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Molecular mechanisms of environmental exposures and human diseases

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Abstract

Genetic underpinnings of diseases are appropriately recognized to be vast and complex. However, a substantial proportion of disease risk is attributable to environmental exposures. Specifically, environmental pollutants cause one in six deaths worldwide and are one of the largest risk factors for premature death in the world. An appreciation of how environmental pollutants interact with our cells to produce deleterious health effects has led to advances in our understanding of the molecular mechanisms underlying the pathogenesis of chronic diseases. Here, we discuss emerging research on interactions of environmental exposures with the human genome and review evidence of the environmental impact on epigenetic mechanisms including DNA methylation, histone modification, and non-coding RNAs. We introduce emerging related areas of investigation including extracellular vesicles, mitochondrial genomics, and epitranscriptomics. Finally, we discuss current challenges and reflect on the exposome and its integration into future environmental health research.

Introduction

Environmental exposures are influential determinants of human health. The modern expansion of industrialization, fossil fuel combustion, and mechanized agriculture has led to a rising global burden of air, water, chemical, and metal pollution.¹ As populations around the world are exposed to rising levels of environmental contaminants,² pollution-related deaths continue to rise. Pollution causes one in six deaths worldwide and is the largest environmental risk factor for premature death in the world.³ Pollution is also the second leading worldwide cause of non-communicable diseases after smoking, contributing to a rising pandemic of respiratory diseases, cardiovascular disease, neurodegenerative diseases, and cancers.⁴ These health consequences emphasize the importance of understanding

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environmental drivers of human health and elucidating how environmental exposures induce deleterious health effects.

Environmental exposures are often linked to disease pathogenesis through molecular pathways that disrupt epigenetic regulators of gene expression.² Epigenetic mechanisms including DNA methylation, histone modification, and non-coding RNAs can modulate gene expression levels without changing the underlying DNA sequence. Many epigenetic modifications are dynamic, reflecting cumulative environmental exposures throughout the lifespan and correlating with aging-related diseases and outcomes.⁵ As a result, beyond providing a mechanistic link between environmental stressors and disease pathogenesis, epigenetic modifications can also function as reliable markers of accelerated aging and subclinical disease.^{6,7} Understanding the ways in which environmental exposures induce epigenetic alterations is therefore critical for the mitigation and prevention of environmentally driven diseases.

In addition to classic epigenetic mechanisms, recent studies have expanded to examine the molecular processes that interact with epigenetic pathways. For example, recent advancements have enabled the isolation and detection of molecular cargo within extracellular vesicles (EVs). EVs are membrane-bound vesicles that are released from multiple organs in the body in response to environmental insults that mediate intercellular communication through complex molecular cargo including microRNA (miRNA).⁸ Similarly, environmental pollutants trigger chemical modification of RNA molecules,⁹ collectively known as the epitranscriptome, which alters phenotypic expression.¹⁰ Environmental insults additionally impact mitochondrial gene activity in a manner that can confer increased disease risk.¹¹ The study of EVs, epitranscriptomics, and mitochondrial genomics offers promising and novel insights into the mechanisms underlying environmental diseases.

In this review, we critically examine the molecular mechanisms linking environmental exposures to human diseases. We discuss the interplay of environmental insults with the human genome and explore how epigenetic modifications underlie the association of environmental exposures with disease pathogenesis. We explore how emerging research related to EVs, epitranscriptomics, and mitochondrial genomics can inform our understanding of environmental contributions to disease risk. Finally, we offer perspectives on new approaches that can further elucidate environmental influences on human health.

Gene-Environment Interactions

Most human diseases arise from the interplay of the human genome and the surrounding environment.¹² Gene-environment interactions reflect the complex ways in which genes interact with environmental factors to influence human traits. When gene-environment interactions exist, environmental exposure-related disease susceptibility differs for individuals with different genotypes.¹³ As a result, evaluating genetic or environmental factors separately may fail to identify high-risk genotypes or vulnerable populations. Gene-environment interaction studies are critical to elucidate biological mechanisms of human disease, risk-stratify patients based on their individual genotypes, and understand the public health implications of prevalent environmental exposures.

Genomic research has demonstrated that certain gene variants predispose individuals to complex diseases. However, genome-wide association studies (GWAS) may fail to identify genotypes with weak or modest effects on disease prevalence but comparatively strong effects on carriers with certain environmental exposures. For example, while asthma is a heritable disease linked to several genetic loci,¹⁴ prior GWAS did not identify an association between glutathione S-transferase (GST) genes and asthma prevalence.¹⁵ In contrast, gene-environment interaction studies showed that carriers of GST null genotypes had increased susceptibility to indoor air pollution and experienced higher rates of asthma compared to participants with functional GST alleles (Figure 1).^{16–18} The GST genes encode detoxifying enzymes that protect against pollution-related oxidative stress and may interact with environmental insults to moderate asthma risk.¹⁹

Beyond identifying novel genomic variants, gene-interaction studies can also strengthen causal inference derived from observational studies.^{20,21} For example, a recent population-based study showed that military personnel who were exposed to a nerve agent and carried a paraoxonase-1 (*PON1*) Q192R polymorphism had increased susceptibility to Gulf War Illness,²⁰ a debilitating syndrome that afflicted military personnel deployed in the Persian Gulf War. Importantly, the study showed that it is possible to demonstrate compositional epistasis in gene-environment interaction studies and to provide stronger evidence for a causal relationship. The study also showed that gene-environment interaction studies may not be impacted by recall bias or confounding by ancestry, which often limit the validity of environmental epidemiologic studies and GWAS. Moving forward, gene-environment interaction studies are essential to identify genotypes that modify disease risk in the presence of key environmental exposures.

While elucidating gene-environment interactions is critical to understanding the genetic liability of human diseases, traditional tests of gene-environment interactions have relatively low power and require strict corrections when comparing multiple genotypes.²² However, evolving analytical frameworks can increase power to detect gene-environment interactions,^{23,24} and resources such as the UK Biobank, the Environmental influences on Child Health Outcomes (ECHO) consortium, and the *All of Us* project may help overcome sample size limitations.^{25,26} With the advent of cutting-edge tools and resources, gene-environment interaction studies have the potential to identify novel genetic loci that contribute to human disease, improve diagnostic methods, optimize prevention strategies, and revolutionize targeted preventive measures in susceptible populations.²⁷

Epigenome

DNA Methylation—DNA methylation occurs when DNA methyltransferase enzymes transfer a methyl group to the C5 position of a cytosine nucleotide forming 5-methylcytosine.²⁸ In contrast, DNA demethylation occurs through a series of deamination and oxidation reactions that remove the methyl group from the cytosine base.²⁹ DNA methylation levels regulate gene expression and exert distinct influences in different genomic regions. In intergenic regions, DNA methylation suppresses potentially harmful genetic elements that can trigger mutation events.³⁰ Similarly, methylation of gene promoter regions often leads to transcriptional silencing.³¹ In contrast, gene body methylation can

increase gene expression.³² Given the intimate connection between DNA methylation and gene activity, understanding how environmental insults impact DNA methylation is critically important.

Particulate matter (PM) air pollution is one of the most ubiquitous pollutants in the world and has been associated with an array of adverse health effects. In particular, PM has been associated with genome-wide differences in DNA methylation in lung and blood cells. Experimental models have shown that when PM is inhaled, small particles disrupt epithelial cell integrity in the lungs, leading to neutrophil chemotaxis and production of reactive oxygen species (ROS).^{33,34} Ultrafine particles pass into the systemic circulation and generate endothelial injury and oxidative stress.³⁵ ROS catalyze DNA demethylation through oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, after which DNA methylation is passively depleted over serial cellular divisions (Figure 2).³⁶ *In vitro* models have also shown that air pollution reduces DNA methyltransferase activity, which is required to maintain DNA methylation levels.³⁷ In addition, pollution-related ROS reduce expression of methionine adenosyltransferase 1A (MAT1A), which decreases availability of biologic methyl donors.³⁸ Together, these pollution-related effects have been associated with genome-wide differences in DNA methylation levels in lung epithelial cells and nucleated blood cells.³⁹

Several population-based studies have confirmed that air pollution exposure was associated with differences in DNA methylation levels in blood leukocytes. One of these studies showed that air pollution exposure was associated with demethylation in the promoter regions of genes in the mitogen-activated protein kinase (MAPK) pathway.⁴⁰ These alterations may hinder DNA methylation-mediated suppression of inflammatory genes and link air pollution exposure to increased cytokine production.⁴¹ In a cross-over trial, PM exposure was associated with reduced methylation in pro-coagulant genes, which may link PM exposure to vascular thrombosis and cardiovascular health.⁴² In a study of non-diabetic men, differential methylation of intercellular adhesion molecule-1 (*ICAM-1*), an inflammatory gene, mediated the relationship between short-term PM exposure and increased diabetes risk.⁴³ A fourth study showed PM was associated with hypomethylation of the aryl hydrocarbon receptor repressor (*AHRR*) gene, which is heavily implicated in the pathogenesis of obstructive lung diseases.⁴⁴ Tobacco smoking is associated with similar differences in DNA methylation patterns,^{45,46} suggesting that these inhaled environmental insults modify respiratory and cardiovascular disease risk through similar mechanistic pathways.

DNA Methylation-Based Biomarkers—Epigenetic changes can function as biomarkers of environmental exposures and disease predisposition. For example, in epigenome-wide association studies (EWAS), tobacco smoking was associated with differences in DNA methylation at thousands of CpG sites in nucleated blood cells.⁴⁶ While smoking cessation was associated with reversion to normative methylation levels at some CpG sites, other sites annotated to genes associated with lung and heart diseases do not return to normative levels even decades after smoking cessation.^{47,48} This is largely because DNA methylation patterns can be maintained in the DNA of daughter leukocyte cells released in the bloodstream through the activity of DNA methyltransferase enzymes. In this context, blood-based

biomarkers that index cumulative smoking-related differences in DNA methylation levels can detect smoking exposures and classify smoking behaviors.⁴⁹ Epigenetic biomarkers can also assess environmentally mediated disease risk in applications that mirror the use of polygenic risk scores to evaluate heritable disease risk.⁵⁰ In a recent population-based study, a DNA methylation-based classifier of tobacco smoke exposure identified former smokers at increased risk of obstructive lung disease and death.⁷ Compared to self-reported smoking exposures, which are subject to recall bias and misreporting,^{51,52} DNA methylation-based smoking indices may capture true smoking-related biologic effects and help identify smokers with increased risk of adverse smoking-related health outcomes.

Beyond reflecting specific environmental insults such as smoking, epigenetic changes can also serve as a proxy for accelerated biological aging. Prior research in different types of human tissue identified age-related changes in DNA methylation that were leveraged to develop epigenetic aging clocks.⁵³ Aging clocks use a collection of CpG sites conserved across mammalian tissue to estimate the “biological age” of human tissue and can reflect age acceleration when the calculated DNA methylation age is higher than the chronological age.⁵⁴ Using these proxy measures, environmental insults have been shown to accelerate aging. In population-based studies, individuals exposed to air pollution and tobacco smoke demonstrated advanced DNA methylation age in blood and lung tissue (Figure 3).^{55–57} Similarly, organochlorine pesticide exposure was associated with accelerated epigenetic aging in peripheral blood leukocytes.⁵⁸ In contrast, another epidemiologic study showed that improved diet and educational attainment were associated with decelerated epigenetic aging in blood leukocytes.^{59,60}

Elucidating environmental factors that impact DNA methylation age is critical because DNA methylation age is predictive of age-related health outcomes. Age-related changes in DNA methylation generate genomic instability and aberrant gene expression that contribute to disease risk. In a population-based study in young adults, epigenetic age acceleration was associated with increased risk of incident type 2 diabetes.⁶¹ In a second prospective cohort study, increased epigenetic age acceleration was associated with increased incidence of coronary artery disease, peripheral artery disease, and heart failure independently of traditional cardiovascular risk factors.⁶² In a third study, epigenetic age acceleration was associated with truncated life expectancy.⁶³ These findings suggest that epigenetic markers serve as an important proxy of aberrant biological aging. Further research is required to determine if epigenetic biomarkers can be applied in clinical settings to facilitate disease risk stratification and enable early diagnosis of environmentally mediated diseases.

Chromatin Remodeling and Histone Modifications—In most cells, chromatin consists of repeating units of nucleosomes. Each nucleosome comprises ~147 DNA base pairs wrapped around eight histone proteins. Each of these “octamer” beads contains two copies each of H2A, H2B, H3, and H4 histones while a single H1 “linker” histone binds the DNA to the octamer.⁶⁴ These core histones have several variants, many of which are tissue-specific, have particular functions, and have been associated with genetic disorders and cancers.⁶⁴ Histones, along with a suite of post-translational modifications including acetylation and phosphorylation, are a critical layer of epigenetic regulation that interacts with DNA methylation and non-coding RNAs to regulate gene expression.^{65,66}

These modifications help define the transcriptional state of the chromatin toward the more transcriptionally active euchromatin or less transcriptionally active heterochromatin. Nucleosome positioning and histone modifications are critical for essentially all biological processes and can affect both the health of the individual⁶⁷ and ensuing generations.^{68,69} Thus, it is critically important to understand how environmental exposures alter histone biology.

Exposure to arsenic, a heavy metal and common environmental pollutant, has been observed to influence several histone modification patterns.^{70,71} It is well-established from both observational and experimental evidence that arsenic alters histone methylation, including global methylation of H2B, H3, and H4.^{70,71} Arsenic also alters methylation at specific sites including H3K4me3, H3K9me3, and H3K27me3.⁷⁰ While the exact mechanisms underlying arsenic-induced changes in histone methylation are unknown, there is some evidence that arsenic activates specific methyltransferase enzymes including G9a.^{72,73} Arsenic may thereby affect the bivalent status of the chromatin.⁷¹ Specifically, simultaneous presence of activating (e.g. H3K4me3) and repressing (H3K27me3) modifications, both of which have been observed with arsenic exposure, is a hallmark of bivalency. This bivalent state may lead to the upregulation of several oncogenes⁷⁴ and may underlie the oncogenic effect of arsenic. Arsenic-induced histone modifications have also been shown to generate oxidative stress, DNA damage, and regulate the cell cycle.⁷¹

Aside from altering histone methylation, arsenic has also been shown to affect the abundance of histone variants via polyadenylation of H3 histone mRNA. H3 polyadenylation disrupts the physiological balance of histone variants and is suspected to be carcinogenic, though confirmatory experimental evidence is necessary.^{75,76} Emerging evidence shows other environmental exposures can trigger posttranslational histone modifications including trace metals,⁷⁷ air pollution,^{78–83} polycyclic aromatic hydrocarbons (PAHs),⁸⁴ pesticides,^{85–87} dioxins,⁸⁸ and plasticizers^{89,90}.

Non-coding RNAs—Non-coding RNAs are key regulators of gene expression at the post-transcriptional level via direct interactions with target genes and coordinated responses with other epigenetic machinery. MicroRNAs (miRNAs) are one class of small non-coding RNAs⁹¹. MiRNAs can directly bind to 3'UTR, 5'UTR and coding sequences to inhibit translation or to promoter regions to induce transcription.⁹¹ Acting in a controlled epigenetic “circuit”, miRNAs can also affect the chromatin state to promote transcription⁹² and are themselves regulated by DNA methylation and histone modifications.⁹³

Despite early evidence that miRNAs are responsive to a range of environmental exposures,⁹⁴ details of the underlying mechanisms have been historically limited.⁹⁵ While oxidative stress and inflammation have long been suspected to play important roles in air pollution-induced miRNA dysregulation, only a few studies have provided experimental evidence.^{96–98} In a cross-over trial, diesel exhaust was shown to affect expression of several miRNA and mRNAs, leading to inflammatory cell recruitment and epithelial cell shedding.⁹⁶ In a recent *in vitro* study, PM-induced ROS downregulated hsa-miR-137, resulting in greater expression of IL-6 and COX-II. This sequence effectively linked air pollution exposure to a pro-inflammatory state that contributed to the pathogenesis of rheumatoid arthritis.⁹⁷

Another *in vitro* showed that PM led to differential expression of miRNAs that were taken up by alveolar macrophages, leading to increased inflammation in the lungs and pulmonary epithelial cell damage.⁹⁸ In the same study, PM_{2.5} increased levels of Peroxiredoxin 6 (Prdx6) via downregulation of mmu-mir-467c-5p, potentially as a protective response to regulate inflammatory injuries.⁹⁸ The connection between PM_{2.5} and miRNA dysregulation has also been implicated in the pathogenesis of Alzheimer's disease.

While miRNAs have historically been the most studied non-coding RNA in environmental health, there is mounting evidence that other non-coding RNAs are modifiable by environmental threats. Circular RNA are single-stranded RNA in a closed continuous loop that derive from protein-coding regions.⁹⁹ Circular RNAs regulate gene expression by acting as miRNA “decoys” or by interacting directly with proteins. Recently, *in vivo* rodent studies showed that air pollutants lead to differentially expressed circular RNAs in the lung^{100,101} and in rodent embryos¹⁰².

Environmental insults likely alter most RNA regulators of gene expression. For example, tRNA fragments are responsive to heat¹⁰³, ultraviolet radiation^{103,104}, and oxidative stress.^{103,105,106} While tRNAs have traditionally been viewed in the context of translation, tRNAs can also regulate gene expression via translation repression and gene silencing.^{107,108} In addition, our growing understanding of how long non-coding RNAs regulate gene expression through modulation of chromatin structure and direct interactions with target genes¹⁰⁹ is accompanied by emerging evidence that air pollution,^{101,110} metals,^{111,112} and other environmental contaminants^{113–115} alter long non-coding RNA expression. These trends affirm that further research elucidating how environmental insults impact expression of non-coding RNAs is critical to expand our understanding of how environmental insults affect human health.

Emerging Areas of Molecular Investigation

Epitranscriptomics—Epitranscriptomics evaluates post-transcriptional modifications of RNA. There are over 100 post-transcriptional modifications across all RNA types that play a critical role in RNA folding, splicing, stability, localization, and translation. The location and quantity of RNA modifications help determine how modifications affect RNA function.¹¹⁶ Similar to epigenetic modifications, RNA modifications are controlled and maintained by a group of “reader”, “writer”, and “eraser” proteins (RWEs).¹¹⁶ The most well-studied and common epitranscriptomic modification is the addition of a methyl group on the sixth nitrogen atom of adenine, also known as N⁶-methyladenosine (m⁶A). The m⁶A modification can be found in all types of RNA¹¹⁷ and helps regulate RNA folding, splicing, stability, and translation.¹¹⁸

Epitranscriptomic changes related to environmental exposures are an underexplored but potentially important pathway through which environmental insults impact human health. For example, m⁶A has been shown to regulate the inflammatory response, activate the adaptive and cellular immune systems, and modulate T-cell homeostasis and differentiation.^{119,120} Similarly, METTL3, an m⁶A methyltransferase (“writer”), is essential for the production of inflammatory cytokines.¹²¹ Experimental research has shown that m⁶A is responsive to external stressors *in vitro*, especially environmental pollutants associated

with oxidative stress including cigarette smoke and PM_{2.5}.^{80,122–125} Accordingly, oxidative stress has been shown to affect m⁶A at hundreds of mRNA transcripts.^{126,127}

Environment-induced alterations in global m⁶A levels may stem in part from altered expression of RWEs. PM_{2.5} exposure leads to hypomethylation of the promoter regions of RWE genes¹²⁸, resulting in differential expression of METTL3^{80,123,124,128} and METTL14.⁸⁰ METTL3 and METTL14 are two “writers” that form the m⁶A methyltransferase complex.¹²⁹ Similarly, cigarette smoke has been shown to alter METTL3 and METTL14 expression via promoter hypomethylation *in vitro*,^{130,131} resulting in higher levels of m⁶A on select miRNAs and long non-coding RNAs. In experimental models, PM_{2.5}-related alterations in METTL3 also induce m⁶A modification of Oxidative Stress-Induced Growth Inhibitor 1 (OSGIN1)¹²⁴ and cadherin 1 (CHD1) mRNAs.¹³² The CHD1 m⁶A modification is part of a mechanism that spans across multiple different biological regulatory networks. Specifically, environmentally-related METTL3 promoter hypomethylation leads to more abundant m⁶A modification on CHD1 that is then recognized by YTHDF2, an m⁶A “reader” that is upregulated via PM_{2.5}-induced downregulation of hsa-miR-494–3p. YTHDF2 in turn inhibits E-cadherin, which can trigger an epithelial-mesenchymal transition characteristic of pulmonary fibrosis (Figure 4).¹³² In human studies, the relationship between air pollution and m⁶A is less clear.^{123,133}

Heavy metals and chemical toxicants have also been shown to alter m⁶A and RWE expression *in vitro*. High levels of arsenic altered global m⁶A levels^{123,134,135} via upregulation of the m⁶A methyltransferase complex and downregulation of FTO, a demethylase.^{134,136,137} This effect was also observed *in vivo* as mice treated with arsenic showed higher global m⁶A levels and downregulation of FTO.¹³⁸ Other metals and chemical toxicants including cobalt,¹³⁹ manganese,¹⁴⁰ chromium,¹⁴¹ cadmium¹⁴², polychlorinated biphenyls (PCBs),^{143,144} PAHs,¹⁴⁵ and other common environmental pollutants^{123,146–148} were also associated with m⁶A modifications and RWE expression levels.

Overall, there is compelling and mounting evidence that RNA modifications are responsive to environmental stimuli in patterns specific to different RNA subtypes. These effects may be driven by changes in RWE expression and activity, which may be mediated by changes in DNA methylation in the promoter regions of these genes. Environmental exposure-related oxidative stress also leads to altered expression of RWEs including m⁶A demethylase FTO¹³⁸ and m⁵C methyltransferase NSUN2.¹⁴⁹ At present, few studies have examined components of the epitranscriptome beyond m⁶A. However, m⁶A is a small part of the epitranscriptomic landscape. As the field evolves, it is imperative to examine other influential RNA abundant modifications.¹⁵⁰

Extracellular Vesicles—EVs are nano-sized membranous vesicles that are released from multiple cell types in the body under physiologic conditions and in response to environmental insults.¹⁵¹ The physiologic state of the parent cell regulates packaging of EV cargo, which includes proteins, nucleic acids, lipids, and metabolites. EVs are released from the parent cell and are endocytosed by recipient cells and alter cell biology through their molecular cargo. For example, EV-encapsulated microRNAs (EV-miRNAs) regulate cellular function by degrading complementary mRNA transcripts.¹⁵² EVs convey molecular

signals between different organs throughout the body and thereby function as a mechanistic link between environmental insults and transcriptional activity.

In population-based studies, exposure to inhaled pollutants triggers lung epithelial cells and alveolar macrophages to release large quantities of EVs into the blood (Figure 5).¹⁵³ For example, it has been shown that in humans, EVs can generate a pro-inflammatory signaling cascade that causes endothelial injury, hypercoagulability, and end-organ dysfunction.¹⁵⁴ Subsequent *in vitro* studies showed that PM-related EVs also triggered hyperresponsiveness of bronchial smooth muscle cells,¹⁵⁵ accelerate vascular thrombosis,¹⁵⁶ and precipitate neurotoxic signaling.¹⁵⁷ EVs thereby mediate the toxic effects of air pollution exposures and contribute to pollution-related risk of chronic lung, heart and neurologic diseases.

Heavy metals^{158–160} and plasticizers including bisphenol-A (BPA) and di(2-ethylhexyl) phthalate (DEHP) have been shown to modulate EV biology and adversely affect the reproductive system. BPA has been detected in the follicular fluid that surrounds oocytes and contributes to oocyte development in women.¹⁶¹ Recent *in vitro* studies showed that BPA altered EV-miRNA expression in primary granulosa cells.¹⁶² Population-based studies in women undergoing *in vitro* fertilization confirmed that phenol and phthalate exposure altered EV-miRNA expression in follicular fluid.^{163,164} In addition, human studies showed that BPA exposure altered protein expression in placenta-derived EVs and impacted pathways known to modulate placental cellular injury.¹⁶⁵ Together, these findings suggest that EVs and their molecular cargo may function as a mechanistic link between chemical exposures and reproductive toxicity.

EVs translate environmental exposures into substantive disease risk through synergistic interactions with miRNAs and other epigenetic mechanisms.¹⁶⁶ EVs may thereby represent viable biomarkers of subclinical disease in humans. For example, in a prospective cohort study, plasma EV-miRNAs functioned as viable biomarkers of lung function impairment.¹⁶⁷ Recent efforts to isolate plasma EVs derived from specific tissue types may further enable accessible liquid biopsies that are predictive of future health outcomes.

Mitochondrial Genomics

Mitochondria facilitate energy metabolism, redox signaling, fat homeostasis, and metabolic regulation.¹⁶⁸ Accordingly, mitochondrial impairments or dysregulation contribute to numerous disease states.¹⁶⁸ Mitochondria are particularly vulnerable to the effects of environmental exposures. The mitochondrion thereby functions both as a sentinel for exposure-induced damage and a central mechanism through which environmental exposures impact human health.¹⁶⁹ As an example, a recent study using a direct assessment of mitochondrial function showed that the neurodevelopmental outcomes associated with prenatal PM_{2.5} exposure are partially mediated by long-term changes in mitochondrial respiration.¹⁷⁰

The number of mitochondrial genomes per cell or the mitochondrial DNA copy number is a commonly used marker of mitochondrial damage. Numerous environmental exposures have been shown to alter mitochondrial DNA copy number including PM air pollution,¹⁷¹ PAHs,¹⁷² heavy metals,¹⁶⁹ and other chemical^{173–175} and occupational exposures.^{176–178}

However, the relationship between environmental stressors and mitochondrial DNA copy number varies depending on the type and duration of the exposure¹⁶⁹ and it is difficult to discern the underlying mechanisms. However, recent work using known mitochondrial toxicants has elucidated more precise molecular mechanisms underlying environment-induced mitochondrial impairment.

Exposure-related excess ROS is a ubiquitous mechanism underlying mitochondrial dysfunction related to PM air pollution¹⁷⁹, heavy metals,¹⁸⁰ PAHs¹⁷², and select pesticides^{181–183}. ROS are typically produced in the matrix of mitochondria in the form of superoxide (O_2^-) and hydrogen peroxide (H_2O_2). However, alterations in this process related to environmental insults can lead to accumulation of electrons and increased ROS production. The accumulation of ROS can lead to altered permeability of mitochondrial membranes,¹⁸⁴ imbalance in calcium homeostasis,¹⁸⁵ increased mitochondrial DNA mutations,¹⁸⁶ and damage to the mitochondrial respiratory chain and ATP production.¹⁸⁵ Mitochondrial dysfunction then triggers systemic effects including inflammasome and inflammatory cytokine release.^{172,179}

Recent research has also identified several specific pollutant-specific pathways. For example, air pollution can lead to aberrant mitochondrial DNA methylation and DNA strand breaks.¹⁷⁹ Dioxins and PAHs can bind aryl hydrocarbon receptors (AhR)¹⁸⁷, which leads to mitochondrial AhR degradation and alterations in cellular respiration.¹⁸⁸ Finally, cyanide and rotenone can inhibit cellular respiration via interactions with complex IV and complex I of the electron transport chain.¹⁸⁹

An area of growing interest in environmental health research is mitochondrial heteroplasmy. Each cell comprises mixed copies of mtDNA genomes and heteroplasmies are mutations that are present in a subset of mtDNA within a cell.¹⁹⁰ Mitochondrial genomes are naturally more susceptible to mutations compared to nuclear genomes and heteroplasmy has been shown to alter mitochondrial gene expression and contribute to several chronic diseases.¹⁹⁰ There is mounting evidence that environmental influences are associated with increased mutation load and mitochondrial heteroplasmy,¹⁶⁹ making mitochondrial heteroplasmy a promising biomarker of both mitochondrial damage and cumulative exposure to environmental exposures.

Current Limitations and Future Perspectives

Need for Multi-Omic Approaches—In order to interpret genomic influences and epigenetic changes in the context of environmental exposures and their impact on human health, it is essential to improve our understanding of the downstream biological consequences of these molecular modifications. At present, most investigations are limited to either a single mechanism (or –omic layer) or use limited sample sizes. However, environmental exposures trigger alterations in multiple regulatory mechanisms that interact to create a systemic response. For example, air pollution triggers changes in DNA methylation, small non-coding RNAs, and epitranscriptomic machinery that collectively influence risk of downstream pulmonary fibrosis.¹⁹¹ There is also an established interplay between non-coding RNA and m⁶A modifications in the context of environmental exposures.^{130,136,142} Given the well-established crosstalk between epigenetic layers,^{192,193}

studies should aim to capture multiple biological mechanisms to better elucidate how environmental pollutants affect human health. This is especially true of major environmental threats including air pollution and heavy metals because they are known to have wide-ranging systemic effects that impact multiple biological systems. Technical limitations related to the analysis of high-dimensional datasets and use of relatively small samples have hindered our ability to conduct multi-omic research. However, emerging analytical tools have facilitated impactful multi-omic research.^{194–196}

High-dimensional genomic and epigenomic discovery studies rely heavily on ontological pathway analyses to elucidate the ways in which molecular changes impact cellular function. While pathway analyses are useful, they are imprecise and carry substantial uncertainty. Currently, these knowledge-based analyses (e.g. KEGG¹⁹⁷, GO¹⁹⁸) are curated from evolving knowledge base, but must make strong assumptions to postulate how epigenomic changes impact cellular function. For example, DNA methylation sites or non-coding RNAs associated with environmental exposures are matched to genes that they putatively regulate. These genes are then used for ontological and pathway-based analyses without explicit evidence that shows how certain molecular changes impact gene expression. The pitfalls of such assumptions are illustrated by the complicated relationship between mRNA and protein abundance: although there is often a correlation between the two, changes in one layer (e.g. mRNA) do not always lead to changes in the other (e.g. proteins).¹⁹⁹ To minimize false discoveries and conclusions from observed epigenetic changes, it is vital that population-based studies start to pair epigenomic changes with downstream functional changes. Such studies, which could be multi-omic or targeted in nature, will facilitate more substantiated interpretation of epigenetic and epitranscriptomic changes. Additionally, it would be helpful to include other epigenetic or molecular layers to accommodate the known interactions between epigenetic machinery.

Tissue Specificity—Due to logistical constraints, human studies are often limited to broad interrogations using heterogeneous cell populations and/or non-specific biomatrices such as blood. While blood-based investigations may reflect systemic changes, these studies often lack the specificity required to understand the pathogenesis of specific diseases. Furthermore, not all cell types will respond to environmental stimuli identically, which makes generalizations difficult. Given the existing restraints and our desire to understand the environmental impact on multiple organ systems, we need to adopt a combination of approaches to address this challenge. Adoption of single-cell sequencing may be one solution to this challenge. Single-cell techniques and approaches can resolve and characterize responses from individual cell types that is commonly found in biospecimen of typical epidemiologic studies. With technical advances and declining costs, this may be the next major step in molecular environmental health studies. Alternatively, deconvolution of multi-tissue samples²⁰⁰ is a useful strategy to estimate the composition of different cell types in a heterogeneous mixture. However, deconvolution alone does not allow us to examine specific cell or tissue types and complementary approaches are necessary. In the context of EVs, experimental strategies can be developed to isolate targets from specific tissues.^{201,202} This approach may allow us to isolate tissue-specific effects that can more clearly convey the impact of environmental exposures on key organ systems. Future research should take

these considerations into account to more clearly elucidate the impact of the environment on human health (Box 1).

Untargeted Discovery and the Exposome—Historically, exposure assessment has lagged behind genetic and epigenetic research with respect to our ability to comprehensively capture an individual’s environmental exposures. This has restricted our ability to monitor and understand environmental impacts on human health. However, just as genome-wide approaches have spurred tremendous advancements in our understanding of complex-trait genetics, implementing a more expansive and comprehensive view of environmental exposures will help elucidate the complex ways in which the environment impacts human health.²⁰³ In practice, we should consider the environment as a high dimensional –omic layer, otherwise known as the exposome.²⁰⁴ Performing untargeted environmental exposure assessments may produce a wealth of data that will more deeply inform our understanding of the complex mechanisms through which the environment shapes human health.

Conclusions

Environmental insults influence epigenetic modifications and related biological systems that interactively shape gene expression. Together, these pathways translate environmental exposures into substantive disease risk. Understanding the influence of environmental stressors on DNA methylation, histone modification, non-coding RNAs, epitranscriptomics, EVs, and mitochondrial genomics is critical to recognize and mitigate the effect of environmental exposures on human health.

Glossary

Biological age

The biological age is impacted by living conditions and lifestyle and refers to the physiological and functional status of an individual; the biological age may be older or younger than the chronological age and serves as a reflection of health and aging.

Compositional Epistasis

Compositional epistasis is a central requirement to a statistical interaction is a mechanistic interaction and requires a study to show that some individuals have the disease of interest if both environmental and genetic exposures are present but will not have the disease of interest if just one exposure is present.

Epigenome-Wide Association Studies

An epigenome-wide association study (EWAS) is a genome-wide study of epigenetic changes such as DNA methylation and their association with a health outcome of interest.

Liquid Biopsy

A liquid biopsy is a peripheral blood test that can detect cells derived from specific types of tissue in the body.

Particulate Matter

Particulate matter consists of microscopic particles of solid or liquid matter that are suspended in the air; fine particles have a diameter of $\leq 2.5 \mu\text{m}$ and are designated PM_{2.5}.

Polygenic Risk Score

A polygenic risk score is an estimate of a person's genetic liability for a disease of interest based upon their genotype.

Crossover trial

A longitudinal study where all participants receive two or more treatments, often in random order and separated by a washout period.

Circular RNAs

Single-stranded RNA in a closed continuous loop that are most often derived from protein-coding regions.

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Box 1.**Keys for Future Molecular Environmental Health Studies**

- Future studies, particularly epidemiologic investigations, should acknowledge the complex interplay across different biological mechanisms (e.g., interactions and feedbacks across histones, DNA methylation, and non-coding RNAs) that are often considered in isolation.
- If possible, studies should strive to expand beyond one single biological mechanism of interest to capture multiple connected systems using the growing number of multi-omic analysis approaches. This is necessary to show, rather than assume, that more “upstream” changes (e.g. DNA methylation) have downstream effects on transcriptional activity and protein expression.
- Investigators should take advantage of studies and programs that have already collected data on multiple molecular mechanisms to conduct both hypothesis-driven and hypothesis-free investigations.
- Studies should consider adopting single cell-based technologies to enrich or purify sample types and capture differential responses from each cell type.
- Studies should consistently acknowledge the uncertainties inherent in ontological and pathway analyses.
- Given recent advancements in exposure assessment and statistical mixture analyses, there is now an opportunity for investigators to consider the environment more holistically, rather than to evaluate discrete exposures independently.
- Beyond better detection of harmful pollutants, there is a need to identify natural exposure patterns and concomitant exposures in a way that would directly address potential for co-exposure confounding.
- While animal studies have demonstrated transgenerational effects of certain environmental pollutants transmitted through epigenetic mechanisms, human studies thus far have been lacking and future studies may consider leveraging multi-generational cohorts to address this gap.

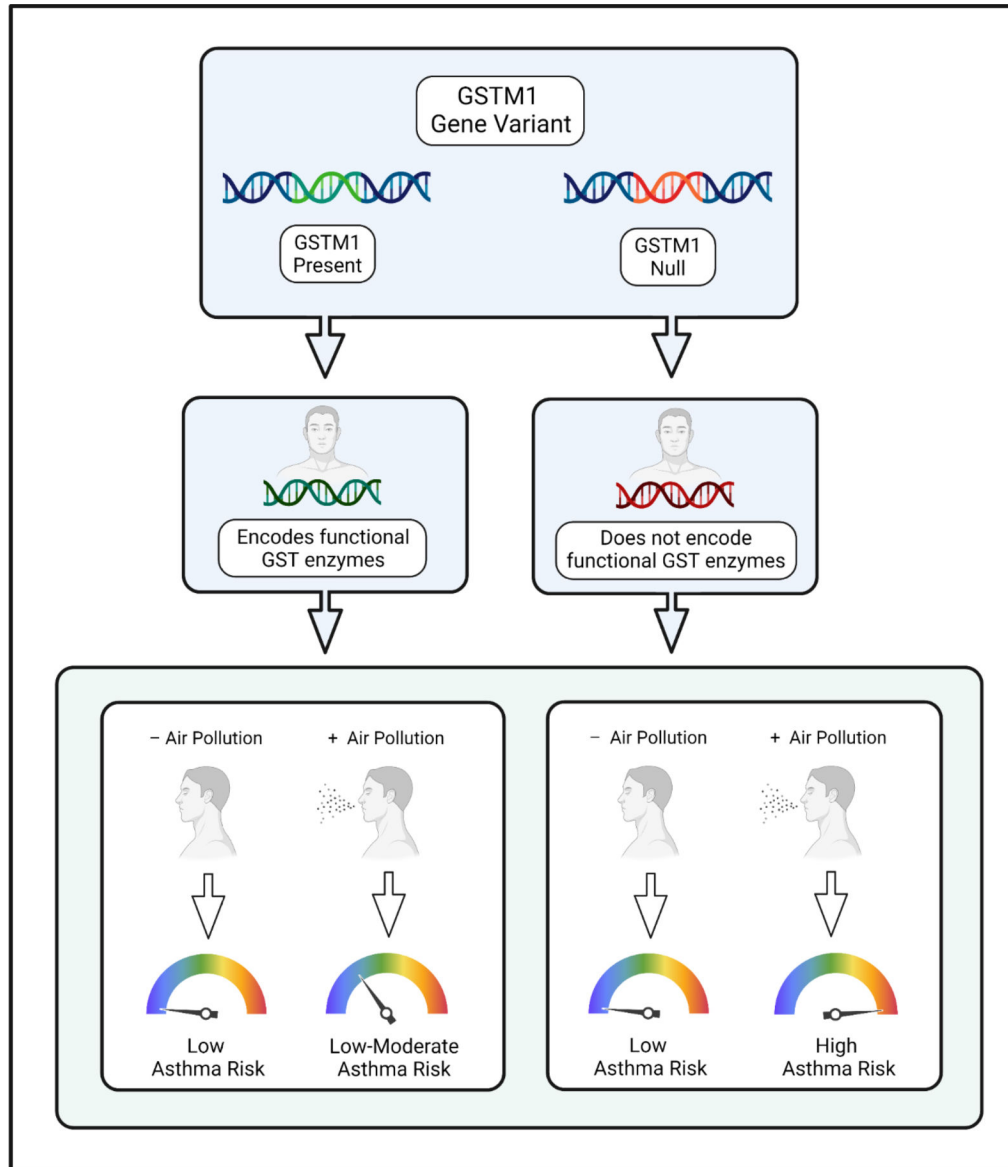


Figure 1. Gene-environment interactions impact disease phenotypes.

When gene-environment interactions exist, environmental exposures confer differing levels of disease risk for individuals with different genotypes. In this example, the glutathione S-transferase gene (*GSTM1*) encodes detoxifying enzymes that defend against oxidative stress. The gene has null alleles that cause loss of enzyme activity. Recent studies have shown that carriers of *GSTM1* null alleles who were exposed to indoor air pollution experienced increased risk of asthma and lung function impairment compared to participants with functional *GSTM1* alleles. This example highlights the importance of evaluating gene-environment interactions to identify SNPs that alter disease risk in the presence of prevalent environmental insults.

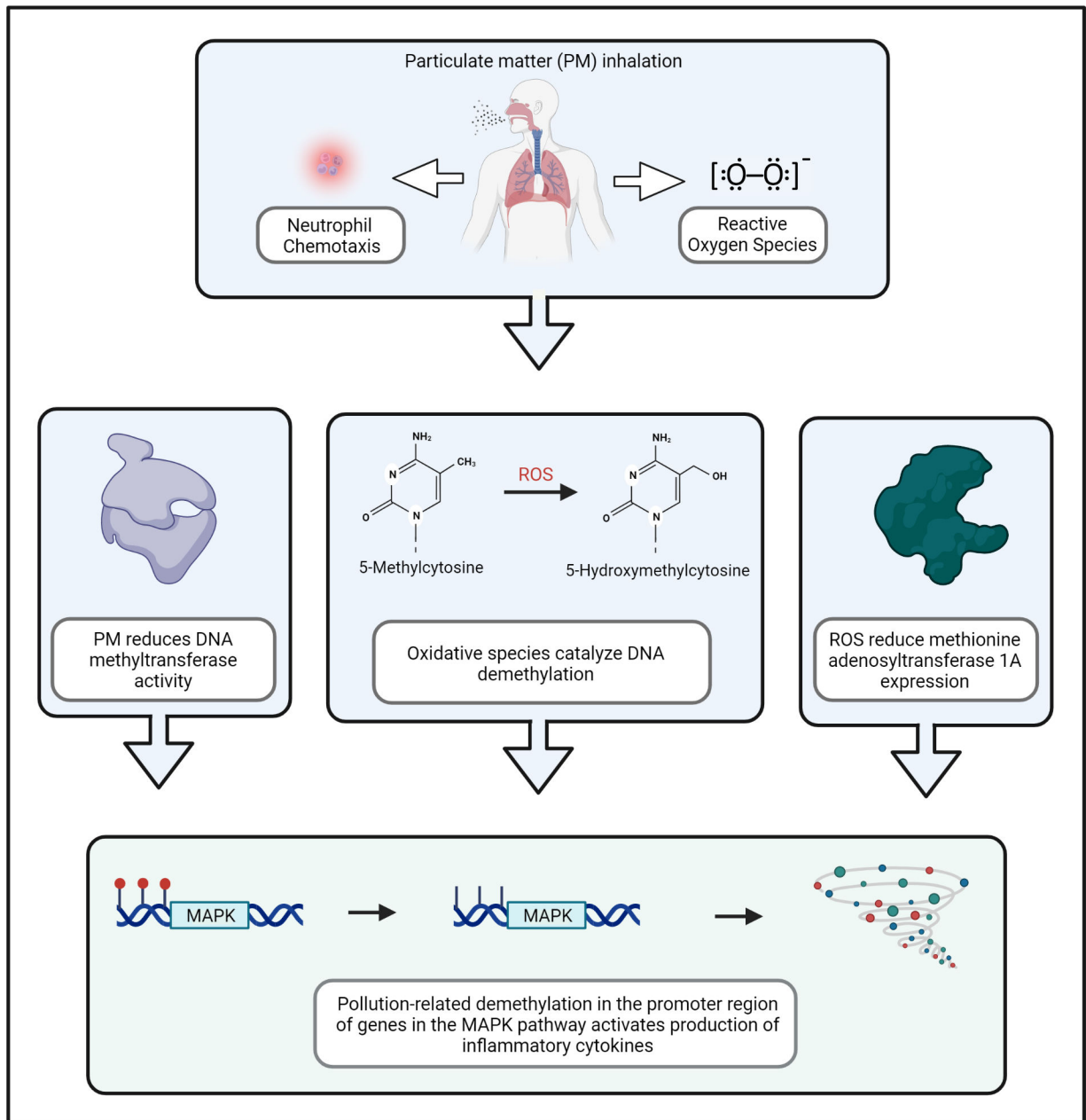


Figure 2. Air pollution alters DNA methylation in genes that regulate expression of inflammatory cytokines.

When particulate matter (PM) air pollution is inhaled into the lungs, small particles disrupt epithelial cell integrity in the lungs and trigger neutrophil chemotaxis and production of reactive oxygen species (ROS). Oxidative species reduce DNA methyltransferase activity, catalyze oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, and decrease expression of methionine adenosyltransferase 1A, thereby reducing availability of biologic methyl donors. These effects lead to pollution-related alterations in DNA methylation, including demethylation in the promoter region of genes in the mitogen-activated protein kinase (MAPK) pathway. These alterations hinder DNA methylation-mediated suppression

of inflammatory genes and link air pollution exposure to the production of pro-inflammatory cytokines.

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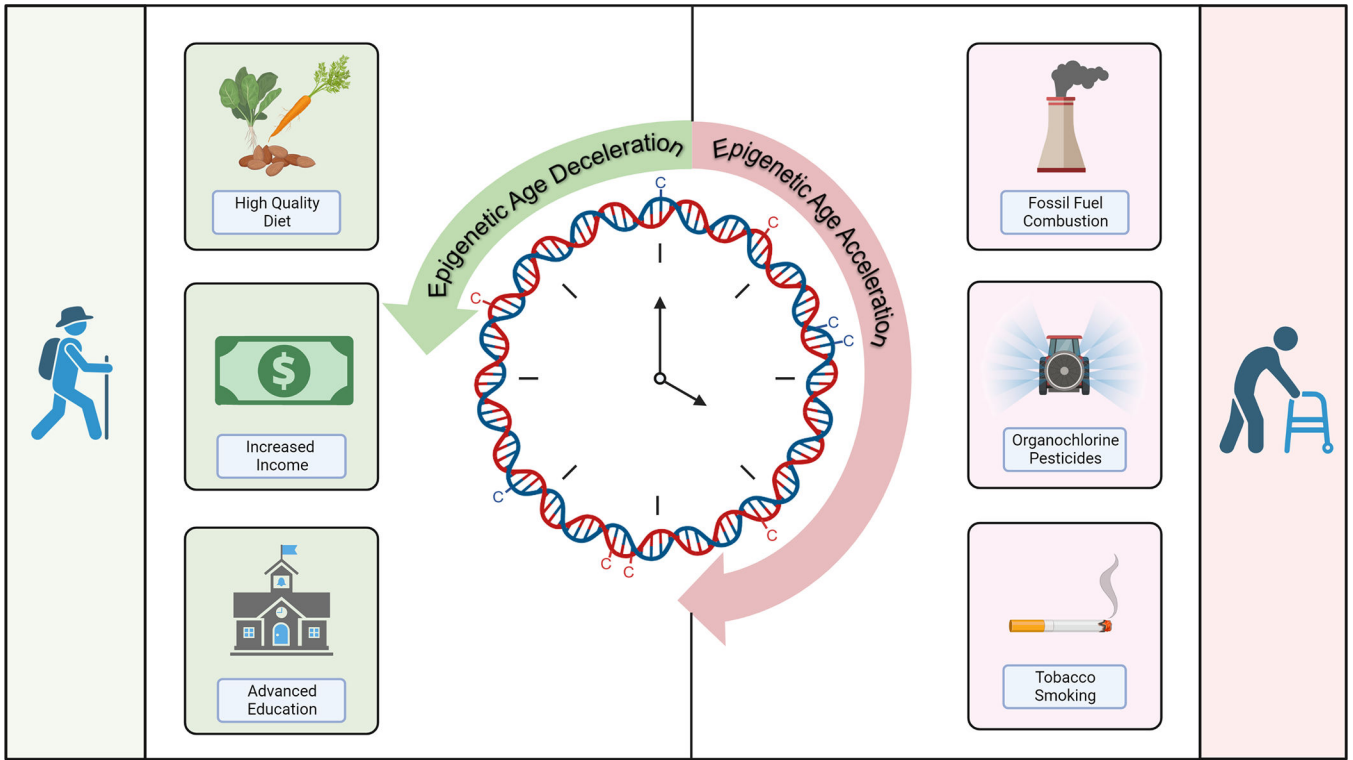


Figure 3. Environmental stressors impact DNA methylation age.

Epigenetic aging clocks encompass CpG sites that estimate DNA methylation age of human tissue. Harmful environmental exposures including fine particulate matter air pollution, organochlorine pesticides, and polycyclic aromatic hydrocarbons are associated with epigenetic age acceleration. In contrast, improved diet quality and higher socioeconomic status are associated with epigenetic age deceleration. Epigenetic age acceleration leads to genomic instability and aberrant gene expression and is associated with aging-related diseases and functional decline.

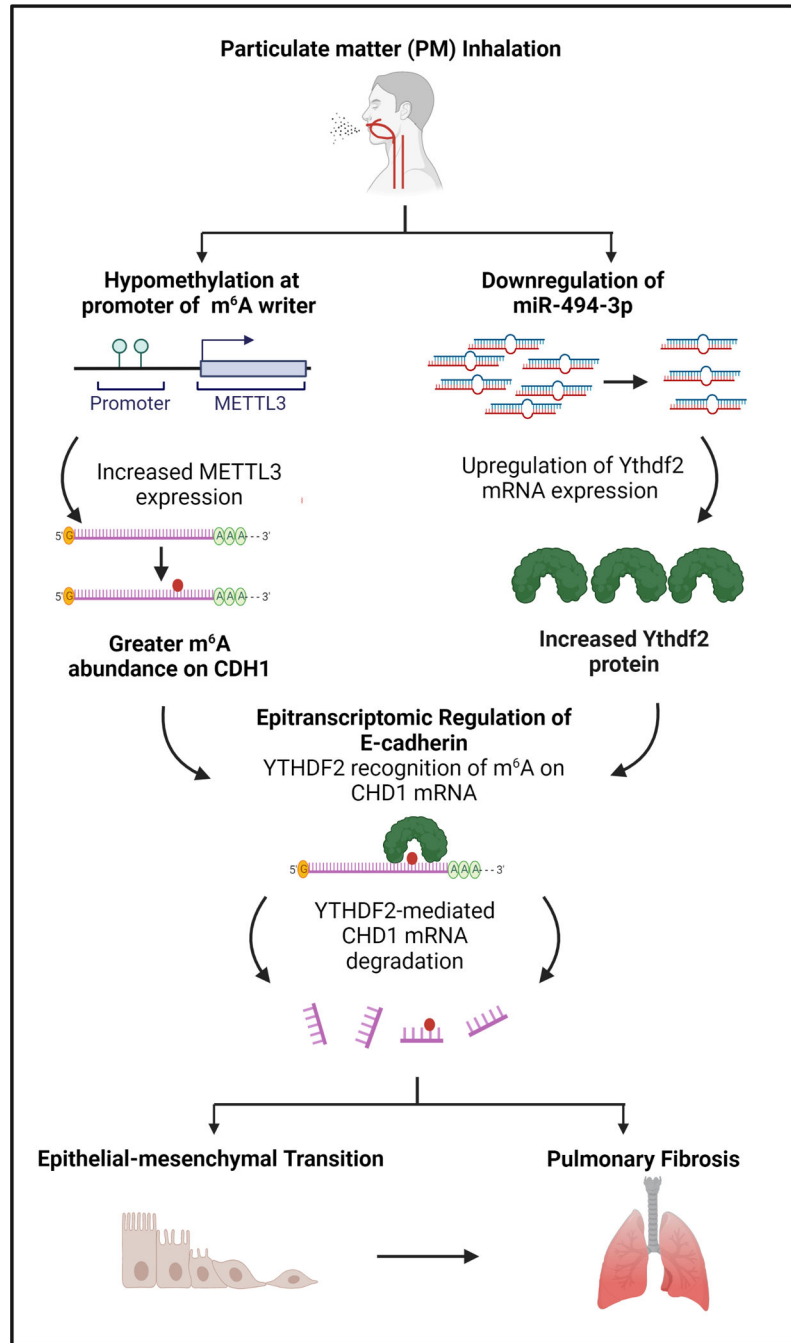


Figure 4. One example of how air pollution can trigger coordinated epigenetic and epitranscriptomic responses that impact human health.

Exposure to particulate matter air pollution can trigger alterations in epitranscriptomic machinery and multiple other regulatory mechanisms that interact to generate a systemic response. In this example, air pollution exposure triggers changes in DNA methylation, small non-coding RNA, and epitranscriptomic machinery that may influence risk of pulmonary fibrosis.

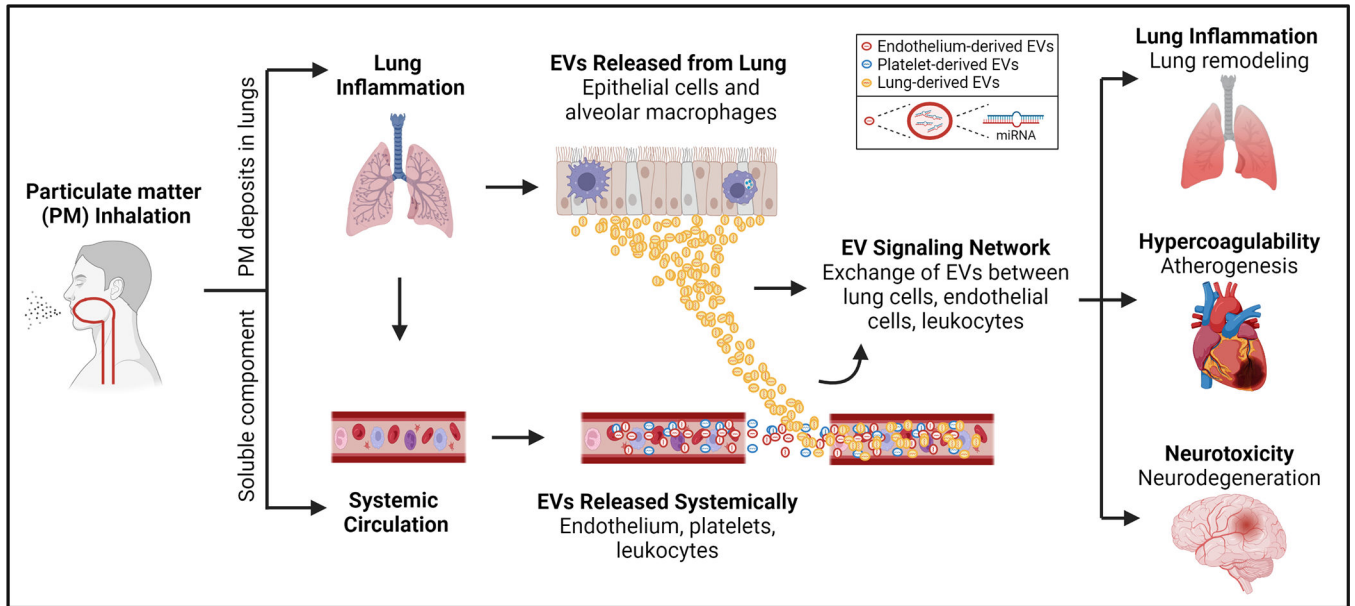


Figure 5. Inhaled environmental exposures trigger EV signaling that mediates systemic inflammation and disease.

Particulate matter inhalation triggers alveolar macrophages and airway epithelial cells to release pro-inflammatory EVs. Ultrafine particles also enter the systemic circulation and trigger EV release from endothelial cells, platelets, and circulating blood leukocytes. EVs amplify production of inflammatory cytokines and enhance recruitment of inflammatory cells. As a result, circulating EVs create a cycle that intensifies inflammation and can lead to end organ dysfunction including lung function impairment, atherogenesis, and neurodegeneration.

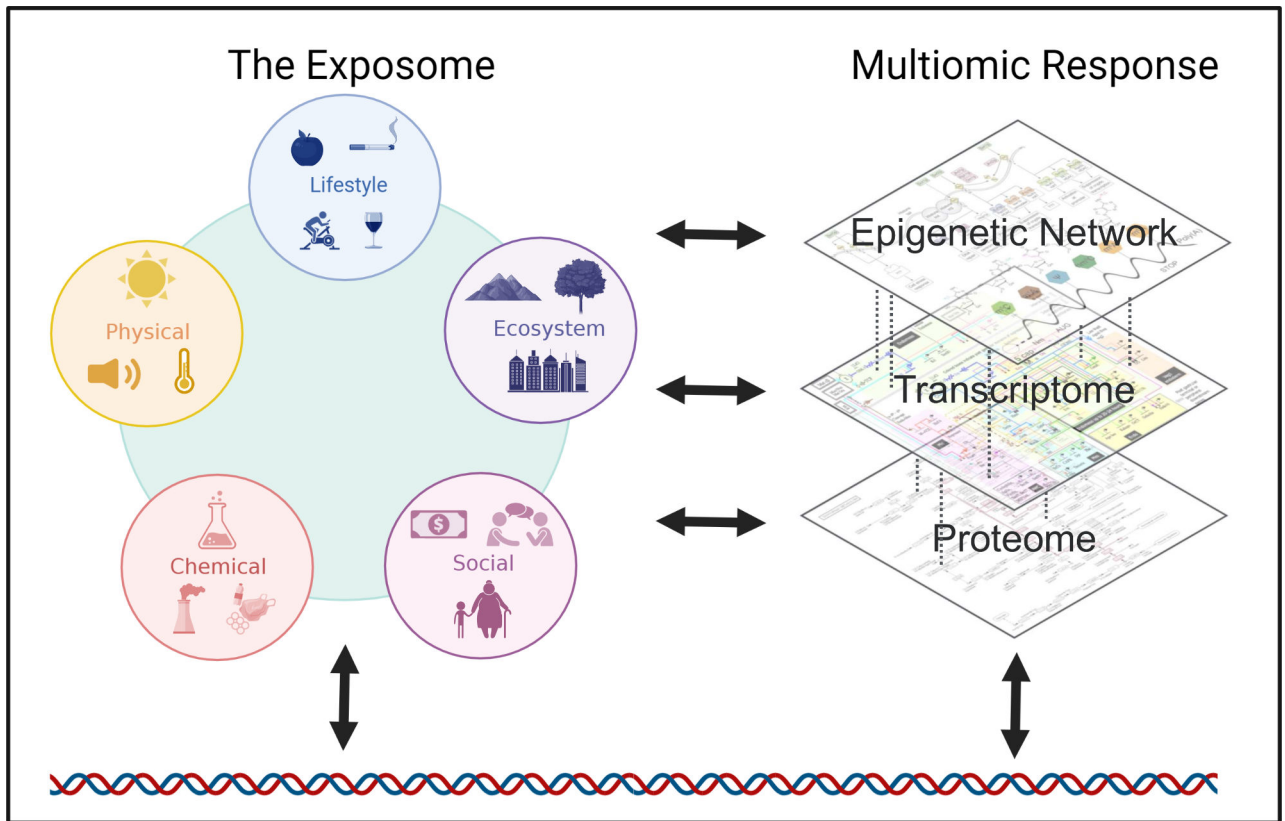


Figure 6. Exposomics and multi-omic responses.

The exposome is the cumulative measure of environmental influences including the external environment, lifestyle, behavior, and diet and the resulting biological and endogenous processes. The exposome induces biological responses at every level and should be integrated into multi-omic studies. Ultimately, the complex non-linear interactions between the environment and our genome, epigenome, transcriptome, metabolome, and proteome drive the aging process and the pathogenesis of chronic diseases.