/OVA-induced experimental asthma. **A**, 8-oxoG challenge alters the expression of genes that were also up-regulated in *A. fumigatus*-/OVA-induced experimental asthma. **B**, 8-oxoG challenge modulates expression of the inflammatory genes induced during *A. fumigatus*-/OVA-induced allergic inflammation and those implicated in **C**, airway epithelial hyperplasia and mucus production; **D**, airway hyper-responsiveness and **E**, airway remodeling. Lungs of naïve mice were challenged with 8-oxoG base, RNA was extracted,

pooled from five mice for each time point, and subjected to RNA-sequence analysis (Illumina HiSeq 1000 sequencing system, UTMB Next-Generation Sequencing Core Facility). For data specificity and validity, three time points were utilized. Genes were hierarchically clustered and heat maps generated using the matrix visualization and analysis platform GENE-E (Broad Institute; Cambridge, MA). The threshold values were set to ±3-fold change in RNA levels to identify genes involved in immuno and pulmonary pathophysiology. Animal experiments were performed according to the NIH Guide for the Care and Use of Experimental Animals and approved by the UTMB IACUC (no. A0807044).

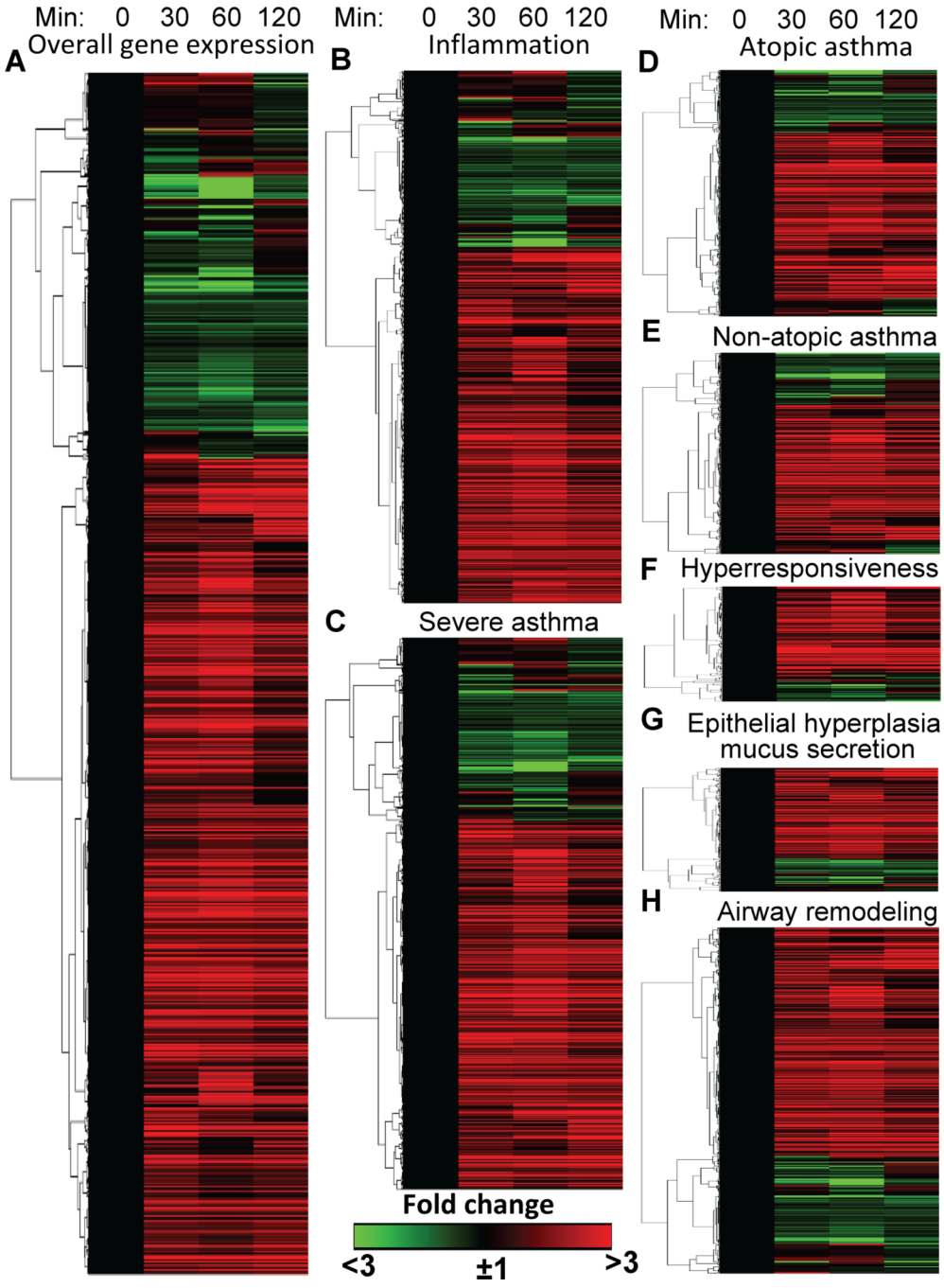

FIGURE 2.

Visual depiction of 8-oxoG challenge-induced alterations in gene expression is similar to the signatures of human asthma. RNA-Seq analysis was carried out as in the legend to Figure 1. Gene sets associated with deregulation of innate/adaptive immune system in asthma (atopic, non-atopic, severe asthma) as well as airway hyperresponsiveness, hyperlasia-mucus secretion and remodeling were defined by GeneCards' database. Genes were hierarchically clustered using the GENE-E analysis platform.

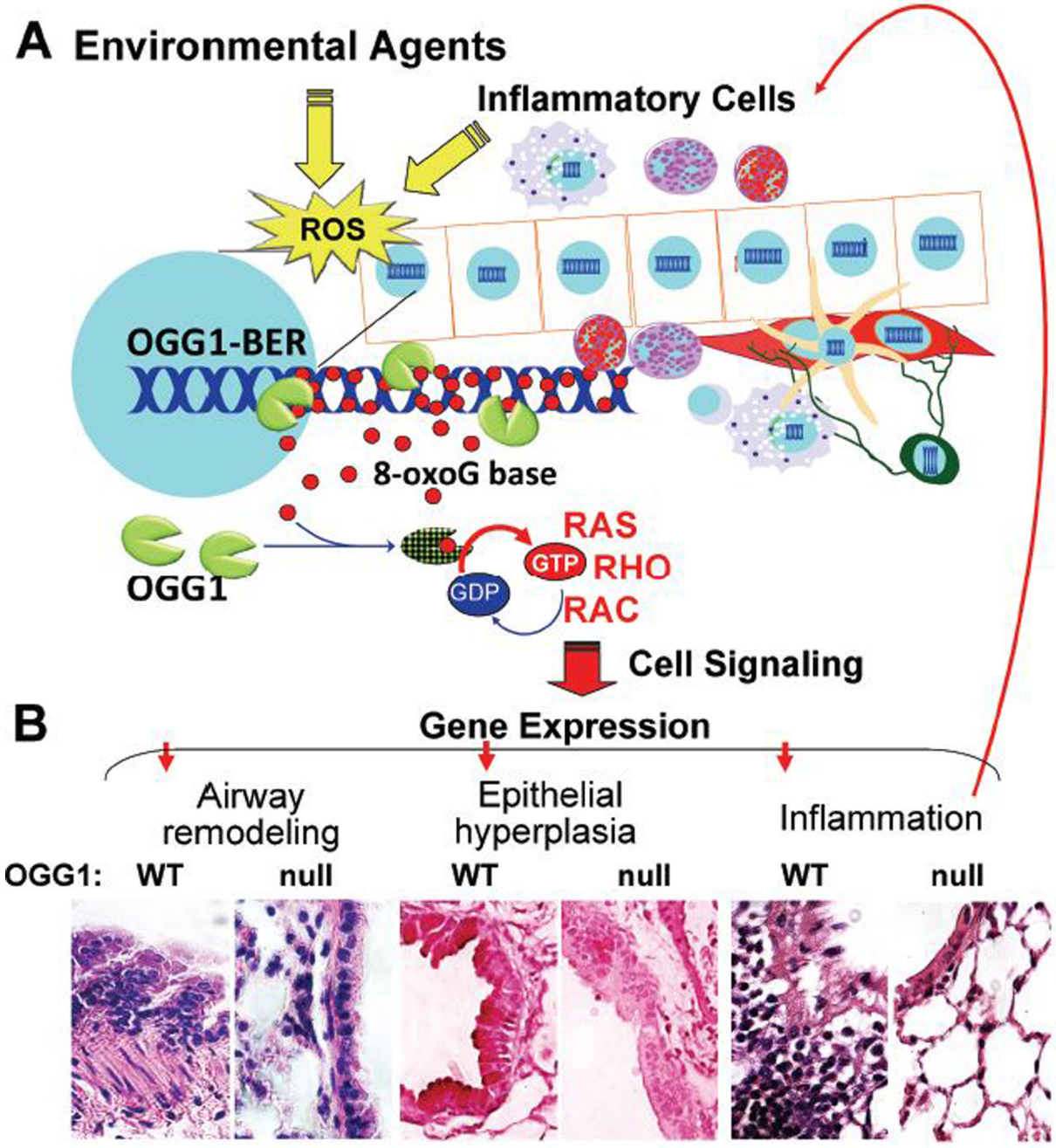

FIGURE 3.

A proposed role of OGG1-initiated DNA BER in the maintenance of chronic inflammatory processes in asthma. Environmental exposures and inflammatory cells generate ROS and intrahelical 8-oxoG. 8-OxoG is repaired via OGG1-BER, then bound by OGG1 to form OGG1•8-oxoG, which activates small GTPases. Signaling downstream from RAS, RHO and RAC induces gene expression implicated in deregulation of the innate and adaptive immune systems. Recruited inflammatory cells generate ROS, and increase DNA damage/repair, leading to a vicious cycle of chronic inflammation. **B**, Patholohistological changes resulting

from OGG1-initiated DNA repair. Sensitized OGG1 null and wild-type mice were RWPE-challenged, their lungs excised, sectioned and stained to demonstrate histological features of experimental asthma. Panel right to left: Inflammatory cells in sub-epithelium, airway epithelial hyperplasia, and increased smooth muscle mass in wild-type (left panels) vs. *Ogg*1^{null} (right panels) mice. WT, wild-type, null, Ogg1^{null}