Epigenetic Regulation in Particulate Matter-Mediated Cardiopulmonary Toxicities: A Systems Biology Perspective

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Abstract

Particulate matter (PM) air pollution exerts significant adverse health effects in global populations, particularly in developing countries with extensive air pollution. Understanding of the mechanisms of PM-induced health effects including the risk for cardiovascular diseases remains limited. In addition to the direct cellular physiological responses such as mitochondrial dysfunction and oxidative stress, PM mediates remarkable dysregulation of gene expression, especially in cardiovascular tissues. The PM-mediated gene dysregulation is likely to be a complex mechanism affected by various genetic and non-genetic factors. Notably, PM is known to alter epigenetic markers (e.g., DNA methylation and histone modifications), which may contribute to air pollution-mediated health consequences including the risk for cardiovascular diseases. Notably, epigenetic changes induced by ambient PM exposure have emerged to play a critical role in gene regulation. Though the underlying mechanism(s) are not completely clear, the available evidence suggests that the modulated activities of DNA methyltransferase (DNMT), histone acetylase (HAT) and histone deacetylase (HDAC) may contribute to the epigenetic changes induced by PM or PM-related chemicals. By employing genome-wide epigenomic and systems biology approaches, PM toxicogenomics could conceivably progress greatly with the potential identification of individual epigenetic loci associated with dysregulated gene expression after PM exposure, as well the interactions between epigenetic pathways and PM. Furthermore, novel therapeutic targets based on epigenetic markers could be identified through future epigenomic studies on PM-mediated cardiopulmonary toxicities. These considerations

collectively inform the future population health applications of genomics in developing countries while benefiting global personalized medicine at the same time.

1. INTRODUCTION: PARTICULATE MATTER-MEDIATED CARDIOPULMONARY TOXICITY

1.1. Particulate Matter

Defined by US Environmental Protection Agency (EPA), particulate matter (PM) air pollution or particle pollution is the mixture of solid particles and liquid droplets existing in the air [1]. Inhalable PM can be divided into three subsets based on size: coarse particles with diameters larger than 2.5 micrometers and smaller than 10 micrometers (i.e., PM10), fine particles with diameters that are 2.5 micrometers and smaller (i.e., PM2.5), and ultrafine particles with diameters less than 0.1 micrometers (i.e., nano-particles - PM0.1). PM contains up to hundreds of different inorganic and organic chemicals including nitrates, carbon, sulfates, metal ions, polycyclic hydrocarbons (PAHs) [2], depending on the sources.

Ambient PM is emitted directly from sources such as construction sites, unpaved roads, fields, smokestacks or fires (primary particles). PM may also form in complicated reactions in the atmosphere of chemicals such as sulfur dioxides and nitrogen oxides that are emitted from power plants, industries and automobiles (secondary particles). The secondary particles make up most of the fine particle pollution in the United States [1].

1.2. CARDIOPULMONARY TOXICITY

Exposure to ambient PM has been proved to contribute to remarkable reductions in life expectancy in the United States. A recent study, which compiled data on life expectancy, socioeconomic status, and demographic characteristics for 211 county

units in 51 U.S. metropolitan areas with matching data on fine-particulate air pollution levels, exhibited that a decrease of 10 mg/m^3 in the concentration of fine particulate matter was associated with an estimated increase of 0.61 year in mean life expectancy. [3]

Epidemiologic studies have consistently demonstrated a strong association between the levels of ambient PM and an increase in emergency room visits and hospital admissions for cardiovascular diagnoses, as well as an increase in cardiovascular mortality [4]. Furthermore, PM exposure has been implicated in a variety of adverse effects including risk for or increasing the severity of asthma [5, 6], chronic obstructive pulmonary disease (COPD) [5, 7], heart rate variability [8, 9], cardiac arrhythmias [10, 11], myocardial ischemia [11], acute myocardial infarction [12, 13], and cerebrovascular accidents (CVA) [14].

Chemically, PM carries high levels of transition metal molecules including iron, nickel, and vanadium [2, 15], which catalyze the generation of reactive oxygen species (ROS) in cytoplasm [16-18]. In addition, PM contains carbon cores which absorb organic compounds including PAHs (polycyclic aromatic hydrocarbons), which dissolve in lung vascular cells after deposition and induce the generation of ROS [19]. PM mediates ROS generation, which is mainly considered as a consequence of mitochondrial dysfunction [20, 21], although some other researchers believe that NADPH oxidase might contribute to the excessive oxidative stress [22, 23]. Since the particle components can be absorbed into pulmonary endothelium and transported to the circulation [24-26], the circulating particles and the induced ROS may mediate

systemic oxidative stress, thereby causing further cellular and molecular injuries including protein oxidation, lipid oxidation, and DNA damages [27, 28].

Extensive epidemiologic studies have demonstrated that PM exposure, as an environmental stimulus, exhibits a closer association with cardiovascular events compared to adverse pulmonary events [5, 29]. The underpinnings for this observation are unclear. However, higher resistance to xenobiotic invasion in lung tissues as well as higher organ levels of phase I enzymes (e.g., cytochrome P450, family 1, subfamily A, polypeptide 1 [CYP1A1]) to oxidize/inactivate xenobiotics [30, 31] are potential contributors to this clinical discrepancy.

2. PM-MEDIATED CARDIOPULMONARY TOXICOGENOMICS

2.1. Gene Expression Dysregulation

With the powerful “-omics” tools (i.e., genome-wide molecular profiling technologies such as microarrays and sequencing) now readily available, multiple aspects of cellular response to PM exposure have been studied: gene transcription (transcriptome) [6, 32-38], DNA methylation (methylome) [39-45], and protein-level expression (proteome) [46-54]. As PM exposure takes place with every inhalation, a life-time exposure to PM occurs for the majority of the general population. Given the fact that air pollution is a significant risk factor for complex human diseases, particularly, the modulation of gene expression has emerged to implicate in PM-related health outcomes such as the risk for cardiopulmonary toxicities.

Several studies utilized cDNA microarrays to study effects of PM exposure on transcriptional gene expression (i.e., mRNA-level) in a variety of models including

endothelial cells, epithelial cells, mouse, rat, and human peripheral blood mononuclear cells [6, 32, 34, 35, 38, 55, 56]. In these studies, PM exposure consistently exhibited strong modifications of gene expression both *in vitro* and *in vivo*, especially in cardiopulmonary systems.

For example, the most common PM-dysregulated pathways include leukocyte extravasation signaling, ERK/MAPK signaling, tight junction signaling, SAPK/JNK signaling, IL-10 signaling, cell cycle/DNA damage/check point regulation, IL-6 signaling, NF-κB signaling, interferon signaling, complement and coagulation cascades, T-cell receptor signaling, and B-cell receptor signaling. The most highly dysregulated genes include: *Irg1*, *Saa3*, *Cxcl9*, *Cxcl2*, *Cxcl10*, *Ccl3*, *Mmp12*, *Dmbt1*, *Clca3*, *Timp1*, *Itlna*, *Arl*, *Cyp1a1*, *Ho1*, *Okl38*, *Pai2*, and *Timp3*. These identified dysregulated pathways and individual genes could potentially serve as genomic markers for PM-mediated diseases or as therapeutic targets.

Notably, active particle components or particles with a suitable size (e.g., ultrafine PM) may penetrate through endothelium to the circulation [24-26], thus exerting regulatory modulation on gene expression in other organs as well (e.g., cardiac tissues and endothelium). Recent research results further revealed that PM exposure could potentiate hypercholesterolemia-mediated endothelial gene regulation to facilitate the synergy in vascular damage in pre-existing conditions [57]. These observations, therefore, demonstrated the cardiovascular effects of PM exposure-induced gene edysregulation, which is correlated with both disease susceptibility and severity [6, 58].

Genetically, gene expression is a quantitative, complex trait that is partially contributed by genetic variation (e.g., through single nucleotide polymorphisms [SNPs]

acting as expression quantitative trait locis [eQTLs]) [59-62]. Epigenetic mechanisms including DNA methylation and histone modifications have emerged to be a novel class of gene regulators that help interpret gene expression variation among normal individuals, as well as gene expression dysregulation under diseased conditions [63, 64]. Since DNA methylation is currently the most studied epigenetic mechanism for gene expression regulation and the availability of convenient high-throughput technologies for profiling genome-wide DNA methylation patterns, we will focus this review on DNA methylation, with a brief introduction to other mechanisms (e.g., histone modifications).

2.2. PM EXPOSURE-INDUCED DNA METHYLATION

PM exhibits a strong interference on DNA methylation, evidenced not only by modulated global DNA methylation (DNA methylation of the entire genome) levels [39], but also by regulated DNA methyltransferase (DNMT) activities [65]. DNA methylation (e.g., the addition of a methyl group to the 5' position of cytosine) is a heritable trait that is essential for various biological processes and the development in higher organisms [66] In adult somatic tissues, DNA methylation in the form of 5-methyl-cytosine (5MeC, or called modified cytosines) typically occurs at CpG dinucleotide sites, while non-CpG methylation is prevalent in embryonic stem cells [67]. Modified cytosines could represent 2-5% of cytosines in mammalian genomes.

DNA Methylation status at promoter regions enriched with CpG sites (or CpG islands) plays a critical role in gene expression regulation, stably silencing gene expression in cells [68]. Reduced levels of DNA methylation have been linked to complex traits and phenotypes including aging and cardiovascular diseases [69, 70].

DNA methylation has also been demonstrated to play a crucial role in the pathogenesis of certain human diseases such as cancer, e.g., gene promoter hypermethylation has been associated with decreased expression of tumor suppressor genes [71]. However, more than 90% of all genomic 5MeCs are located in transposable repetitive elements or transposons, outside of promoter regions and may not directly contribute to gene expression regulation, though their role in gene regulation (e.g., as *trans*-acting regulators) can not be completely ruled out. In contrast, transposon DNA methylation can be used to estimate global genomic DNA methylation content, e.g., based on Alu and LINE-1 (Long Interspersed Nuclear Element-1) repeated elements [39].

Recent studies have established associations between DNA methylation and PM exposure. ROS generated by transitional metals or other PM-contents represent a unifying mechanism to account for these findings. Traditional explanation is that oxidative DNA damage can interfere with the ability of DNMT to interact with DNA, thus resulting in a generalized altered methylation of cytosine residues at CpG sites [72]. Acute exposure (1-7 days) to ambient PM leads to a consistently reduced global DNA methylation in both human or experimental animal studies [39, 45, 73], whereas chronic exposure may mediate complex and conflicted outcomes [73-75], possibly due to variations in particle type, gender, and differential species response. PM-containing chemical components may exert direct or indirect influences on DNA methylation patterns.

For example, cadmium, nickel, vanadium and iron stimulate the generation of ROS [76], thus altering DNA methylation; nickel [77] contributes to a persistent silence of gene expression via augmented DNA methylation with an alternative mechanism

other than ROS; and arsenic [78] suppresses global DNA methylation via suppression of intracellular SAM (S-adenosyl methionine), the methyl group donor implicated in the DNA methylation pathway. Here we provide more details of several important PM components that have strong effects on DNA methylation.

Arsenic

Arsenic is a metalloid element ubiquitously existing in the nature. The common oxidation numbers of arsenic are +5 (V_2O_5) and +3 (V_2O_3), which are the natural existing forms of arsenic in the environment, especially in PM. Arsenic was known as a potent toxin thousands of years ago. [79] Arsenic induces mitochondrial dysfunction and a rapid decline of mitochondrial membrane potential, with a consequence of uncontrolled formation and release of ROS to the cytosol.

The extensive oxidative stress induced by arsenic exposure modulates DNA structure and function including DNA bases oxidation, DNA strand break, DNA adduct formation, and DNA methylation. A large body of evidence has accumulated to suggest that arsenic-mediated chronic diseases could be mediated by DNA hypomethylation or hypermethylation. Besides the primary cellular mechanism of oxidative stress-mediated dysregulation through altered DNA methylation, arsenic has also been found to regulate cellular levels of SAM to modulate DNA methylation. In addition, arsenic directly promotes expression of DNMT1 and DNMT3a with unknown molecular mechanisms.

Cadmium

Cadmium is a transition heavy metal which is poorly excreted by the human body. Cadmium inhibits DNMT activity and mediates a global DNA hypomethylation, while prolonged exposure to cadmium induces an upregulation of DNMT expression, thus promoting DNA methylation. The mechanism of the biphasic regulation of DNA methylation is unknown, while cadmium-induced oxidative stress via Femton-like reaction might contribute to this observation.

Nickel

Nickel is wildly used in industry and daily life with a considerable level in the ambient PM. Environmental nickel compounds were found to induce DNA hypermethylation to silence genes *in vitro* (e.g., Chinese hamster G12 cell lines), and *in vivo*. It is thought that nickel might induce *de novo* DNA methylation by a mechanism of magnesium substitution in the phosphate backbone of DNA.

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons, or PAHs, are a group of organic chemicals commonly found in PM formed from the incomplete combustion of certain materials. PAHs are found to mediate significant cardiovascular toxicities. After exposure, PAHs are metabolized to produce phenolic products and reactive epoxides, which can bind to DNA to form DNA adducts. Several recent studies have shown that prenatal PAH exposure may be associated with global DNA hypomethylation and the presence of detectable PAH-DNA adducts. In contrast, CpG site-specific hypermethylation in certain genes by PAH exposure has also been identified to be associated with DNA damage

and internal exposure of PAH. Although the mechanism of PAH-induced DNA hypomethylation is not completely understood, intracellular ROS induced by PAH exposure might take a leading role. Interestingly, Fang et al demonstrated that PAH might dysregulate SAM bioavailability via glycine N-methyltransferase to exert systemic effects on global DNA methylation.

Table 1. Effects of Several Major Particulate Matter Components on DNA Methylation

Chemical	Effects on DNA Methylation	Putative Mechanism	Reference
Arsenic	global DNA hypermethylation	Oxidative stress; DNMT1 and DNMT3a up-regulation	[80-82]
Cadmium	global DNA hypermethylation and global de novo DNA hypomethylation	Oxidative stress; DNMT3A/3B up-regulation	[83, 84]
Nickel	DNA hypermethylation	Magnesium substitution in DNA backbone	[85, 86]
PAHs	global DNA hypomethylation	ROS. SAM bioavailability regulation	[87, 88]

PAH - polycyclic aromatic hydrocarbons; DNMT - DNA methyltransferase; ROS - reactive oxygen species.

Novel theories (triple-step) have been utilized to explain the altered DNA methylation by particle air pollutants, especially fine and ultrafine PM (**Figure 1**), which may carry heavier organic content and penetrate endothelium to enter the circulation to exert systemic epigenetic effects. PM suppresses the intracellular levels of SAM, the methyl donor for DNMT via direct oxidation and inactivation of SAM by ROS [89]. In addition, PM up-regulates catechol-O-methyltransferase (COMT) [90], another

methyltransferase utilizing SAM, which also degrades catecholamines such as dopamine, epinephrine, and norepinephrine [91].

PM-induced up-regulation of COMT depletes the SAM intracellular pool, therefore indirectly inhibiting DNMT. Depletion of SAM leads to a sudden increase of intracellular SAH (S-adenosyl homocysteine), which further inhibits DNMT that mediates the transcriptional activation of downstream genes. This is especially true in cardiomyopathy patients with abnormal baseline levels of COMT, contributing to the higher susceptibility of cardiovascular patients to PM-induced sudden death. Further characterization of the complex mechanism and identification of specific target genes could help advance future development of therapeutic strategies.

2.3. Histone Modifications

Histones participate in gene regulation via acetylation and deacetylation of lysine residues in the N-terminal tail and on the surface of the nucleosome core. Typically, these reactions are catalyzed by histone acetyltransferase (HAT) or histone deacetylase (HDAC) [92]. Acetylation of the lysine residues at the N terminus of histone proteins removes positive charges, thereby reducing the affinity between histones and DNA. Histone acetylation makes RNA polymerase and transcription factors easier to access the promoter region [92], thus enhancing transcription, while histone deacetylation represses transcription.

PM exposure was found to increase the activity of HAT, as well as the level of acetylated histone H4 in the nuclei of PM10-exposed cells [93]. PM exposure also promotes inflammatory cytokine release, which is enhanced by co-treatment with the

HDAC inhibitor [94], indicating that remodeling histone acetylation on chromatin is involved in PM10-mediated pro-inflammatory responses [93]. Some reports indicate that PM-containing environmental contaminants may contribute to dysregulated histone acetylation. For example, nickel reduces acetylation of histones H2A, H2B, H3, and H4 [95], and chromium reduces acetylation of histones H3 and H4 [96]. In contrast, PAHs, the major PM organic components, promote the acetylation of histones H3 and H4 via AHR (aryl hydrocarbon receptor) transactivation [97]. The final outcomes of alterations in histone modifications via ambient PM exposure remains unclear and likely dependent upon PM composition. The extent to which PM-mediated histone modifications is involved in gene dysregulation, requires further investigation.

3. A NOVEL SYSTEMS BIOLOGY APPROACH TO ENVIRONMENTAL TOXICOGENOMICS ON DNA METHYLATION

Since DNA methylation is currently the most investigated epigenetic mechanism and the recent availability of high-throughput technologies for profiling genome-wide DNA methylation levels, we will discuss the epigenomic approach to environmental toxicogenomics by focusing on DNA methylation, though it must be emphasized that a more comprehensive understanding of PM-mediated cardiopulmonary toxicities would only be possible by integrating all of the relevant mechanisms including, e.g., genetic variation in the form of SNPs, as well as other epigenetic factors (e.g., microRNAs, histone modifications).

From the current studies on PM-induced gene dysregulation, PM-mediated epigenetic effects are likely to be on multiple genes and pathways. A comprehensive,

systemic profiling of DNA methylation would greatly enhance our knowledge of the extent of PM-mediated DNA methylation, as well as the gene targets/pathways affected by these epigenetic effects. Particularly, recent progress in biotechnology in the area of whole-genome approaches to profiling DNA methylation levels, has begun to allow comprehensive study of the roles of DNA methylation in environmental toxicogenomics. For example, DNA methylation can be detected using bisulfite conversion, methylation-sensitive restriction enzyme (MSRE) digestion, methyl-binding proteins and antimethylcytosine antibodies (**Table 2**). Combining these techniques with DNA microarrays and/or high-throughput sequencing (e.g., using the next-generation sequencers) has made the mapping of DNA methylation feasible on a genome-wide scale [98].

Table 2. Common approaches to profiling DNA methylation

Approach	**Mechanism**	**Genome-wide Implementation**
bisulfite conversion	bisulfite conversion of cytosine to uracil	microarray, e.g., the Illumina HumanMethylation 450K array; sequencing
MSRE	utilizing methylation-specific restriction enzymes	microarray; sequencing
methyl-binding proteins and antimethylcytosine antibodies	enriching methylated DNA fragments through ChIP	microarray, i.e., ChIP-on-chip; sequencing

MSRE - methylation-sensitive restriction enzyme digestion; ChIP - chromatin immunoprecipitation.

Chemically, bisulfite conversion is based on treatment of DNA with bisulfite, a chemical that results in the conversion of cytosine to uracil, but leaves 5-methylcytosine residues unaffected, thus introducing specific changes (i.e., C to T transitions) in the DNA sequence that depend on the methylation profiles of cytosine residues. The methylation profiles then can be deduced by techniques such as direct sequencing [99, 100], pyrosequencing [101] or microarray-based approaches [102]. The deducted methylation profiles can be used in genome-wide analyses for their relationships with phenotypes of interest (e.g., disease risk). Particularly, microarrays may be designed using oligonucleotide pairs targeting CpG sites of interest, with one complementary to the unaltered methylated sequence, and the other to the C-to-T converted unmethylated sequence [103].

For example, after bisulfite conversion, the new Illumina Infinium HumanMethylation450 BeadChip (Illumina 450K array) (Illumina, Inc., San Diego, CA) can be used to comprehensively profile the DNA methylation in samples under different conditions. The Illumina 450K array allows researchers to interrogate >485,000 methylation sites (e.g., methylation sites distributed across the promoters, first exons, gene bodies, and untranslated regions) per sample at single-nucleotide resolution with a coverage of 96% of CpG islands and 99% of RefSeq genes [104]. An earlier version of this platform (i.e., Illumina 27K array) has been successfully used to profile genome-wide DNA methylation in human cell lines [63]. In contrast, the method of MSRE

digestion uses methylation-specific restriction enzymes such as HpaII to cleave DNA at specific methylated-cytosine residues. Similarly, whole-genome oligonucleotide microarrays may then be used to differentiate the MSRE-digested products and background (e.g., DNA fragments digested by non-methylation-sensitive restriction enzymes such as MspI) [105]. Furthermore, the methods based on methyl-binding proteins and anti-methylcytosine antibodies use specific methyl-group binding proteins or methylcytosine antibodies to enrich methylated DNA fragments through chromatin immunoprecipitation (ChIP) [106]. Whole-genome oligonucleotide microarrays and/or direct sequencing may be used to interrogate the methylation profiles of the ChIP products (i.e., ChIP-on-chip).

In spite of specific limitations such as reduced coverage of the MSRE digestion approach to only CpG sites [105] and incomplete conversion and degradation of DNA during bisulfite treatment [107], these whole-genome approaches have begun to allow genome-wide analyses of DNA methylation in humans [99]. For example, in a previous study, using the bisulfite sequencing approach, the CpG methylation profiles of 43 samples derived from 12 different human tissues (e.g., heart muscle, skeletal muscle) and various primary cell types (e.g., dermal fibroblasts, CD4+ and CD8+ lymphocytes) were determined for chromosomes 6, 20, and 22 [99].

These whole-genome or epigenomic approaches provide promising tools to profile the variation in DNA methylation and to illustrate the relationship between DNA methylation and gene expression. The integration of genomic and epigenomic data using a systems biology approach may benefit the research of environmental toxicogenomics by greatly expanding the scale of the investigative targets. By

integrating this information with data from a detailed map of human genetic variation, for example the recently launched 1000 Genomes Project [108] data on more than 1,000 world-wide samples from a variety populations (>17 million SNPs), it is reasonable to expect increased understanding of the contribution of both genetics and epigenetics to the PM-mediated toxicities. Future investigations from a systems biology view could help elucidate the comprehensive relationships between PM exposure and the complex networks of genetic/epigenetic factors, which in combination implicating the PM-mediated health outcomes including cardiopulmonary toxicities.

CONCLUSIONS AND FUTURE OUTLOOK

PM is known to exert epigenetic regulations with similar epigenetic alterations found in patients with cardiopulmonary diseases. Epigenetic dysregulation, particularly alterations in DNA methylation by PM is likely to contribute to air pollution-mediated health effects. Future prospective studies are likely to clarify individual genes dysregulated by PM exposure via epigenetic pathways. Since gene expression is a quantitative trait (partially controlled by both genetic and epigenetic factors) with significant variation between human populations [59, 62, 109], using these novel genomic and epigenomic approaches would allow comprehensive evaluation of the relative genetic and epigenetic contribution to the PM-mediated toxicities. A systems approach to PM-mediated cardiopulmonary toxicities integrating DNA methylation and other relevant information would be useful for identifying novel biomarkers and therapeutic targets for attenuating PM-mediated adverse health effects.

These considerations collectively inform the future population health applications of genomics in developing countries while benefiting global personalized medicine at the same time.

List of abbreviations

AHR	aryl hydrocarbon receptor
5MeC	5-methyl-cytosine
ChIP	chromatin immunoprecipitation
COPD	chronic obstructive pulmonary disease
COMT	catechol-O-methyltransferase
CVA	cerebrovascular accidents
DNMT	DNA methyltransferase
EPA	Environmental Protection Agency
HAT	histone acetylase
HDAC	histone deacetylase
MSRE	methylation-sensitive restriction enzyme
LINE-1	Long Interspersed Nuclear Element-1
PAH	polycyclic hydrocarbon
PM	particulate matter
ROS	reactive oxygen species
SAH	S-adenosyl homocysteine
SAM	S-adenosyl methionine

CONFLICT OF INTERESTS

None declared/applicable.

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Figure Legends

Figure 1. PM activates gene expression via suppression of DNA methylation (Triple-step theory). PM induces ROS generation and COMT up-regulation. ROS inactivates SAM, the methyl-donor for DNA methylation. Up-regulated COMT depletes SAM intracellular pool, and increases the reaction product SAH, which serves as a DNMT inhibitor. Reduced SAM and elevated SAH inhibit DNMT and mediate hypomethylation of DNA. Hypomethylation in the CpG island located in gene promoter leads to the transcriptional gene activation.

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