

RESEARCH NOTE

Particulate matter air pollution exposure disrupts the Nrf2 pathway in sinonasal epithelium via epigenetic alterations in a murine model

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Funding information

SB (U01ES026721), MR (R01AI143731).

KEYWORDS

air pollution, biomarker, chronic rhinosinusitis

1 | INTRODUCTION

Exposure to airborne environmental pollutants including fine particulate matter (PM_{2.5}) has been linked to respiratory disease, including chronic rhinosinusitis (CRS), allergies, and anosmia.¹ Recent work has established a link between PM_{2.5} exposure and the development of nonallergic CRS.¹ In addition, CRS symptom severity is influenced by air pollutant exposure, and chronic PM_{2.5} exposure has been found to induce sinonasal epithelial barrier dysfunction and type 2 eosinophilic rhinosinusitis in

a novel mouse model.² Molecular mechanisms of airway responses to these environmental stimuli, including possible epigenomic reprogramming, remain unknown.

PM_{2.5} contains redox cyclic chemicals which induce oxidative stress upon exposure, causing inflammation.³ One endogenous mechanism that detoxifies these effects is the nuclear erythroid 2-related factor 2 (Nrf2) pathway.^{4,5} Upon activation by environmental stressors such as PM_{2.5}, Nrf2 (a transcription factor) translocates to the nucleus and upregulates the expression of several antioxidant cytoprotective genes: heme oxidase (HMOX-1), glutamate

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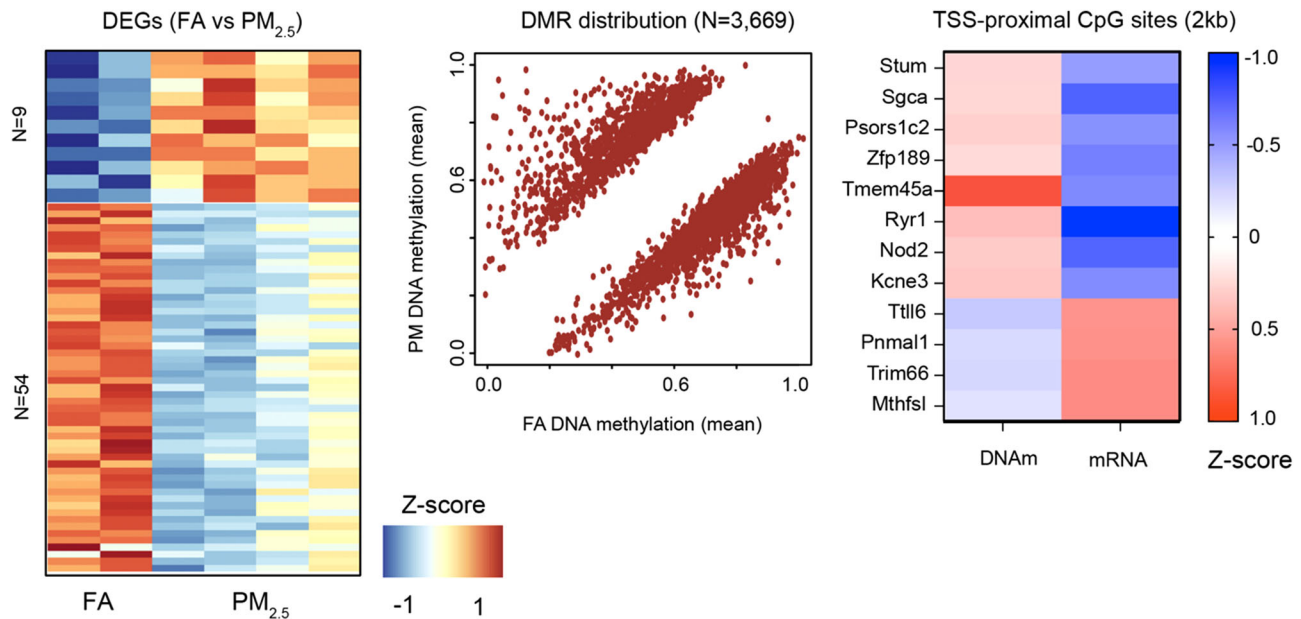


FIGURE 1 RNA-seq analysis of nasal mucosa samples uncovered differentially expressed genes (DEGs) related to $PM_{2.5}$ exposure. Left: heatmap indicating up- and downregulated genes in $PM_{2.5}$ -exposed mice (filtered air [FA] $n = 2$, particulate matter [PM] $n = 4$) with a 1.5-fold change cutoff (false discovery rate [FDR] < 0.05). Middle: scatter plot depicting differentially methylated regions (DMRs) related to $PM_{2.5}$ exposure according to whole-genome bisulfite sequencing (WGBS) analysis of nasal mucosa samples. Right: heatmap of DMR and mRNA expression in transcription start sites (TSS)-proximal CpG sites (2 kb). Increased DNA methylation is associated with repression of downstream mRNA transcription (e.g., *Stum*, *Sgca*, *Psors1c2*, etc.), and decreased DNA methylation is associated with upregulation of mRNA transcription (*Ttil6*, *Pnmal1*, *Trim66*, *Mthfs1*)

cysteine ligase catalytic subunit (GCLC), and glutamate cysteine ligase modulatory subunit (GCLM).⁵ Through such responses, Nrf2 activation restores in vitro sinonasal epithelial barrier permeability that was restricted by $PM_{2.5}$ and other toxicants.⁵ In this study, we utilized next-generation sequencing (NGS) approaches to identify altered expression pathways and epigenetic modifications that arise in response to chronic sinonasal $PM_{2.5}$ exposure in a mouse model.

2 | METHOD AND RESULTS

Adolescent male C57BL/6 mice (with Institutional Animal Care and Use Committee [IACUC] protocol approval) were exposed to either $PM_{2.5}$ ($n = 4$, 70–100 $\mu\text{g}/\text{m}^3$, comparable to extreme exposures in major urban cities in Asia) or filtered air ($n = 2$) for 14 weeks as previously described, and RNA from sinonasal tissue samples were isolated as previously described² and prepared for sequencing (Illumina TrueSeq). We quantified gene expression using a TaRGETII uniform pipeline,⁶ and raw counts were measured with featureCounts. We identified 64 differentially expressed genes (DEGs) in sinonasal tissue from $PM_{2.5}$ -exposed mice compared with the filtered-air controls (Figure 1, false discovery rate [FDR] < 0.05). Inter-

estingly, nine of the 53 downregulated genes were within the Nrf2 oxidative stress response pathway (Table 1). These genes include *hmx-1* (FA 83.34 CPM vs. $PM_{2.5}$ 32.37 CPM), *Gclc* (*Gclc*; 561.02 vs. 222.17), *Akr1b8* (16.43 vs. 7.44), *Gpx2* (111.85 vs. 60.7), *catalase* (252.98 vs. 137.52), *glutathione S-transferase 1* (89.85 vs. 51.48), *sulfiredoxin* (67.25 vs. 40.47), *glutamate-cysteine ligase modifier subunit* (*Gclm*; 237.6 vs. 147.13), and *Pparg1a* (62.23 vs. 40.86). No common pathways were identified among the 11 upregulated genes, although two olfactory receptor genes were identified (*Olfr140* and *Olfr455*) of uncertain significance.

In order to explore whether chronic $PM_{2.5}$ exposure created epigenomic modifications, we then applied bisulfite treatment to genomic DNA extracted from the same sinonasal tissues via the Accel-NGS Methyl-Seq DNA library kit (Swift Biosciences). Differentially methylated regions (DMRs) and sites were identified with a minimum of five CpG sites per region and at least > 5% difference in methylation (p -value < 0.01) using the BSmooth package. In this exploratory analysis, we observed global hypomethylation in intergenic/intron sites and hypermethylation in promoters (total DMR = 3.6K) in mice exposed to $PM_{2.5}$ compared with controls (Figure 1). We queried these locations for known Nrf2 binding sites contained in the Cistrome database and identified 13 DMRs of transcription start sites (TSS) (< 2 kb from TSS) that correspond

TABLE 1 Up-/downregulated genes after PM_{2.5} exposure and Nrf2 pathway related differentially expressed genes (DEGs) (fold change > 1.5, false discovery rate [FDR] < 0.05)

Upregulated	Fold change	Downregulated	Fold change
Gm5130	6.34	Odam	30.54
Olf140	6.17	Smtnl1	9.84
Gucylb2	6.13	Myh2	9.58
Vmn1r28	5.12	Nrap	9.32
Olf1455	4.71	Ttn	9.09
Gm17484	3.23	Neb	8.42
Rims3	2.33	Mmp12	7.97
Tspoap1	1.89	Tcap	7.51
Rd3	1.88	Myo18b	7.12
Dlgap3	1.78		
Impdh1	1.75		
		Nrf2-regulated	...
		Hmox1	2.56
		Gclc	2.49
		Gpx2	1.82
		Cat	1.82
		Gstpl	1.72
		Gclm	1.60

to Nrf2 target genes (Table 1).⁷ These data suggest that PM_{2.5} exposure results in epigenetic reprogramming of Nrf2 antioxidant response genes in the sinonasal mucosa.

3 | DISCUSSION

Here, we report for the first time that the Nrf2 pathway is globally downregulated in the sinonasal mucosa after long-term exposure to airborne PM_{2.5} in mice. These findings are analogous to the chronic obstructive pulmonary disease (COPD) literature, where Nrf2 levels are also downregulated, making Nrf2 supplementation or enhancement a therapeutic option. We also report the unique findings of epigenetic modifications of the Nrf2 gene in the sinonasal mucosa, demonstrating that chronic PM_{2.5} exposure may have long lasting, potentially irreversible effects.

Few studies have examined the link between PM_{2.5} stimulation and Nrf2 expression. A study by Piao et al. demonstrated that PM_{2.5} exposure alone for 5 weeks caused a decrease in Nrf2 signaling with limited sinonasal inflammation.⁸ These findings are in contrast to previous cell culture studies stimulated with acute exposures to PM_{2.5} which demonstrated increased Nrf2 expression. Based on our findings, we postulate that chronic PM_{2.5} exposures are necessary to overcome the acute oxidative

stress phase to create downregulation of Nrf2 with robust eosinophilic inflammation.

Disruption of key antioxidant responses at the mucosal interface may represent a critical mechanism by which air pollution causes CRS. In prior research, activation of the Nrf2 pathway was shown to strengthen the sinonasal epithelial barrier and mitigate the type 2 inflammatory response and eosinophilia in the nose and sinuses of mice.^{5,9} Indeed, Nrf2 activation via *Keap1* deletion attenuates sinonasal inflammation and improves epithelial barrier function in mice, while deletion of *Nrf2* exacerbates inflammation and creates epithelial barrier dysfunction.¹⁰ Thus, our data in this pilot study with a limited sample size, demonstrating that chronic PM_{2.5} exposure creates eosinophilic inflammation and epigenetic reprogramming of Nrf2 antioxidant response genes in the sinonasal mucosa, suggests that the Nrf2 pathway may be a useful therapeutic target for treatment of PM_{2.5}-induced or exacerbated CRS. Studies of this phenomenon in larger samples and in translational studies in humans could provide greater insight into the molecular mechanisms that underlie environmentally mediated inflammatory disease in the upper airway.

ACKNOWLEDGEMENTS

We would like to acknowledge Jinhu Yin, PhD and Daifeng Wang, PhD for their technical and analytical expertise.

DISCLOSURES

N. London holds stock in Navigen Pharmaceuticals and was a consultant for Cooltech Inc., neither of which are relevant to the study. The other authors declare no relevant conflict of interest.

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How to cite this article: Park B, London NR, Tharakan A, et al. Particulate matter air pollution exposure disrupts the Nrf2 pathway in sinonasal epithelium via epigenetic alterations in a murine model. *Int Forum Allergy Rhinol.* 2022;12:1424–1427. <https://doi.org/10.1002/alr.23010>