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Air pollution and the epigenome: A model relationship for the exploration of toxicoepigenetics

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Abstract

The field of toxicoepigenetics is rapidly emerging to provide new insights into the relationship between environmental factors, the epigenome, and public health. Toxicoepigenetic data have the potential to revolutionize our understanding of environmental exposure effects and susceptibility. Studies in recent years have demonstrated that exposure to air pollution alters epigenetic modification states; however, continued advancement of the field is limited by the intrinsic complexity of the epigenome and inherent limitations of different types of studies (epidemiological, clinical, and *in vitro*) that are used in toxicoepigenetics. Overcoming these challenges will require a concerted and collaborative effort between molecular and cellular biologists, toxicologists, epidemiologists, and risk assessors to develop a thorough and practical understanding of the relationship between air pollution exposure, the epigenome, and health effects. Here we review the current state of air pollution epigenetics and discuss perspectives on the necessary steps to move the field forward to determine the role that the epigenome plays in air pollution exposure effects and susceptibility.

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1. Introduction

The epigenome consists of a complex set of modifications to DNA and histone proteins that primarily serves to provide instructions for the regulation of chromatin structure and thus directs gene expression, DNA replication/repair, and other cellular functions [1–3]. These instructions are written in an alphabet that includes >130 histone modifications [4], as well as DNA methylation (5-methylcytosine) and its derivatives 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine(5-fC), and 5-carboxylcytosine (5-caC), which play varying roles in the regulation of gene expression that are yet to be fully characterized [reviewed in 5]. The patterns of these modifications can be stable and inherited across mitotic and meiotic generations; however, they are also dictated by intrinsic (*e.g.*, age, sex, and genotype) and extrinsic (*e.g.*, stress, diet, and toxic exposures) factors, collectively referred to as "environmental factors" [reviewed in 6]. These environmental factors shape the epigenome and mediate exposure outcomes from the molecular level [7]. They can also influence short- and long-term disease susceptibility by modifying the baseline epigenetic state and "priming" cells, tissues, and organisms for a given response. Understanding the complex relationship between environmental factors, the epigenome, and the effects of air pollutant exposure has the potential to lead to the identification of transformative biomarkers of susceptibility and effect that will drive the next generation of risk assessment. While promising, moving forward will require that critical questions be addressed and that challenges be overcome.

The effects of air pollution have been widely investigated *in vitro* (cell lines and primary cells) and *in vivo* (animal models, controlled human exposures, and epidemiological studies) [8,9]. These studies have shown that pollutants such as ozone and particulate matter (PM) are important contributors to cardiopulmonary morbidity and mortality; however, only a small fraction of these studies have included epigenetic endpoints. This relatively small number of studies has demonstrated an association between air pollution exposures and epigenetic changes, and has been the subject of several reviews in recent years [10–17]. Moving forward from these founding observations will require the establishment of a causative relationship between air pollution exposures, health effects, and the epigenome; however, accomplishing that goal will require researchers to develop innovative approaches to acquire and integrate data describing how exposures influence levels of a diverse array of epigenetic modifications, determine the utility and applicability of surrogate tissues, and identify the mechanisms through which

epigenetic changes/differences lead to exposure-associated disease, among others. Here we provide a brief history of studies that serve as the foundation for our understanding of the association between air pollutant exposure and the epigenome. We discuss challenges, data gaps, and strategies to examine the putative causative role for the epigenome in the adverse effects of environmental exposures, using air pollution as a model. As recommended by Burris and Baccarelli [14], the perspectives expressed here were developed through a cross-specialty collaboration between an epigeneticist/molecular toxicologist, an epidemiologist, a risk assessor, and an environmental health scientist that specializes in clinical research.

2. Linking air pollution exposure and the epigenome

Early studies linking air pollution exposure and epigenetic changes focused around the effect of PM exposures (black carbon, $PM_{2.5}$, and PM_{10}) on DNA methylation levels within either repetitive elements (*Alu* and LINE-1) — which serve as markers of broader-scale epigenetic changes throughout the genome — or the inducible nitric oxide synthase (iNOS/NOS2) gene — which plays a role in the modulation of oxidative stress and inflammation following air pollution exposure [18—21]. While still primarily focused on the effects of air pollution exposure on epigenetic endpoints in peripheral leukocytes in large study populations, more recent approaches have expanded to global profiling of site-specific DNA methylation by incorporating bead chip technology, which allows for the simultaneous interrogation of >480,000 (450k array) or >850,000 (EPIC array) individual CpG loci. When paired with large, highly documented populations (*e.g.*, KORA and the Normative Aging Study) with matched local or regional air pollution data these studies have revealed associations between methylation of CpG sites linked to a range of genes and different air pollutants [22—25], health outcomes [26,27], and epigenetic aging [28—32]. More focused exploration of small sets of exposure- or effect-associated loci in these studies has also demonstrated an association between PM exposure and coagulation, inflammation, and endothelial function. For example, altered methylation of inflammatory markers such as, ICAM-1 and IL-6, were observed with increased exposure to black carbon [33], and showed some modification by psychological factors [34]. Further, mediation analysis found that the methylation status of ICAM-1 mediated a small portion (9%) of the association of 28-day $PM_{2.5}$ with fasting blood glucose levels [35]. Overall, these studies illustrate the complexity of the interrelationship between environmental exposures, such as air pollution, and intrinsic factors such as fasting blood glucose and anxiety.

While the majority of studies examining the relationship between air pollution exposure and the epigenome focus on DNA methylation, several have linked exposures with bulk changes in histone modifications in peripheral leukocytes of exposed humans [36,37], blood and total lung tissue in rats [38], and cultured human airway epithelial cells [39,40]. To the best of our knowledge, only one study to date has explored the air pollution exposure-associated changes in histone modifications at specific loci. Liu et al. [41] associated high levels of $PM_{2.5}$ exposure with changes in H3K27ac within the regulatory regions of genes associated with immune function in peripheral leukocytes; however, the small study population ($n = 4$; with 2 high- and 2 low-exposed individuals) may limit the broader interpretation of their findings. Studies such as those described above serve as proof-of-principle that exposure to a range of air pollutants is associated with changes in both DNA methylation and histone modifications; however, the field has yet to address key questions that must be answered to provide the depth of understanding required for the use of epigenetic data in practical applications, such as risk assessment and adverse outcome pathway development (Box 1). Doing so will involve coordinated effort between a broad range of disciplines including: molecular biologists/toxicologists, who can demonstrate biological plausibility and supply mechanistically relevant epigenetic profiles, clinical researchers, who can validate the putative biomarkers in humans and infer causality, and epidemiologists, who can assess relationships between the candidate biomarkers and exposures in real-world scenarios. Finally,

Box 1. Moving forward in toxicoepigenetics.

Primary Questions:

1. Is there a relationship between exposure-related epigenetic changes and alterations in gene expression?
2. How do DNA methylation and histone modifications work together to influence exposure effects and susceptibility?
3. Do changes/differences in epigenetic modification states cause air pollutant exposure effects or are they just associated?
4. Is peripheral blood a suitable surrogate for measuring the impact of air pollution exposure on the epigenome when access to the affected tissue is impractical?

Secondary Questions:

1. How persistent are epigenetic modifications that are associated with exposure effects and/or susceptibility?
2. Does exposure-induced epigenetic reprogramming exhibit latency between the exposure and altered susceptibility?
3. Are the epigenetic changes resulting from air pollutant exposure adaptive, adverse, or neither?
4. How do we define a subset of the >130 epigenetic modifications that are informative for identifying individuals/populations that are susceptible to discrete or mixed air pollutant exposures?
5. Are exposure-induced changes in epigenetic states markers of effect, susceptibility, or both?