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Review

Particulate Matter Toxicity Is Nrf2 and Mitochondria Dependent: The Roles of Metals and Polycyclic Aromatic Hydrocarbons

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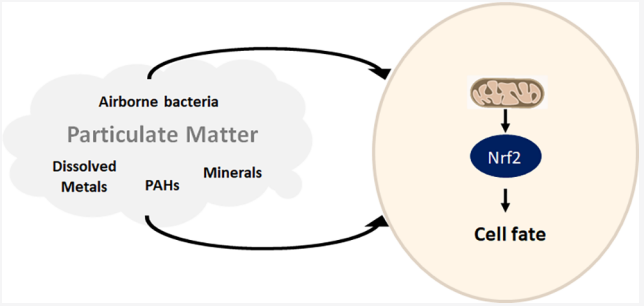
ABSTRACT: Particulate matter (PM), an important component of air pollution, induces significant adverse health effects. Many of the observed health effects caused by inhaled PM are associated with oxidative stress and inflammation. This association has been linked in particular to the particles' chemical components, especially the inorganic/metal and the organic/polycyclic aromatic hydrocarbon (PAH) fractions, and their ability to generate reactive oxygen species in biological systems. The transcription factor NF-E2 nuclear factor erythroid-related factor 2 (Nrf2) is activated by redox imbalance and regulates the expression of phase II detoxifying enzymes. Nrf2 plays a key role in preventing PM-induced toxicity by protecting against oxidative damage and inflammation. This review focuses on specific PM fractions, particularly the dissolved metals and PAH fractions, and their roles in inducing oxidative stress and inflammation in cell and animal models with respect to Nrf2 and mitochondria.



INTRODUCTION

Ambient air pollution is a global health risk factor.[1−3] It increases mortality and morbidity and has been identified as a leading risk factor for the global burden of disease, contributing to an estimated 4.1 million deaths in 2016,[3−5] especially in low-/middle-income countries.[6] Air pollution mainly affects the respiratory system,[7] and numerous studies have correlated exposure to ambient fine particulate matter (PM) with an aerodynamic diameter smaller than 2.5 μm ($PM_{2.5}$) with various health-related outcomes, such as premature death, cardiovascular diseases (CVD), lung diseases, stroke, asthma, and chronic obstructive pulmonary disease (COPD).[3,4,6−13] Furthermore, the International Agency for Research on Cancer (IARC) has declared outdoor air pollution carcinogenic to humans, as it increases cancer incidence.[14]

Air pollution is an airborne mixture of substances including gases, particles, and biological components, in the Earth's atmosphere. $PM_{2.5}$ in particular has been implicated in adverse health effects. $PM_{2.5}$ is composed of either primary particles that are emitted directly into the atmosphere or secondary particles produced *in situ* by atmospheric chemical reactions between precursor gases or between any gas-phase species and primary particles. Primary $PM_{2.5}$ can originate from both natural sources (dust storms,[15] forest fires[16]) and anthropogenic sources (biomass burning, fossil fuel combustion, consumer products,[17,18] cigarette smoke [CS]), resulting in a complex chemical mixture of solids and liquids.[19−24] Secondary $PM_{2.5}$ is generated by chemical reactions and physical interactions that involve sulfuric acid, nitric acid, and volatile organic species from anthropogenic and/or biogenic sources. These components react with ozone, hydroxyl radicals, nitrate radicals, and other reactive agents to form secondary inorganic aerosols (SIAs) and secondary organic aerosols (SOAs).[13,25−27] On a global scale, emissions of biogenic volatile organic compounds (VOCs) from surface vegetation, oceans, and soils are greater than anthropogenic emissions.[26] On regional and urban scales, anthropogenic VOCs from fuels, industry activities, and consumer products may compose the majority of secondary PM formation.[17]

The health effects caused by inhaled $PM_{2.5}$ are associated with the particle sizes and shapes, the chemical composition of the mixture, and the ability of the particles to absorb and retain toxic and carcinogenic compounds.[28−30] The toxicity of $PM_{2.5}$ particles depends on their sizes and masses which influence their ability to penetrate into and deposit in the lungs. $PM_{2.5}$ particles are particularly important as they account for the majority of the deposited mass in the deeper lung, leading to clear correlation between $PM_{2.5}$ and epidemiological evidence for health effects. However, it remains unclear whether $PM_{2.5}$ components of the same mass elicit the same biological effects

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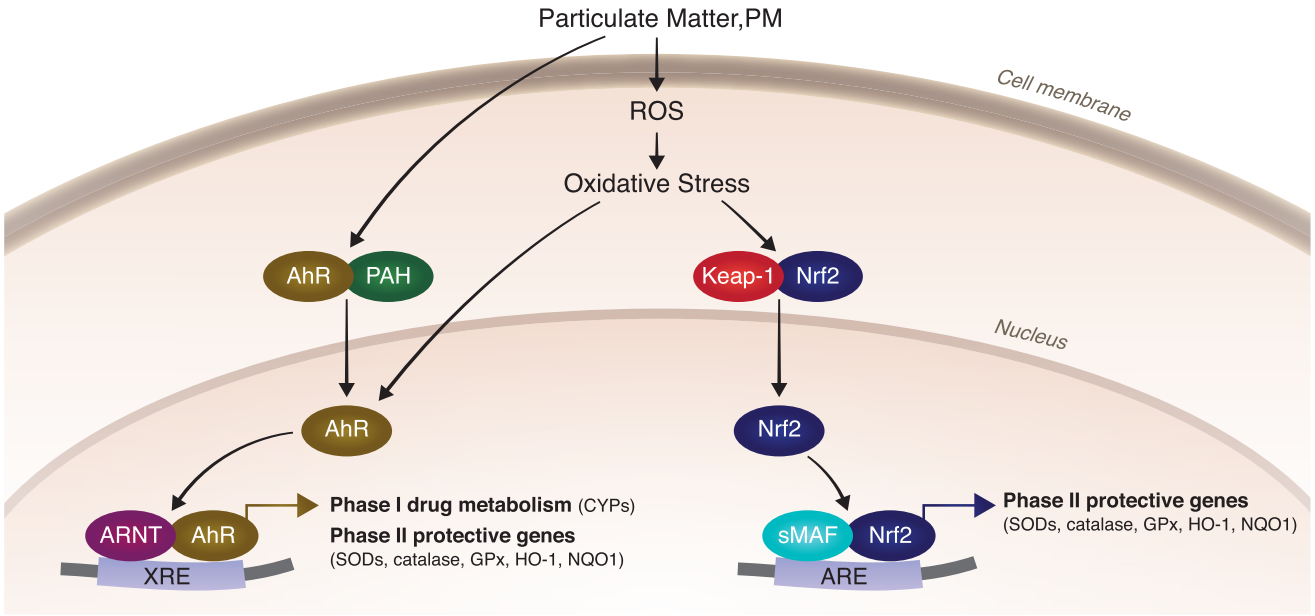

Figure 1. Activation of Nrf2 and AhR signaling pathways. Inorganic components of PM, such as metals, cross the cell membrane by facilitated diffusion/membrane transport proteins, where they then induce oxidative stress. Organic components such as PAHs are able to span the cells' membrane due to their lipophilic characteristics and can also act as AhR ligands. Nrf2 is bound to KEAP-1 in the cytosol; a change in redox homeostasis leads to conformational changes, leading to translocation of Nrf2 to the nucleus. Activation of the Nrf2 pathway is mediated by the ARE enhancer sequence in target genes. AhR can be directly activated by PAHs or naturally occurring compounds and endogenous ligands, which bind to AhR and facilitate AhR translocation to the nucleus, where it binds to XRE and activates phase I/II enzyme genes. Activation of the AhR leads to oxidative stress due to metabolism of the ligand and induction of CYP enzymes.

and whether individual components of $PM_{2.5}$ cause different health-related effects; furthermore, the main mechanisms of toxicity have not been elucidated.

The chemical composition of $PM_{2.5}$ may be broadly divided into three groups: (1) minerals from different origins, including common resuspended minerals (e.g., quartz) and minerals that form through chemical and physical reactions;[31] (2) dissolved metals (V, Fe, Pb, Zn, Cd, Mn, Co, Cu), which are present in trace concentrations in fossil fuels but are also emitted from biomass burning, combustion processes, vehicle traffic, brake/tire wear and dust, etc.; and (3) organic components generated from primary and secondary sources, including toxicants such as polycyclic aromatic hydrocarbons (PAHs), oxygenated/nitrated PAHs, radicals and persistent organic radicals, phenols, and atmospheric humic-like substances (HULISs). Organic compounds with aromatic rings form through incomplete combustion of coal, petrol, and wood from residential, industrial, and mobile sources.[19,20,25,32] The chemical composition of PM is thought to be an important determinant of health outcomes.[8,9,30,33−39] However, the specific characteristics or components of PM that are harmful to human health and the actual mechanisms by which they affect health are still not well understood. Recent and continuing advances in atmospheric analytical chemistry and biological research techniques enable researchers to narrow the knowledge gaps and resolve atmospheric pollution compositions,[24] so that specific atmospheric components can be connected to the health effects that they cause.

The main mechanisms of PM-induced health effects are thought to involve oxidative stress and inflammation. Various components of PM, including environmentally persistent radicals, peroxides, aromatic compounds, and dissolved metals, can generate reactive oxygen species (ROS), leading to oxidative stress and consequently enhancing various biological processes such as inflammation and cell death.[7,40−44] The mitochondria are major locations for ROS production as well as cellular targets of the damaging effects of PM,[45−47] suggesting a key role for the mitochondria in PM-induced toxicity. Previous studies have shown that the transcription factor NF-E2 p45-related factor 2 (Nrf2), which regulates the expression of phase II detoxifying enzymes, plays a key role in preventing PM-induced toxicity by protecting against oxidative damage and inflammation. Thus, phase II detoxifying enzymes can influence the courses of cell and tissue responses.[39,48−54] This paper provides an overview of how exposure to PM fractions, particularly inorganic and organic fractions (specifically dissolved metals and PAHs), can induce biological responses *in vivo* (in animals) and *in vitro* (in cultured cells) with respect to the Nrf2 system and to mitochondria.

PM INDUCES REDOX-SENSITIVE NRF2-DEPENDENT RESPONSE

Exposure to $PM_{2.5}$ is a major global health concern.[3,6,10,11] The respiratory system, the major exposure pathway, is inevitably affected; it is exposed to various inhaled toxicants (e.g., environmental gases, PM and radicals that are present in $PM_{2.5}$).[13,51,55] The unique characteristics and anatomy of the lungs generate an oxidative microenvironment that requires constant redox homeostasis. Exposure to environmental pollutants (gases and PM) can disturb the lungs' redox homeostasis by elevating ROS levels and by reducing antioxidation capacity, thus leading to oxidative stress.[43,52] Cells and tissues contain antioxidants that respond to and counteract oxidative stress. These molecules are abundant in the lung epithelial fluid and lung tissue.[52] The antioxidant system includes antioxidant enzymes, such as superoxide dismutases (SODs), catalase, and glutathione peroxidase (Gpx), indirect phase II protective genes, such as gluta-

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thione-S-transferase isozymes, γ-glutamyl cysteine ligase catalytic and modifier subunits, and NADP(H):quinone oxidoreductase (NQO1), and small antioxidant molecules (GSH). The protective genes are induced at the transcriptional level through a process mediated by a *cis*-acting element called an antioxidant response element (ARE).[56−58] Activation of gene transcription through AREs is mediated by Nrf2.[59] In addition, the phase II protective genes are mainly regulated by Nrf2.[48,57] Nrf2 belongs to the cap'n'collar family of transcription factors, each member of which has a highly conserved basic-region leucine zipper structure.[60] Nrf2 activity is regulated by Kelch-like ECH-associated protein-1 (KEAP-1), an adaptor protein that binds to Nrf2 in the cytoplasm to induce its proteasomal degradation. Activation of Nrf2 in response to stress releases Nrf2 to translocate to the nucleus, where it affects gene transcription.[59] Thus, Nrf2 is involved in the oxidative and inflammatory responses induced by exposure to environmental pollution, especially those caused by acute and chronic exposure to $PM_{2.5}$.[39,48−54,58,61] (Figure 1).

One major question is to what degree specific molecules or particle components (directly or indirectly) drive the oxidative stress response. With regard to particle toxicology, the oxidative stress paradigm includes oxidative effects that originate from the particles or particle components and assumes that the biological reactivity of a particle is due to its oxidative potential (OP) and to secondary ROS production in exposed cells and tissues.[8,62,63] ROS derivatives (hydrogen peroxide, organic hydroperoxides, quinones, semiquinones, environmentally persistent radicals) are molecules that can induce oxidative stress.[17,19,24,25,34,64−67] Additionally, other molecules can change their oxidation states and promote the formation of ROS, thus exacerbating oxidative stress.[39,53,68−71] The two main components of $PM_{2.5}$ that will be discussed in this review are the metals and the PAHs. We focus on these components because although they are not the most abundant components in PM, they have the greatest potential to generate ROS in biological systems.

Role of Metals in Nrf2 Activation. $PM_{2.5}$ can contain metals that originate from vehicles (brake and tire wear),[70,72] combustion,[15] construction, and urban and mineral dust.[69] It has been shown that exposure to water-soluble metals present in urban PM can induce the activation and expression of Nrf2/antioxidants/phase II detoxifying enzymes, possibly as part of the protection system against oxidative stress.[53,55,70,72−74] Metals present in PM, particularly Fe, Zn ,and Cu,[70,72] have been found to activate a Nrf2-dependent antioxidant response that protects exposed human bronchial epithelial cells from apoptosis.[68] Removal of these soluble metals by chelation significantly diminishes the pulmonary Nrf2 response.[72] A combination of exposure to aqueous extracts of PM containing dissolved metals and feeding of an obesogenic diet has been found to elicit DNA methylation in a tissue-specific manner and to especially affect the expression of catalase and Nrf2.[75] Exposure of HL-1 cardiomyocytes to residual oil fly ash particles from oil combustion that also contain trace metals such as V, Al, Si, and Fe[76] induces Nrf2 activation and nuclear translocation that lead to a protective antioxidant response.[53]

Role of the Organic Fraction/PAHs in Nrf2 Activation. A major fraction of the global $PM_{2.5}$ mass is composed of organic compounds from anthropogenic and biogenic origins. $PM_{2.5}$ from combustion and biomass burning can contain a high proportion of PAHs and other aromatics such as phenols from lignin pyrolysis and HULIS.[17,19,20,25,32] Indeed, it has been shown that PAH-rich particles that form in premixed flames, such as those from vehicle exhaust, can induce oxidative stress and activate Nrf2 in rat lungs.[50] Urban pollution particles with high levels of PAHs and polychlorinated biphenyls from Buenos Aires have been found to induce Nrf2 activation in cardiomyocytes.[53] Furthermore, positive associations have been observed between traffic-related pollutants ($PM_{2.5}$ PAHs, black carbon, and NOx) and Nrf2-gene expression in a Los Angeles cohort (elderly subjects with coronary artery disease).[54] Exposure of mice to different concentrations of PM from biodiesel burning have been found to activate the Nrf2/heme oxygenase (HO)-1 and inflammation via the nuclear factor kappa-B (NF-κB)/tumor necrosis factor (TNF)-α pathways.[49,77] In addition, exposure of endothelial cells residing at the air−liquid interface (ALI) to standard diesel exhaust PM (SRM2975) induced significant activation and nuclear translocation of Nrf2.[78]

In the atmosphere, PAHs can further react and form oxygenated or nitrated derivatives by atmospheric aging processes.[13,19,27,32,79−82] Atmospheric oxidation of PAHs and other organic compounds generates SOAs, which account for a substantial mass fraction of aerosols in the atmosphere.[24,25,27,65,80] SOAs can also affect the Nrf2 gene expression pathway. For example, in one study, human airway epithelial cells (BEAS-2B cells) were exposed to isoprene SOAs in an ALI exposure system, and the most affected genes belonged to the Nrf2 pathway.[38,83,84] In addition, a study in which BEAS-2B lung cells were exposed to isoprene-derived SOA constituents (from isomeric isoprene epoxydiols, IEPOXs) showed that 33 target gene-miRNA pairings were associated with Nrf2-oxidative stress pathways.[36] In another ALI study, Nrf2 levels were found to increase following exposure of lung epithelial cells (A549 cells) to aged naphthalene SOAs.[34]

Therefore, both trace metals and PAHs in $PM_{2.5}$ can trigger the Nrf2/antioxidant defense response upon exposure. However, whether Nrf2-mediated induction of antioxidant genes protects against chemical air pollutants and whether organic and inorganic compounds activate Nrf2 via the same mechanism of action are not yet clear.

PM-INDUCED NRF2 REDOX SIGNALING: THEORY, DOSE RESPONSE, AND RELATED MECHANISMS

Exposure to $PM_{2.5}$ has been implicated in both oxidative stress and inflammation, which are underlying mechanisms of lung damage and CVD.[10−12,42,53−55,72,85] The oxidative stress paradigm suggests that long-term exposure to low levels of environmental ROS or oxidative stressors induces antioxidant production to restore redox homeostasis. When this protection is insufficient,[86,87] the increased stress induces other mechanisms, such as inflammation.[43,48,88,89] Finally, when all defense systems are overwhelmed, they shut down, leading to cell death.[85] These responses depend on pro-/antioxidant balance, which varies from one organism to another.[86,87] Under physiological conditions, the basal levels of the transcription factor Nrf2 in the cytosol are low, since Nrf2 is bound to KEAP-1. Elevation in oxidative stress leads to translocation of Nrf2 to the nucleus, where it binds to AREs[56−58] and activates phase II protective genes that help to maintain homeostasis (Figure 1).

Chronic exposure to environmental PM affects lung homeostasis, suggesting that Nrf2 activation depends on the PM exposure dose. A recent study compared the impact of a

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single exposure to PM extract with the impact of repeated exposures to PM extract of the same dose and from the same source in mouse lungs.[55] After a single exposure, Nrf2 levels increased, and the lungs remained intact. However, following five repeated exposures, oxidative damage in the lungs and a systemic inflammatory reaction were observed. The lung mRNA levels of Nrf2/antioxidant system phase II detoxifying enzymes decreased following repeated exposures. Disruption of the lung tissue oxidant/antioxidant (inflammatory/defense) balance was evidenced by increased levels of lipid and protein oxidation.[55] These results support a phenomenon in which low-dose or single acute exposure to PM induces the expression of Nrf2-related antioxidant genes to counteract the increased ROS levels in tissue. Under high-level exposure conditions or in response to multiple chronic exposures, the antioxidant system may fail to be activated and protect against the oxidative burst, thus leading to lung tissue oxidative damage. Other findings that support this concept were obtained in a study on mice exposed to biodiesel PM. The exposed mice showed increased protein expression levels of Nrf2, p-NF-κB, and HO-1; however, the Nrf2 levels were higher in the low-dose exposure group than in the high-dose exposure group.[49] In another study, Nrf2/phase II protective gene expression was directly related to the number of exposures at the lowest $PM_{2.5}$ dose ($2\,\mu g/cm^2$) but, surprisingly, inversely related to the number of exposures at the highest dose ($10\,\mu g/cm^2$). Again, this response may have been attributable to a compromised capacity to activate the protective Nrf2 tissue defense system under high-dose exposure.[90]

Other factors can also activate the Nrf2/antioxidant system. These include direct and indirect mechanisms by which $PM_{2.5}$ exerts its effects. The direct pathways are related to the penetration of $PM_{2.5}$ particles or their components into the pulmonary system to directly affect lung cells and lung and other tissues.[91] The indirect pathways are related to mechanisms by which exposure of the respiratory system to PM causes the release of inflammatory cytokines that circulate through the bloodstream and induce a systemic reaction that can affect remote tissues.[7,92,93] In any case, the lungs, as the primary organ that encounter the particles, are exposed to the highest masses/doses of the particles.[7] Oxidative stress and inflammation have been detected in the lungs and liver of mice exposed to water and organic extracts of urban $PM_{2.5}$ collected in Beijing, China. Nrf2/phase II protective enzymes are activated in the liver of these mice but suppressed in the lungs. These findings suggest that toxic components from PM circulate in the bloodstream from the primary organs (lungs) to secondary organs (such as the liver) that can have different susceptibilities to exposure.[94] In addition, organic pollution extracts with high PAH content have been found to cause damage to the liver than to the lungs, as PAHs can trigger signaling agents such as inflammatory cytokines or can accumulate in other tissues, especially fatty tissues such as the liver,[94] where they induce toxicity. In a similar manner, different basal expression levels and metabolic rates in different tissues may result in organ-specific Nrf2 responses. In one study, the effects of concomitant metal-rich $PM_{2.5}$ extract exposure and high-fat diet feeding on remote metabolic tissues, such as the liver and white/brown adipose tissues, were investigated. Exposure to the $PM_{2.5}$ metal extract led to opposite responses (of select genes related to the Nrf2 pathway) in the lung and liver. Compared to a normal diet, the high-fat diet increased Nrf2 and catalase gene expression in the liver.[75] Together, the data presented for lungs, liver, and adipose tissues provide general insights into the systemic and tissue-selective impacts of PM exposure and the possible metabolic implications.

Mechanisms of Action of Metals on Nrf2 Signaling. The diverse characteristics of the $PM_{2.5}$ chemical components may trigger different signaling pathways to activate Nrf2. Initially, metals cross the cell membrane via facilitated diffusion or transport through membrane proteins.[95] It has been suggested that the main mechanism of action of metals from $PM_{2.5}$ involves induction of oxidative stress and ROS formation.[35,96,97] Metals can elicit the formation of ROS through Fenton and Haber−Weiss reactions and can subsequently induce oxidative stress. These pro-oxidant factors can change the cellular redox state.[44,96]

Metals can also evoke an inflammatory response that occurs when tissues or cells are damaged by $PM_{2.5}$. Immune cells and other molecular mediators are involved in this protective response.[48] In humans, exposure to aqueous extracts of $PM_{2.5}$ containing high concentrations of dissolved metals has been found to cause an inflammatory response, increasing IL-8 and TNF-α levels in the lower respiratory tract and bronchoalveolar lavage fluid. Moreover, $PM_{2.5}$ can stimulate secondary ROS generation as part of an inflammatory response.[48,61,98,99] The studies reviewed so far show the connections between dissolved metals from $PM_{2.5}$ and the induction of Nrf2, thus supporting the involvement of metals in both oxidative stress and inflammation related to Nrf2 activation. The effects of the metals are likely mediated by enhanced ROS formation or cytokine secretion.

Mechanisms of Action of PAHs on Nrf2 Signaling. The organic component of $PM_{2.5}$, which is partially made up of PAHs, quinones, peroxides, and radicals, can contribute to oxidative injury in the lungs.[13,34,81,82] The mechanisms of the effects of the organic fraction may involve ROS formation and other processes. Due to their lipophilic character, PAHs can cross the cell membrane, but they can also act as ligands for aryl hydrocarbon receptor (AhR).[100] AhR is expressed in all tissues but is highly expressed in the liver, adipose tissues, and bronchial epithelial cells.[101] Activation of AhR upregulates cytochrome P450 (CYP) metabolizing enzymes that can transform toxicants into less toxic forms (thus providing protection). PAHs are often metabolized into quinones, which can be further metabolized into semiquinones.[66] When semiquinones are reduced back into quinones in a process called redox cycling, they can form additional ROS[67,96] that further aggravate $PM_{2.5}$ toxicity.[100,102] On the one hand, this dual action of AhR activation may contribute to the amplification of some diseases; on the other hand, it may alleviate others.[103] It has been shown that ambient urban dust that contains PAHs induces proinflammatory T cell and dendritic cell responses via AhR in naive CD4+ T cells purified from male/female adult mice.[102]

PM that contains environmentally persistent free radicals can also induce AhR activation and cytokine secretion in human bronchial epithelial cells.[104] Crosstalk between AhR (phase I) and Nrf2 (phase II) protective/metabolizing enzymes[98] may exist, because both types of proteins control the expression of xenobiotic-metabolizing enzymes (XRE).[56,88] For example, the expression of NQO1 depends on both AhR and Nrf2 (Figure 1). Exposure to PAHs induces Nrf2 activation in the mouse hepatoma cell line 1c1c7, but this

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effect can be abolished by transfection with a small interfering RNA (siRNA) targeting AhR,[105] thus indicating the existence of a link between Nrf2 and AhR. In addition, exposure of rat progenitor cells to PAHs induces the expression of CYP1A1, CYP1B1, and Nrf2 genes in the WB-F344 cell line.[106] Another study has revealed that exposure to extracts from PAH-rich $PM_{2.5}$ collected in Beijing, China, increases Nrf2 and CYP gene (CYP 1a1 and 2e) expression in the liver, but not in the lungs.[94] This finding implies that PAHs can exert their toxic effects through two different mechanisms: by increasing ROS formation and thus increasing oxidative stress and by binding to AhR, which may directly or indirectly activate Nrf2. It is yet to be determined whether AhR is activated in correlation with Nrf2 in lung tissue and whether this induction enhances or aggravates protection through Nrf2 signaling

PAHs can also generate strong proinflammatory responses, for example, the release of the proinflammatory cytokines IL-1β, IL-6, and TNF-α from macrophages increases after exposure to $PM_{2.5}$ organic extracts[40,81,107] In addition, exposure to a PM mixture of diesel exhaust together with an urban-like pollutant mixture has been found to produce a significant IL-8 inflammatory response in A549 lung cells.[77,108] Acrolein and *p*-tolualdehyde (representative VOCs) elicit an inflammatory response and cellular damage in A549 cells.[109] Furthermore, mouse macrophages exposed to PAHs show induction of IL-1β, Toll-like receptor, and NF-κB, indicating an increased inflammatory response. This response is induced by organic extracts but not water extracts, implying that inflammation is the main mechanism of action of PAHs.[110] Whether PAHs function through oxidative stress or inflammatory processes is not clear. It is possible that both mechanisms are activated in response to exposure to PM organic/PAH extracts and that a strong decrease in Nrf2 expression after exposure to PAHs[39,40,111] amplifies the inflammatory response.

MITOCHONDRIA ARE INVOLVED IN PM-INDUCED TOXICITY

ROS generation and subsequent increases in oxidative stress have been recognized as major contributors to cell damage, cell death, DNA damage, and inflammation due to $PM_{2.5}$ exposure.[39,41,112,113] As ROS are produced mainly in the mitochondria as byproducts of cellular respiration,[113,114] disruption of mitochondrial electron transport (oxidative phosphorylation) can further augment ROS production and amplify oxidative stress.[41,114] Mitochondria are highly sensitive to environmental toxicants,[47] and $PM_{2.5}$ has been shown to accumulate within mitochondria[112,115] and further disrupt mitochondrial membrane potential,[39] damage mitochondrial structure and function,[39,45,112] alter mtDNA (through strand breaks and methylation),[39,47] and activate mitochondrial programmed apoptosis in pulmonary tissues.[41,116] However, studies on the specific connections between PM exposure and mitochondria are limited; thus, these connections require further investigation. Several studies have suggested that metals and PAHs exert their toxicity through different mechanisms involving mitochondria.[37,99,112,116−118]

Roles of Metals in Mitochondria-Related Toxicity. The specific effects of metals from PM on mitochondria have been shown in several studies. Metals found in oil fly ash, especially V, Fe, and Ni, impair mitochondrial function and increase apoptosis in lung epithelial cells.[116] PM-bound metals that penetrate into cells and enter mitochondria can disturb mitochondrial membrane potential, disrupt mitochondrial structure, induce intracellular and mitochondrial ROS production, change calcium homeostasis, and induce apoptosis. All of these processes are also correlated with cancer progression.[115] Examination of lung epithelial cells exposed to PM samples collected in Milan containing high concentrations of transition metals (Ni and Zn) and PAHs has shown the occurrence of cell membrane lysis and mitochondrial ultrastructural disruption with increased ROS production, suggesting that metals (but also PAHs) contribute to ROS production and toxicity.[117] Pretreatment of alveolar epithelial cells with Fe chelators protects the cells from $PM_{2.5}$-induced mitochondrial dysfunction, DNA damage, and apoptosis.[99] In addition, a recent study has shown that exposure to metals from PM results in increased ROS production in lung epithelial cells and reduced mitochondrial function.[39] Collectively, these findings suggest that the cytotoxicity exerted by metals is driven by changes in oxidative status that induce mitochondrial dysfunction and apoptosis.

Roles of PAHs in Mitochondria-Related Toxicity. PAHs toxicity has also been shown to involve mitochondria. It has recently been shown that exposure of bronchial epithelial cells to $PM_{2.5}$ leads to PAHs penetration into the cells; activation of AhR, which influences mitochondrial membrane potential and apoptosis.[37] Another study has demonstrated that ultrafine particles (UFPs), especially organic carbon and PAHs, from $PM_{2.5}$ collected in Los Angeles induce oxidative stress and mitochondrial damage, suggesting a role of organic agents in generating redox activity.[112] Another study has shown that organic extracts containing high concentrations of PAHs increase mortality, reduce ROS production, and reduce mitochondrial function in lung epithelial cells.[39]

ROS, oxidative stress, and other sources of genotoxic damage[45,119] also influence the regulation of mitochondrial copy number (mtDNAcn), which is involved in PAH toxicity. In a study on Polish workers exposed to PAHs, the workers had considerably different mtDNAcns than the control subjects.[120] Conversely, decreased mitochondrial DNA content is associated with exposure to PAHs in human TK6 cells,[118] and similar findings have been obtained by Pardo et al.,[39] who showed that extracts rich in PAH reduce mtDNAcns in lung bronchial cells.

PM-Induced Effects on the Nrf2 Signaling Pathway and Mitochondria. A direct link between PM, mitochondria, and Nrf2 has been found in several recent studies. For example, Leclercq et al.[90] observed mitochondrial dysfunction after exposure of human bronchial cells to PM. This dysfunction was accompanied by an increase in Nrf2 (gene expression and binding activity) and a dose-dependent increase in NF-κB. In another study, $PM_{2.5}$ collected in Los Angeles induced mitochondrial damage and increased HO-1 expression related to the Nrf2 pathway.[112] In a study that investigated the mechanism by which Nrf2 exerts its protective effect against $PM_{2.5}$-induced toxicity in lung cells, Nrf2 was silenced in lung cells. The Nrf2-silenced cells demonstrated increased susceptibility to various PM extracts: Water extracts rich in metals increased mitochondrial ROS production and oxidative stress levels, while organic extracts containing high levels of PAHs increased mortality and reduced ROS production. Changes in mitochondria were also observed in Nrf2-silenced cells that obtained higher basal mitochondrial respiration rates than control cells. Mitochondrial respiration was increased following exposure to the water extracts but not following exposure to

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the organic extracts. The Nrf2-silenced cells exposed to the organic extracts showed reduced mitochondrial membrane potentials and reduced mtDNAcns.[39] These findings suggest that ROS overproduction induces oxidative damage and activates the Nrf2 signaling pathways. However, prolonged and repeated exposure or exposure to PAHs induces an oxidative boost[8,67,86,87,102,104] that can partially inactivate the Nrf2 signaling pathway and critically impair mitochondrial redox homeostasis, thereby producing persistent mitochondrial dysfunction and reducing the cell energy supply (Figure 2).

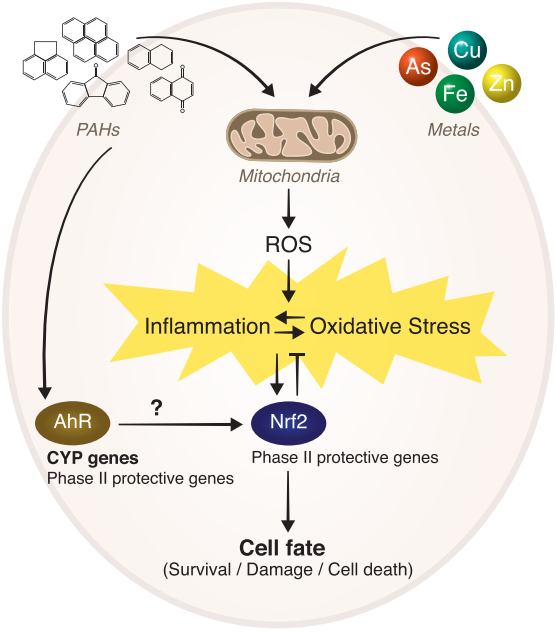

Figure 2. PM components (inorganic and organics, mostly metals and PAHs, respectively) induce oxidative stress and inflammation. Exposure to PM enhances ROS formation and alters mitochondrial function, which may lead to inflammation. The disturbance of redox homeostasis alters the activation of redox-sensitive signaling pathways such as Nrf2. Additionally, PAHs activate the AhR pathway, contributing further to the physio-pathological inflammatory effects of PM.

CONCLUSIONS AND FUTURE PERSPECTIVES

Despite cumulative data suggesting that there are different mechanisms of action for different PM components, there is still a lack of knowledge regarding the mechanisms that control PM-induced responses. Due to differences in chemical properties, the inorganic and organic fractions, that is, metals and PAHs, of PM seem to exert not only synergistic but also different biological effects. Both inorganic and organic components can enhance ROS generation, and since ROS are generated primarily in the mitochondria, the mitochondria may be the primary organelles affected by changes in redox status. Inorganic compounds, especially dissolved metals, elicit the formation of ROS, causing oxidative stress and consequent inflammation. PAHs may additionally activate the AhR signaling pathway, thus contributing to a pathophysiological inflammatory effect in some cases. PAHs generate a stronger inflammatory response than metals, which could be explained by the activation of several signaling pathways at the same time. We suggest that Nrf2 signaling may coordinate the response linking mitochondrial signaling and cell fate following $PM_{2.5}$ exposure. We therefore propose that future studies should focus on the crosstalk between mitochondrial signaling and Nrf2 signaling. Additional research is needed to more thoroughly characterize the role of the Nrf2-mediated oxidative stress response in PM-induced toxicity. In addition, other components of PM, such as airborne toxins and biomass burning-related particles, should be studied for their involvement in Nrf2-related PM toxicity.

The doses of toxicants deposited in the lungs are important for the effects of metals and PAHs discussed here. UFPs, which comprise all particles smaller than 100 nm and whose health effects are currently under intense investigation and discussion, do not contribute significantly to the deposited mass of metals and PAHs deep in the lungs. Thus, UFP toxicology might be mediated via other pathways, including particle translocation and particle surface-mediated mechanisms. This is another important topic for future research.

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Notes

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Xinghua Qiu received his B.Sc. degree in applied chemistry and Ph.D. degree in environmental science, both from Peking University, China. After postdoctoral work with Ronald Hites at Indiana University in Bloomington, IN, USA, he joined Peking University as an associate professor at the College of Environmental Sciences and Engineering. His research interests lie in the toxic organic components of particulate matter, in particular, polycyclic aromatic hydrocarbons and the derivatives and their health effects.

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Yinon Rudich is a Professor and Dean of Chemistry at the Weizmann Institute, Rehovot, Israel. Prof. Rudich studies the effect of changing chemical composition of aerosols on their optical and toxicological properties. Specifically, he studies aerosols optical properties, ice nucleation by dust and proteins, the microbial communities in transported aerosols, and the health effects of aerosols. Yinon Rudich is a PI in the Weizmann-Helmholtz International Lab aeroHEALTH.

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■ ABBREVIATIONS

AhR, aryl hydrocarbon receptor; ARE, antioxidant response element; COPD, chronic obstructive pulmonary disease; Gpx-1, glutathione peroxidase 1; GSH, glutathione; mtDNAcn, mitochondrial DNA copy number; NF-κB, nuclear factor kappa B; PAH, polycyclic aromatic hydrocarbon; Nrf2, nuclear factor erythroid 2-related factor 2; PM, particulate matter; ROS, reactive oxygen species; SOA, secondary organic aerosols; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha; UFP, ultrafine particle; IL-8, interleukin 8; XRE, xenobiotic-metabolizing enzymes

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