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Review article

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Molecular mechanisms of environmental exposures and human disease

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Abstract

A substantial proportion of disease risk for common complex disorders is attributable to environmental exposures and pollutants. An appreciation of how environmental pollutants act on our cells to produce deleterious health effects has led to advances in our understanding of the molecular mechanisms underlying the pathogenesis of chronic diseases, including cancer and cardiovascular, neurodegenerative and respiratory diseases. Here, we discuss emerging research on the interplay of environmental pollutants with the human genome and epigenome. We review evidence showing the environmental impact on gene expression through epigenetic modifications, including DNA methylation, histone modification and non-coding RNAs. We also highlight recent studies that evaluate recently discovered molecular processes through which the environment can exert its effects, including extracellular vesicles, the epitranscriptome and the mitochondrial genome. Finally, we discuss current challenges when studying the exposome — the cumulative measure of environmental influences over the lifespan — and its integration into future environmental health research.

Sections

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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 cancer and cardiovascular, neurodegenerative and respiratory diseases[4]. These health consequences emphasize the importance of understanding environmental drivers of human health and elucidating how environmental exposures induce deleterious health effects.

The exposome is defined as the cumulative measure of environmental influences over the lifespan and is known to induce biological responses in every layer of human biology[5] (Fig. 1). 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 RNA, 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 ageing-related diseases and outcomes[6]. As a result, beyond providing a mechanistic link between environmental stressors and disease pathogenesis, epigenetic modifications can function as reliable markers of accelerated ageing and subclinical disease (that is, the presence of disease before the onset of observable symptoms)[7,8]. 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 examined other molecular processes that interact with epigenetic pathways. For example, extracellular vesicles are released from multiple organs in response to environmental insults and can mediate intercellular communication through complex molecular cargo, such as microRNAs (miRNAs)[9]. Moreover, environmental pollutants can trigger the chemical modification of RNA molecules[10], collectively known as the epitranscriptome, which alters phenotypic expression[11]. Additionally, environmental insults can have an impact on mitochondrial gene expression, which can increase disease risk[12].

In this Review, we examine the molecular mechanisms through which environmental pollutants have an impact on common complex diseases. We discuss the interplay of environmental insults with the human genome and explore how epigenetic modifications underlie the association between environmental exposures and disease pathogenesis. We review how emerging research related to extracellular vesicles, 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.

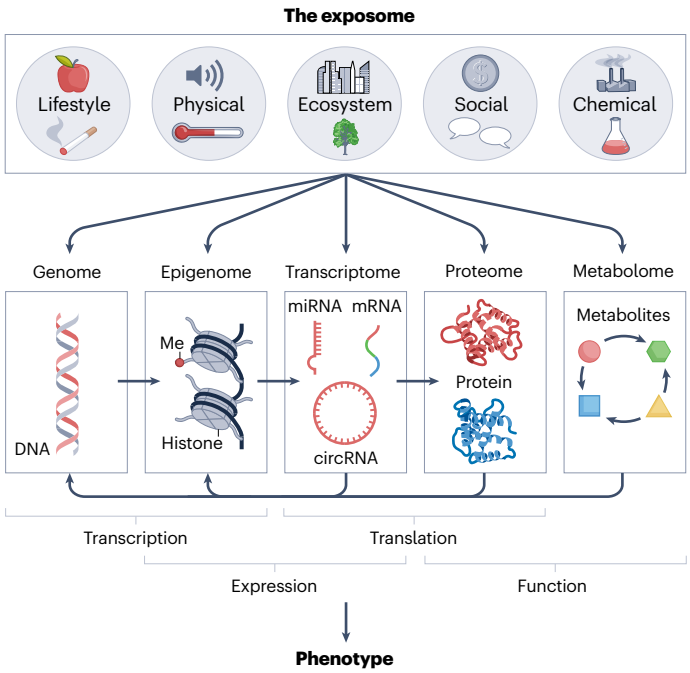

Fig. 1 | The exposome and multi-omic responses. The exposome is the cumulative measure of environmental influences, including the external environment, lifestyle, behaviour and diet, and the resulting biological and endogenous processes. The exposome induces biological responses at every level and should be integrated into multi-omic studies. Ultimately, the complex nonlinear interactions between the environment and our genome, epigenome, transcriptome, metabolome and proteome drive the ageing process and the pathogenesis of chronic diseases. circRNA, circular RNA; Me, methylation; miRNA, microRNA.

Gene–environment interactions

Although some rare diseases are caused by mutations in a single gene and have high genetic heritability, most common complex disorders, such as asthma, arise from the interplay of multiple genes and environmental stressors[13]. 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 depending on genotype[14] (Fig. 2). As a result, the separate evaluation of genetic and environmental factors can fail to identify high-risk genotypes or vulnerable populations. Gene–environment interaction studies are critically needed to elucidate the 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 genetic variants predispose individuals to complex diseases. However, genome-wide association studies (GWAS) can fail to identify genotypes with weak or modest effects on disease prevalence but comparatively strong effects on carriers with certain environmental exposures. For example, although asthma is a heritable disease linked to several genetic loci[15], previous GWAS did not identify an association between *GST* genes and asthma prevalence[16]. By 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 than participants with functional *GST* alleles[17–19] (Fig. 2). The *GST* genes encode glutathione *S*-transferases, which are detoxifying enzymes that protect against pollution-related oxidative stress and can interact with environmental insults to moderate asthma risk[20].

Beyond identifying novel genomic variants, gene–environment interaction studies can strengthen causal inference derived from observational studies[21,22]. For example, a recent population-based study

showed that individuals carrying a p.Glu192Arg polymorphism in the gene *PON1*, which encodes paraoxonase-1, had increased susceptibility to Gulf War Illness when exposed to a nerve agent[21]. 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 affected by recall bias or confounding by ancestry, which often limit the validity of environmental epidemiologic studies and GWAS[23]. Moving forward, gene–environment interaction studies are essential to identifying genotypes that modify disease risk in the presence of key environmental exposures.

Although elucidating gene–environment interactions is critical to understanding the genetic liability of human diseases, statistical tests of gene–environment interactions have fairly low power and require strict corrections when comparing multiple genotypes[24]. However, evolving analytical frameworks can increase the power to detect gene–environment interactions[25,26], 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[27,28]. 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[29].

Epigenome–environment interactions

Environmental insults influence epigenetic modifications that shape gene expression. Epigenetic modifications thereby translate environmental exposures into substantive disease risk. Understanding the influence of environmental stressors on DNA methylation, histone modification and non-coding RNAs is important to recognize and mitigate the effects of environmental exposures on human health.

DNA methylation

Given the intricate connection between DNA methylation and gene activity, understanding how environmental insults can have an impact on DNA methylation is critically important. DNA methylation occurs when DNA methyltransferase enzymes transfer a methyl group to the C5 position of a cytosine nucleotide, forming 5-methylcytosine[30]. By contrast, DNA demethylation occurs through a series of deamination and oxidation reactions that remove the methyl group from the cytosine base[31]. DNA methylation levels exert distinct influences in different genomic regions. In intergenic regions, DNA methylation suppresses potentially harmful genetic elements that can trigger mutation events[32]. Similarly, methylation of gene promoter regions often leads to transcriptional silencing[33]. Conversely, gene body methylation can increase gene expression[34].

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 epithelial cells and nucleated blood cells[35]. 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)[36,37]. Ultrafine particles pass into the systemic circulation and generate endothelial injury and oxidative stress[38]. ROS catalyse DNA demethylation through oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, after which DNA methylation is passively depleted over serial cellular divisions[39] (Fig. 3). In vitro models have also shown that air pollution reduces DNA methyltransferase activity, which is required to maintain DNA methylation levels[40]. In addition, pollution-related ROS reduce expression of methionine adenosyltransferase 1A (MAT1A), which decreases availability of biologic methyl donors[41].

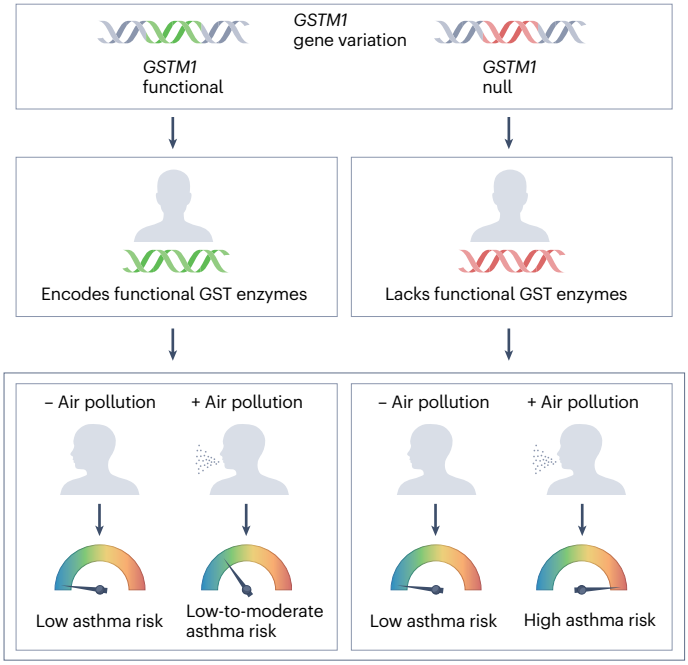

Fig. 2 | Gene–environment interactions have an impact on disease phenotypes. When gene–environment interactions exist, environmental exposures confer differing levels of disease risk for individuals with different genotypes. In this example, the glutathione *S*-transferase gene *GSTM1* encodes detoxifying 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[17]. This example highlights the importance of evaluating gene–environment interactions to identify SNPs that alter disease risk in the presence of prevalent environmental insults.

Several population-based studies have confirmed the association between air pollution exposure and 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[42]. These alterations may hinder DNA methylation-mediated suppression of inflammatory genes and link air pollution exposure to increased cytokine production[43]. In a crossover trial, PM exposure was associated with reduced methylation in pro-coagulant genes, which may link PM exposure to vascular thrombosis and cardiovascular health[44]. In a study of non-diabetic men, differential methylation of *ICAM-1*, an inflammatory gene, mediated the relationship between short-term PM exposure and increased diabetes risk[45]. A fourth study showed that PM was associated with hypomethylation of the *AHRR* gene, which is heavily implicated in the pathogenesis of obstructive lung diseases[46]. Tobacco smoking and PM exposure are associated with similar differences in DNA methylation patterns[47,48], suggesting that these inhaled environmental insults modify respiratory and cardiovascular disease risk through similar mechanistic pathways.

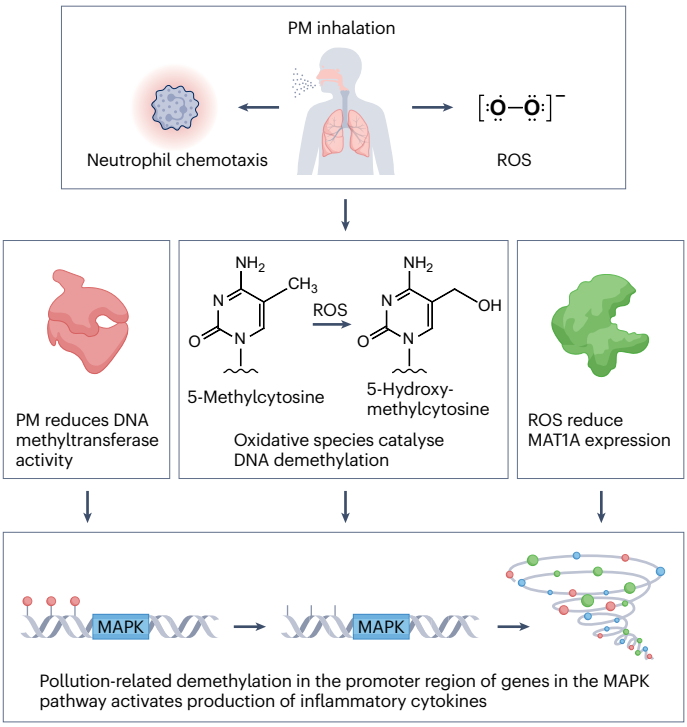

Fig. 3 | Air pollution alters DNA methylation in genes that regulate expression of inflammatory cytokines. When particulate matter (PM) air pollution is inhaled into the lungs, small particles disrupt epithelial cell integrity and trigger neutrophil chemotaxis and production of reactive oxygen species (ROS). Oxidative species reduce DNA methyltransferase activity, catalyse oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and decrease expression of methionine adenosyltransferase 1A (MAT1A), 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.

DNA methylation-based biomarkers

Epigenetic changes can function as biomarkers of environmental exposures and disease predisposition. For example, in epigenome-wide association studies, tobacco smoking was associated with differences in DNA methylation at thousands of CpG sites in nucleated blood cells[48]. Although 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 did not return to normative levels even decades after smoking cessation[49,50]. 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 behaviours[51].

Epigenetic biomarkers can also assess environmentally mediated disease risk in applications that mirror the use of polygenic risk scores to evaluate heritable disease risk[52]. 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[8]. Compared to self-reported smoking exposures, which are subject to recall bias and misreporting[53,54], 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 ageing. Previous research in different types of human tissue identified age-related changes in DNA methylation that were leveraged to develop epigenetic 'ageing clocks'[55]. Ageing clocks leverage machine learning to estimate the biological age of a DNA source based on a collection of CpG sites conserved across mammalian tissues. This estimate can reflect epigenetic age acceleration, that is, when the calculated DNA methylation age is higher than the chronological age[56]. Using these proxy measures, environmental insults have been shown to accelerate ageing. In population-based studies, individuals exposed to air pollution and tobacco smoke demonstrated advanced DNA methylation age in blood and lung tissue[57–59] (Fig. 4). Similarly, organochlorine pesticide exposure was associated with accelerated epigenetic ageing in peripheral blood leukocytes[60]. By contrast, another epidemiologic study showed that improved diet and educational attainment were associated with decelerated epigenetic ageing in blood leukocytes[61,62].

Elucidating environmental factors that have an impact on 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 mellitus[63]. 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[64]. In a third study, epigenetic age acceleration was associated with shortened life expectancy[65]. These findings suggest that epigenetic markers serve as an important proxy of aberrant biological ageing. Further research is required to determine whether epigenetic biomarkers can be applied in clinical settings to facilitate disease risk stratification and enable early diagnosis of environmentally mediated diseases.

Chromatin remodelling and histone modifications

Chromatin is packaged into repeating units of nucleosomes, which comprise ~147 DNA base pairs wrapped around eight histone proteins. Each 'octamer' bead contains two copies of H2A, H2B, H3 and H4 histones, with a single H1 'linker' histone binding the DNA to the octamer[66]. These core histones have several variants, many of which are tissue specific, have particular functions and have been associated with genetic disorders and cancers[66]. 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[67,68]. These modifications help define the transcriptional state of the chromatin towards the more transcriptionally active euchromatin or less transcriptionally active heterochromatin. Nucleosome positioning and histone modifications are thus essential for all biological processes and can affect both the health of the individual[69] and that of later generations[70,71]. 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 histone modification patterns[72,73].

Glossary

Biological age
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 ageing.

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

Compositional epistasis
A central requirement to demonstrate that there is a mechanistic gene–environment interaction that requires a study to show that some individuals will 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.

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

Epigenome-wide association study
A genome-wide study of epigenetic changes such as DNA methylation and their association with a health outcome of interest.

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

Particulate matter
Microscopic particles of solid or liquid matter that are suspended in the air; fine particles have a diameter of ≤2.5 μm and are designated $PM_{2.5}$.

Polygenic risk score
An estimate calculated as a weighted sum of many trait-associated alleles to summarize a person's genetic liability of developing a disease of interest based on their genotype.

It is well established from both observational and experimental evidence that arsenic alters histone methylation, including higher levels of genome-wide methylation of H2B and lower levels of genome-wide methylation of H3 and H4[72,73]. Arsenic also alters methylation at specific sites including histone 3 lysine 4 trimethylation (H3K4me3), H3K9me3 and H3K27me3 in a sex-dependent and tissue-dependent manner[72]. Although the exact mechanisms underlying arsenic-induced changes in histone methylation are unknown, there is some evidence that arsenic activates specific methyltransferase enzymes, including G9a[74,75]. Arsenic may thereby affect the bivalent status of chromatin[73]. Specifically, the simultaneous presence of activating (for example, H3K4me3) and repressing (such as H3K27me3) modifications, both of which have been observed with arsenic exposure, is a hallmark of bivalency. This bivalent state can lead to the upregulation of oncogenes[76] and may underlie the oncogenic effect of arsenic. Arsenic-induced histone modifications have also been shown to generate oxidative stress, damage DNA and regulate the cell cycle[73].

Aside from altering histone methylation, arsenic has 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[77,78], although confirmatory experimental evidence is necessary. Emerging evidence shows other environmental exposures can trigger post-translational histone modifications including trace metals[79], air pollution[80–85], polycyclic aromatic hydrocarbons (PAHs)[86], pesticides[87–89], dioxins[90] and plasticizers[91,92].

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. miRNAs are one class of small non-coding RNAs[93] that can directly bind to 3′ untranslated regions, 5′ untranslated regions and coding sequences in mRNAs to inhibit translation or to promoter regions to induce transcription[93]. Acting in a controlled epigenetic 'circuit', miRNAs can also affect the chromatin state to promote transcription[94] and are themselves regulated by DNA methylation and histone modifications[95].

Despite early evidence that miRNAs are responsive to a range of environmental exposures[96], details of the underlying mechanisms have been limited[97]. Although 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[98–100]. In a crossover trial, diesel exhaust was shown to affect expression of several miRNA and mRNAs, leading to inflammatory cell recruitment and epithelial cell shedding[98]. 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 links air pollution exposure to a pro-inflammatory state that contributes to the pathogenesis of rheumatoid arthritis[99]. In another in vitro study, PM exposure 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[100]. 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[100]. The connection between $PM_{2.5}$ and miRNA dysregulation has also been implicated in the pathogenesis of Alzheimer disease[101].

Although 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. For example, recent in vivo rodent studies showed that air pollutants lead to differentially expressed circular RNAs in the mouse lung[102,103] and in rat embryos[104]. As another example, tRNA fragments are responsive to heat[105], ultraviolet radiation[105,106] and oxidative stress[105,107,108]. Although tRNAs have traditionally been viewed in the context of translation,

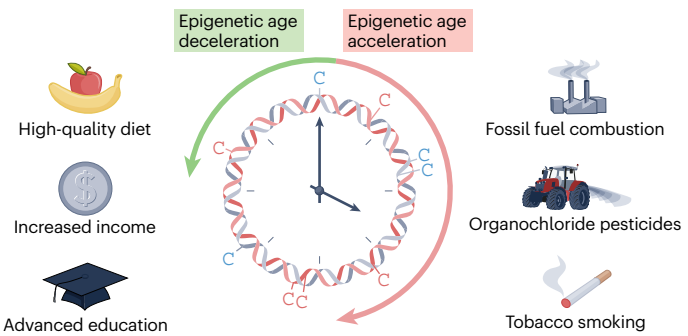

Fig. 4 | Environmental stressors affect DNA methylation age. Epigenetic ageing clocks encompass CpG sites that can be used to estimate the DNA methylation age of human tissue. Harmful environmental exposures including fine particulate matter air pollution, organochlorine pesticides and polycyclic aromatic hydrocarbons are associated with epigenetic age acceleration. By 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 ageing-related diseases and functional decline.

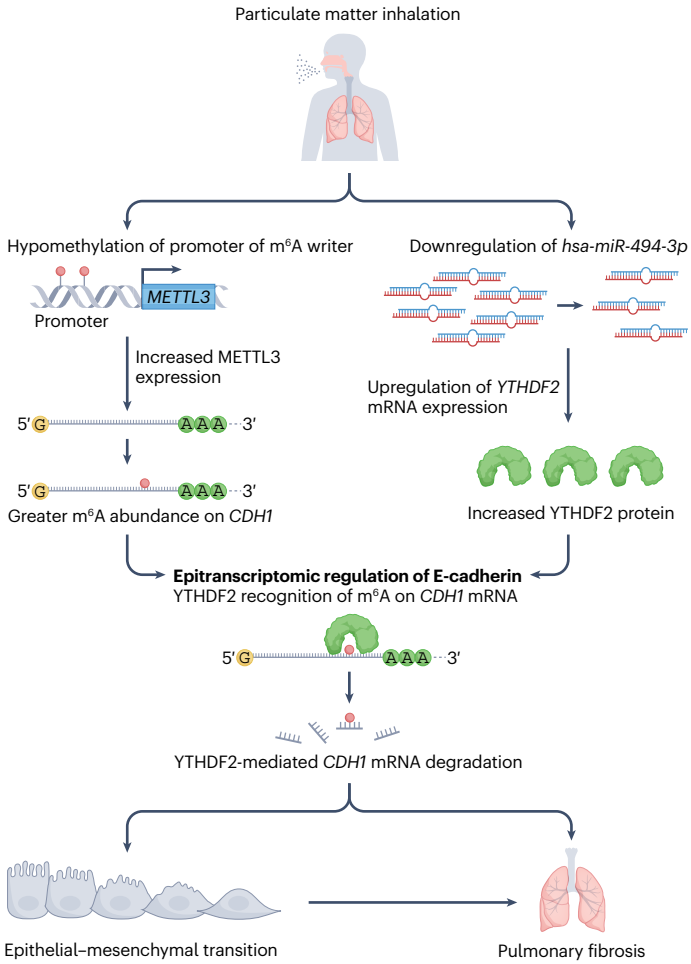

Fig. 5 | Illustrative example of how air pollution can trigger coordinated epigenetic and epitranscriptomic responses that affect human health. Exposure to particulate matter air pollution can trigger alterations in epitranscriptomic machinery and multiple other regulatory mechanisms that interact to 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. Specifically, environmentally related *METTL3* promoter hypomethylation leads to more abundant m^{6}A modification on *CDH1*, which encodes cadherin-1 (also known as epithelial cadherin or E-cadherin). The m^{6}A modification on *CDH1* is then recognized by YTHDF2, an m^{6}A 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[134].

tRNAs also regulate gene expression via translation repression and gene silencing[109,110]. In addition, our growing understanding of the mechanisms by which long non-coding RNAs regulate gene expression through modulation of chromatin structure and direct interactions with target genes[111] is accompanied by emerging evidence that air pollution[103,112], metals[113,114] and other environmental contaminants[115–117] alter long non-coding RNA expression. These trends affirm that further research elucidating how environmental insults modify the expression of non-coding RNAs is critically needed to expand our understanding of how environmental insults affect human health.

Emerging areas of investigation

Epitranscriptomics

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[118]. Similar to epigenetic modifications, RNA modifications are controlled and maintained by a group of 'reader', 'writer' and 'eraser' proteins (RWEs)[118]. 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 N^6-methyladenosine (m^{6}A). The m^{6}A modification can be found in all types of RNA[119] and helps regulate RNA folding, splicing, stability and translation[120].

Epitranscriptomic changes related to environmental exposures are an underexplored but potentially important pathway through which environmental insults can have an impact on human health. For example, m^{6}A has been shown to regulate the inflammatory response, activate the adaptive and cellular immune systems and modulate T cell homeostasis and differentiation[121,122]. Similarly, METTL3, an m^{6}A methyltransferase (that is, writer), is essential for the production of inflammatory cytokines[123]. Experimental research has shown that m^{6}A is responsive to external stressors in vitro, especially environmental pollutants associated with oxidative stress such as cigarette smoke and $PM_{2.5}$[82,124–127]. Accordingly, oxidative stress has been shown to affect m^{6}A on hundreds of mRNA transcripts[128,129].

Environment-induced alterations in global m^{6}A levels may stem in part from changes in the expression of RWEs. $PM_{2.5}$ exposure leads to hypomethylation of the promoter regions of RWE genes[130], resulting in differential expression of METTL3[82,125,126,130] and METTL14[82]. METTL3 and METTL14 are two writers that form the m^{6}A methyltransferase complex[131]. Similarly, cigarette smoke has been shown to alter METTL3 and METTL14 expression via promoter hypomethylation in vitro[132,133], resulting in higher levels of m^{6}A on select miRNAs and long non-coding RNAs. In experimental models, $PM_{2.5}$-related alterations in METTL3 also induce m^{6}A modification of *OSGIN1*[126] and *CHD1* mRNAs[134]. The CHD1 m^{6}A modification is part of a mechanism that spans across multiple different biological regulatory networks (Fig. 5). In human studies, the relationship between air pollution and m^{6}A is less clear[125,135]. A weak association was observed between 8-h black carbon exposure and global m^{6}A levels, but no associations were observed with $PM_{2.5}$ or PM_{10}[135]. Furthermore, the study did not find associations between air pollutant levels and mRNA expression of six m^{6}A RWEs[135]. However, the same study did identify decreased global m^{6}A levels associated with smoking, which is consistent with previous experimental studies. In addition, a Polish cohort study found that ambient $PM_{2.5}$ was associated with greater RWE gene expression[125].

Heavy metals and chemical toxicants have also been shown to alter m^{6}A and RWE expression in vitro. High levels of arsenic increased global m^{6}A levels[136,137] via upregulation of the m^{6}A methyltransferase complex and downregulation of FTO, a demethylase[136,138,139]. This effect was also observed in vivo, as mice treated with arsenic showed higher global m^{6}A levels and downregulation of FTO[140]. Other metals and chemical toxicants including cobalt[141], manganese[142], chromium[143], cadmium[144], polychlorinated biphenyls (PCBs)[145,146], PAHs[147] and other common environmental pollutants[125,148–150] were also associated with m^{6}A 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 the m^6A demethylase FTO[140] and m^5C methyltransferase NSUN2[151]. At present, few studies have examined components of the epitranscriptome beyond m^6A. However, m^6A is a small part of the epitranscriptomic landscape[152]. As the field evolves, it is imperative to examine other influential RNA abundant modifications.

Extracellular vesicles

Extracellular vesicles are nano-sized membranous vesicles that are released from multiple cell types in the body under physiologic conditions and in response to environmental insults[153]. The physiologic state of the parent cell regulates packaging of extracellular vesicle cargo, which includes proteins, nucleic acids, lipids and metabolites. Extracellular vesicles are released from the parent cell and are endocytosed by recipient cells, and they alter cell biology through their molecular cargo. For example, extracellular vesicle-encapsulated miRNAs (EV-miRNAs) regulate cellular function by degrading complementary mRNA transcripts[154]. Extracellular vesicles 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 extracellular vesicles into the blood[155] (Fig. 6). For example, it has been shown that in humans, extracellular vesicles can generate a pro-inflammatory signalling cascade that causes endothelial injury, hypercoagulability and end organ dysfunction[156]. Subsequent in vitro studies showed that PM-related extracellular vesicles also triggered hyper-responsiveness of bronchial smooth muscle cells[157], accelerated vascular thrombosis[158] and precipitated neurotoxic signalling[159]. Extracellular vesicles thereby mediate the toxic effects of air pollution exposures and contribute to pollution-related risk of chronic lung, heart and neurologic diseases.

Heavy metals[160–162] and plasticizers including bisphenol-A (BPA) and di(2-ethylhexyl) phthalate (DEHP) have been shown to modulate extracellular vesicle 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[163]. Recent in vitro studies showed that BPA decreased EV-miRNA expression in primary granulosa cells[164]. Population-based studies in women undergoing in vitro fertilization confirmed that phenol and phthalate exposure increased expression of some EV-miRNAs and decreased expression of other EV-miRNAs contained in follicular fluid[165,166]. In addition, human studies showed that BPA exposure increased expression of some proteins and decreased expression of other proteins in placenta-derived extracellular vesicles and affected pathways known to modulate placental cellular injury[167]. Together, these findings suggest that extracellular vesicles and their molecular cargo may function as a mechanistic link between chemical exposures and reproductive toxicity.

Extracellular vesicles translate environmental exposures into substantive disease risk through synergistic interactions with miRNAs and other epigenetic mechanisms[168]. Extracellular vesicles 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[169]. Recent efforts to isolate plasma extracellular vesicles derived from specific tissue types may further enable accessible liquid biopsies that are predictive of future health outcomes.

Mitochondrial dysfunction

Mitochondria facilitate energy metabolism, redox signalling, fat homeostasis and metabolic regulation[170]. Accordingly, mitochondrial impairments or dysregulation contribute to numerous disease states[170]. Mitochondria are particularly vulnerable to the effects of environmental exposures. The mitochondrion thereby functions both

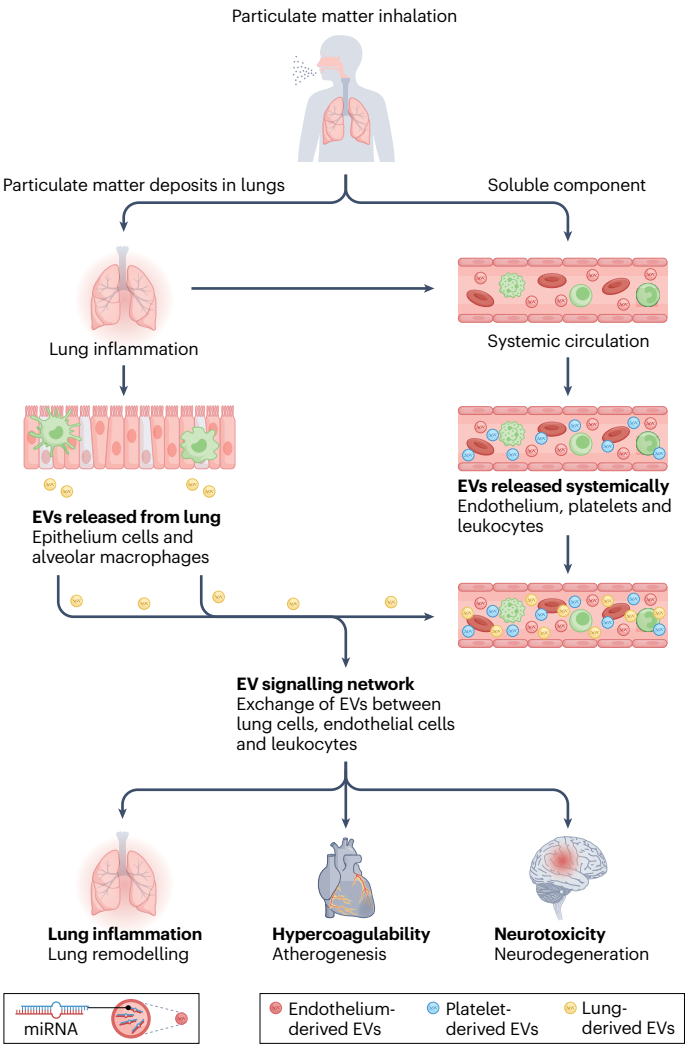

Fig. 6 | Inhaled environmental exposures trigger extracellular vesicle signalling that mediates systemic inflammation and disease. Particulate matter inhalation triggers alveolar macrophages and airway epithelial cells to release pro-inflammatory extracellular vesicles (EVs). Ultrafine particles also enter the systemic circulation and trigger EV release from endothelial cells, platelets and circulating blood leukocytes. EVs contain short, non-coding microRNAs (miRNAs) that modify transcriptional activity in recipient cells. EVs thereby amplify production of inflammatory cytokines and enhance recruitment of inflammatory cells. As a result, circulating EVs and their encapsulated molecular cargo create a cycle that intensifies inflammation and can lead to end organ dysfunction including lung function impairment, atherogenesis and neurodegeneration.

Box 1

Keys for future molecular environmental health studies

- Future studies, particularly epidemiologic investigations, should acknowledge the complex interplay across different biological mechanisms (for example, interactions and feedbacks across DNA methylation, histone modifications and non-coding RNAs) that are often considered in isolation.
- Where 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 'upstream' changes (for example, DNA methylation) have 'downstream' effects on transcriptional activity and protein expression.
- Investigators should take advantage of studies and programmes 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 needing 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.
- Although 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.

as a sentinel for exposure-induced damage and as a central mechanism through which environmental exposures can have an impact on human health[171]. 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[172].

The number of mitochondrial genomes per cell or the mitochondrial DNA (mtDNA) copy number is a commonly used marker of mitochondrial damage. Numerous environmental exposures have been shown to alter mtDNA copy number including PM air pollution[173], PAHs[174], heavy metals[171] and other chemical[175–177] and occupational exposures[178–180]. However, the relationship between environmental stressors and mitochondrial DNA copy number varies depending on the type and duration of the exposure[171], and it is difficult to discern the underlying mechanisms. Nevertheless, 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 pollution[181], heavy metals[182], PAHs[174] and select pesticides[183–185]. 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[186], imbalance in calcium homeostasis[187], increased mtDNA mutations[188] and damage to the mitochondrial respiratory chain and ATP production[187]. Mitochondrial dysfunction then triggers systemic effects including inflammasome and inflammatory cytokine release[174,181].

Recent research has also identified several pollutant-specific pathways. For example, air pollution can lead to aberrant mitochondrial DNA methylation and DNA strand breaks[181]. Dioxins and PAHs can bind aryl hydrocarbon receptors[189], which leads to degradation of these receptors in mitochondria and alterations in cellular respiration[190]. Finally, cyanide and rotenone can inhibit cellular respiration via interactions with complex IV and complex I of the electron transport chain[191].

An area of growing interest in environmental health research is mitochondrial heteroplasmy. Each cell contains many copies of mtDNA, and heteroplasmies are mutations that are present in a subset of mtDNA within a cell[192]. Mitochondrial genomes are naturally more susceptible to mutations than are nuclear genomes, and heteroplasmy has been shown to alter mitochondrial gene expression and contribute to several chronic diseases[192]. There is mounting evidence that environmental influences are associated with increased mtDNA mutation load and heteroplasmy[171], making mitochondrial heteroplasmy a promising biomarker of both mitochondrial damage and cumulative exposure to environmental exposures.

Current limitations and future perspectives

Multi-omics approaches

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 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 (Fig. 1). For example, air pollution triggers changes in DNA methylation, small non-coding RNAs and epitranscriptomic machinery that collectively influence the risk of pulmonary fibrosis[193]. There is also an established interplay between non-coding RNA and m^6A modifications in the context of environmental exposures[132,138,144]. Given the well-established crosstalk between epigenetic layers[194,195], 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 such as air pollution and heavy metals because they are known to have wide-ranging systemic effects that have an impact on multiple biological systems. Technical limitations related to the analysis of high-dimensional datasets and use of fairly small samples have hindered our ability to conduct multi-omic research. However, emerging statistical tools[196], such as LUCID[197] and xMWAS[198], have facilitated impactful multi-omic research.

High-dimensional genomic and epigenomic discovery studies rely heavily on ontological pathway analyses to elucidate the ways in which molecular changes affect cellular function. Although pathway analyses are useful, they are imprecise and carry substantial uncertainty. Currently, resources for pathway analyses, such as KEGG[199] and GO[200], are curated from the evolving knowledge base but rely on strong assumptions to predict how epigenomic changes influence 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 ontology-based and pathway-based analyses without explicit evidence showing how certain molecular changes regulate 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 (that is, mRNA) do not always lead to changes in the other (that is, proteins)[201]. 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

Owing to logistical constraints, human studies are often limited to broad interrogations using heterogeneous cell populations and/or nonspecific biomatrices such as blood. Although blood-based investigations may reflect systemic changes, these studies often lack the specificity required to reveal the pathogenesis of specific diseases. Furthermore, not all cell types will respond to environmental stimuli identically, which makes generalizations difficult. Given existing constraints 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 technologies may be one solution to this challenge. Single-cell approaches can resolve and characterize responses from individual cell types commonly found in a biospecimen of typical epidemiologic studies[202]. With technical advances and declining costs, this may be the next major step in molecular environmental health studies. Alternatively, deconvolution of multi-tissue samples is a useful strategy to estimate the composition of different cell types in a heterogeneous mixture[203]. However, deconvolution alone does not allow us to examine specific cell or tissue types, so complementary approaches are necessary. In the context of extracellular vesicles, experimental strategies can be developed to isolate targets from specific tissues[204,205]. This approach may allow us to isolate tissue-specific effects, potentially using a liquid biopsy-like approach in future environmental health studies to identify epigenetic, mitochondrial and epitranscriptomic signals 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. 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 mechanisms through which the environment shapes human health[206]. In practice, we should consider the environment as a high-dimensional omic layer, otherwise known as the exposome[5]. Performing non-targeted environmental exposure assessments, such as the use of non-targeted high-resolution mass spectrometry coupled with large spectral databases for chemical identification[207], may produce a wealth of data that will more deeply inform our understanding of 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 impact of environmental stressors on the genome and epigenome is thus critical to recognizing and mitigating the effects of environmental exposures on human health.

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