



Genomic tools for environmental epigenetics and implications for public health

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Abstract

Epigenetics refers to the study of mitotically heritable and potentially reversible changes in gene expression unrelated to the DNA sequence itself, influenced by epigenetic marks including chromatin modifications, noncoding RNA (ncRNA), and alterations to DNA itself via methylation and hydroxymethylation. Epigenetics has taken center stage in the study of diseases such as cancer, diabetes, and neurodegeneration; however, its integration into the field of environmental health sciences and toxicology (e.g. toxicoepigenetics) is in its infancy. This review highlights the need to evaluate surrogate and target tissues in the field of toxicoepigenetics as the National Institute of Environmental Health Sciences multiphased Toxicant Exposure and Response by Genomic and Epigenomic Regulators of Transcription consortia make headway and the emergence of ncRNA biomarkers. The review also discusses lead (Pb) as a potential toxicoepigenetic exposure, where prenatal and postnatal Pb exposure is associated with reprogramming of DNA methylation, histone modifications, and microRNA expression, representing potential biomarkers or predictors for Pb-induced health outcomes. Finally, new advances in epigenome editing, highlighting the potential of small ncRNA, will be explored for environmental health sciences research.

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Toxicoepigenetics, Lead (Pb), DNA methylation, Noncoding RNA, piRNA, circRNA.

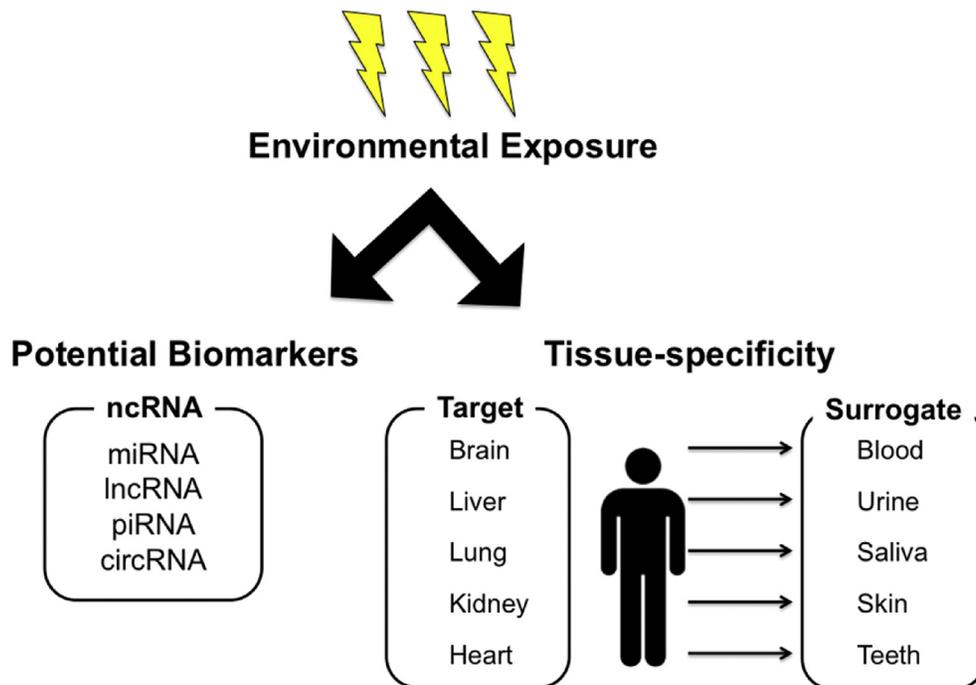
Introduction to the role of the environmental epigenetics in health and disease

The genetic material of every organism exists within the context of regulatory networks that govern gene expression, collectively called the epigenome. Epigenetics has taken center stage in the study of diseases such as cancer, diabetes, and neurodegeneration; however, its integration into the field of environmental health sciences and toxicology is in its infancy. Increasing the presence of epigenetics in toxicological research (e.g. toxicoepigenetics) will promote more in-depth understanding of important aspects of toxicology, including the mechanisms underlying toxicant-mediated health effects and the role of the environment and lifestyle in modulating individual susceptibility. Despite a growing interest in the integration of epigenetics into the field of toxicology, recent advances of the field have not yet fully incorporated toxicoepigenetics into research or risk assessment programs. Here, we provide a primer on the fundamental principles and practices critical to understanding the role of the epigenome in regulating gene expression and the resulting response of cells, tissues, and individuals to toxicant exposures. We first discuss the complexity of tissue and cell type in epigenomics research by highlighting the National Institute of Environmental Health Sciences' (NIEHS) multiphased Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription (TaRGET) Program ([Fig. 1](#), right side) [1]. We then focus on the emerging need to expand beyond DNA methylation in toxicoepigenetics research to also include noncoding RNAs (ncRNAs) as biomarkers of exposures and mechanisms of disease risk ([Fig. 1](#), left side). Finally, new advances in epigenome editing, highlighting the potential of small ncRNA, will be explored for environmental health sciences research.

Toxicoepigenetics and the critical need to evaluate surrogate and target tissues

Epigenetics refers to the study of mitotically heritable and potentially reversible changes in gene expression unrelated to the DNA sequence itself. Epigenetic marks include chromatin modifications (e.g. histone protein acetylation and trimethylation), noncoding long and small RNA (e.g. lncRNA, miRNA), and alterations to DNA itself (e.g. DNA methylation, DNA

Fig. 1



Environmental exposure impact on the epigenome involves the evaluation of target versus surrogate tissues and potential of noncoding RNAs as biomarkers. ncRNA, noncoding RNA; miRNA, microRNA; lncRNA, long noncoding RNA; piRNA, PIWI-associated small RNA; circRNA, circular RNA.

hydroxymethylation of cytosines in cytosine-guanine (CpG) contexts) [2,3]. Given the urgent need for a better understanding of the effects of environmental exposures on the epigenome, the NIEHS established the multiphased, multiinstitutional TaRGET consortium in 2012. The first phase of the consortium, TaRGET I, yielded novel insights into how environmental factors such as endocrine-disrupting chemicals (EDCs) or arsenic affect disease susceptibility via perturbation of the complex interconnected epigenetic pathways that regulate gene expression [4,5].

In 2016, the TaRGET II consortium was subsequently established to address additional ongoing challenges in environmental epigenomics studies [1]. First, current human population-based environmental epigenetics studies are limited to easily obtainable sources of DNA (hair, blood, and saliva). It is currently unclear, however, to what extent epigenomic changes in these surrogate tissues reflect changes that occur in the disease-relevant, but often inaccessible, target tissues. Second, whether toxicant-induced changes in the epigenome persist in target or surrogate tissues over time after exposure cessation is unclear. Third, it is increasingly evident that the effects of exposures are highly sex specific, adding another layer of complexity to the interpretation of population-based studies. Thus, the TaRGET II consortium was formed to investigate

the conservation of toxicant-induced epigenomic changes across tissues and time, in both males and females, after a variety of developmental environmental exposures. These studies include the measurement of DNA methylation and hydroxymethylation, histone modifications, ncRNAs, chromatin structure, and gene expression. The investigation of multiple epigenetic factors in parallel is a key strength of TaRGET II. Indeed, it is well established that multiple epigenetic mechanisms work in concert to regulate gene expression [6]. The data obtained from TaRGET II will be used to inform the design of human population-based studies. The third and fourth phases, TaRGET III and IV, will be aimed at the analysis and integration of genomic and epigenomic data in human population-based studies.

Lead as a representative toxicoepigenetic exposure

Exposure to heavy metals such as lead (Pb) remains a significant public health concern, particularly in poorer urban areas. Pb is a well-established environmental toxicant linked to adverse neurologic, cardiovascular, and metabolic outcomes [7,8]. Prenatal and postnatal Pb exposure is associated with reprogramming of DNA methylation, histone methylation and acetylation, and miRNA expression [9–13]. These epigenetic changes may represent biomarkers of Pb exposure, predictors of Pb-induced health outcomes, or mechanistic mediators

of toxicity. Thus, establishment of disease-specific epigenetic markers of Pb exposure may allow identification of individuals at high risk of adverse health outcomes, as well as lend mechanistic insight into Pb-mediated toxicity. These findings, in turn, may inform the development of dietary, pharmacologic, or social interventions for high-risk individuals. As Pb target tissues in human populations are typically limited to small numbers of postmortem specimens, identification of appropriate surrogate tissues for Pb exposure is critically important.

As part of the TaRGET II consortium, we recently used our established mouse model of perinatal environmental exposures [14] to determine whether common DNA methylation signatures of Pb exposure exist between blood and liver. Two weeks before mating, dams were assigned to control or lead-acetate (32 ppm) water [14]. Pb exposure proceeded through gestation/lactation and was discontinued at weaning when the animals were 3 weeks of age. Between 3 weeks and 5 months of age, offspring were administered standard chow and Pb-free tap water. Mice were sacrificed at 5 months of age, and blood and liver samples were collected in accordance with protocols established by the TaRGET II consortium [1]. To assess Pb-mediated programming of DNA methylation, we conducted enhanced reduced representation bisulfite sequencing in the blood and liver samples. We hypothesized that Pb exposure would lead to stable reprogramming of DNA methylation in both the liver and blood and that a subset of sites would overlap between the two tissues. Indeed, although Pb exposure ceased at 3 weeks of age, this analysis revealed thousands of stably modified, sex-specific differentially methylated regions (DMRs) in the adult blood and liver of exposed females and males. Overall, 3 DMRs overlapped between blood and liver in females and 4 in males. Thus, perinatal exposure to Pb induced sex-specific changes in DNA methylation that persisted into adulthood, with newly identified signatures overlapping between blood and liver (Accepted abstract, Society of Toxicology 2019 Annual Meeting).

Challenges and opportunities in toxicoepigenetic studies

The identification of suitable surrogate tissues in environmental epigenomics studies presents several challenges. Indeed, comparisons of basal [15] and toxicant-induced changes in DNA methylation [16] across surrogate tissues (cord blood, placenta, umbilical artery, and saliva) have revealed low correlations among the tissues, suggesting that extrapolation of epigenetic changes in surrogate tissues to target tissues should be carried out with caution. Blood is the most frequently used tissue given that it is easily accessible and can be collected at multiple time points and in large sample sizes [17]. However, blood cells undergo rapid turnover,

as well as exposure-, age-, and disease-dependent changes in cellular composition [18–20]. Recent work using living human brain biopsies demonstrated that a small percentage (7.9%) of CpGs showed a significant correlation in DNA methylation between blood and brain [21]. Interestingly, however, comparison of saliva and blood demonstrated that DNA methylation signatures in saliva were more similar, on average, to several brain regions than blood samples [22]. With regard to liver, comparison of age-associated changes in DNA methylation between blood and liver demonstrated that 67.8% of age-associated changes in the liver overlapped and occurred in the same direction as those in white blood cells [23]. Thus, the utility of blood as a surrogate may depend on several factors, including the environmental exposure, age, and target tissue(s) of interest.

In our work, it is interesting that a relatively small number of DMRs overlapped between blood and liver. Given the significant heterogeneity in cell types in the liver and blood, it is plausible that Pb-mediated epigenetic changes are limited to subpopulations of cells. Single-cell epigenomic approaches currently in development will likely shed light on this important question [24]. It is also plausible that blood may not be an appropriate surrogate tissue for Pb-induced changes in hepatic DNA methylation. Given the highly tissue-specific nature of epigenetic patterns [25], surrogate tissues from the same embryonic germ layer as the target tissue may exhibit more overlap in basal and environment-induced epigenetic changes. Indeed, a recent comparison of neonatal cord blood and tissue to 25 primary tissues/cell types demonstrated that cord blood and tissue are ideal surrogates for target tissues of mesodermal origin [26]. As the liver and the epithelial layer of the gut are both derived from the embryonic endoderm, stool samples, which capture cells shed from the epithelial layer of the gut, may be ideal surrogates for liver tissue.

ncRNA in the field of toxicology

With the completion of the human genome project, the scientific community was quite puzzled by the discovery of ~1.5% of the human genome that consists of protein coding mRNA [27], while nearly 80% of the remainder includes ncRNAs that participate in functionally important biochemical activities [25]. These ncRNAs are also critical for epigenetic regulation and have therefore gained much attention in the field of environmental epigenetics, toxicology, and risk assessment [28]. The ncRNA molecules longer than 200 nucleotides are classified as long ncRNAs (lncRNAs), which are essential for genomic imprinting, X-chromosome inactivation, and development [29,30]. In contrast, ncRNAs shorter than 200 nucleotides are termed small ncRNAs (sncRNA) and include constitutive/housekeeping RNA species such as transfer RNA, small nucleolus RNA

(snoRNA), small nuclear RNA (snRNA), and ribosomal RNA, as well as regulatory RNA species such as microRNA (miRNA), endogenous siRNA (endo-siRNA), and PIWI-associated small RNA (piRNA). The snRNA is the most popular class of ncRNA in the field of environmental toxicology and risk assessment, with the greatest likelihood of serving as a mode of intervention due to its wide applicability. Since its discovery in 1991 [31], another ncRNA species, circular RNA (circRNA) is becoming increasingly popular among researchers due to its high abundance in disease stages ranging from cancer to development [32], thus serving as a promising candidate for future studies in environmental toxicology.

Emergence of ncRNA biomarkers in toxicology

The advancement of toxicoepigenetic research shows much promise in understanding the genetic and environmental factors, which often influence human health outcomes. The fast-evolving fields of transcriptomics, bioinformatics, and sequencing technology have contributed to the robust development and breadth of RNA biology. Thus, the identification of potential ncRNA biomarkers in a broad range of human disease pathologies contribute to ncRNA-based diagnostic and therapeutic approaches in translational research [33,34]. The lncRNA, snRNA, and circRNA biomarkers are particularly interesting candidates because they specifically bind to DNA, RNA, or both to regulate gene transcription, translation, and epigenetic modifications such as DNA methylation.

In contrast to lncRNAs, snRNAs and circRNA biomarkers are small, versatile, resistant to degradation, and are available in a wide array of human biospecimens including saliva, urine, and blood [35]. The most extensively studied ncRNA biomarkers are miRNAs, which are 21–23 bp in length and involved in transcriptional and posttranscriptional gene repression via specific base pairing with their gene targets [36,37]. Emerging research in the field of environmental toxicology indicates evidence for miRNA biomarkers associated with exposure to EDCs, metals, pollution, and several human diseases [13,38–43]. For example, hsa-miR-146a was overexpressed and associated with bisphenol A accumulation in human placentas [38] and decreased expression of hsa-miR-575, hsa-miR-4286 was associated with Pb exposure in the cervix of women during their second trimester of pregnancy [13], and 54 circulating miRNAs were identified in association to short-term exposure to traffic-related air pollution with tissue-specific biomarkers [39].

Some recent studies have shown lncRNA response to environmental stressors such as EDCs, metals, cigarette smoke extracts, and genotoxic agents [34,44–46]. For instance, lncRNAL20992 was upregulated in a Pb-

exposed neuronal-injury cell model, whereas lncRNA profiles of lung tissues derived from cigarette smokers and nonsmokers identified two lncRNAs associated to chronic obstructive pulmonary disease [45]. In contrast to miRNAs, lncRNAs regulate gene expression by complex, often times by unknown mechanisms. Thus, future research in lncRNAs is necessary to elucidate their functional and toxicoepigenetic roles in response to environmental exposure.

Future directions for ncRNA biomarkers

Only a handful of studies indicate the involvement of other snRNA species such as piRNAs and circRNAs in toxicological research [47–49]. When compared to miRNAs, piRNAs are typically 24- to 32-bp-long snRNAs that contain a 5' uridine signature, an adenosine signature at the 10th position, and a 2'-O-methylation modification at the 3' ends for stability. piRNAs are responsible for transposon silencing via stable, DNA methylation induction and are highly expressed from the germline [50,51]. Thus, piRNAs represent a fascinating adaptive mechanism and 'ready-made' tool for innovation in locus-specific repression, which may be used as a potential tool to develop a novel epigenome editing technique for diagnosis and functional inhibition [52,53]. Recent toxicology research indicates that piRNAs are associated with environmental and chemical exposures [47,49,54,55]. For example, piRNAs were the most altered snRNA upon investigating the epigenetic transgenerational inheritance of dichlorodiphenyltrichloroethane exposure in rat sperm [49] and dichlorodiphenyltrichloroethane/vinclozolin exposure in ovarian disease-associated rats' ovarian granulosa cells [47]. The piRNAs may be considered potential biomarkers in future studies because of their high stability, expression in human blood, and tissue specificity [50,56]. Unfortunately, many toxicology studies that investigate potential snRNAs associated with environmental/chemical exposures tend to disregard analyzing snRNAs such as piRNAs, which may lead to oversight in potential discovery. Thus, researchers should consider analyzing piRNAs in future toxicoepigenetic studies or reanalyzing previous data that involved germline tissues.

circRNAs, on the other hand, are a product of back-splicing of linear pre-mRNA which join together a donor site with an upstream acceptor site and are well conserved across mammals [57]. circRNAs are remarkable biomarkers due to several characteristics: stability; resistance to exonucleases such as RNase R; universality/high abundance; presence in liquid biopsies (i.e. blood, saliva, urine, and cerebrospinal fluid); and strong specificity depending on tissue, developmental stage, and age [58,59]. In some cases, circRNAs serve as molecular sponges that regulate transcription by removing miRNAs [58]. They are associated with cancer, Alzheimer's disease, diabetes, cellular stress, and aging

[60,61]. Moreover, testis derived from atrazine-exposed developing *Xenopus laevis* indicated 44 upregulated and 361 downregulated circRNAs associated with testicular degeneration [48]. Thus, many circRNAs are becoming increasingly popular candidates for diagnosis and clinical interventions and may serve as a potential ncRNA of interest in future research in toxicology [59]. The study of less popular ncRNA species such as piRNAs and circRNA may lead to identification of potential biomarkers related to human health and disease, which may contribute to personalized, targeted RNA interventions.

Conclusions

In summary, identification of suitable surrogate tissues in environmental epigenomics studies will likely be dependent on the toxicant, target tissue, dose, duration of exposure, and the epigenetic mark being tested. A comprehensive understanding of tissue-specific epigenomic changes after exposure to a variety of common toxicants is currently lacking. The TaRGET II consortium seeks to fill this knowledge gap, and findings from this study will yield important insight into the utility of DNA, chromatin, and ncRNA-based biomarkers of toxicant exposure. Epigenetic biomarkers of disease can be combined with other approaches, such as the measurement of toxicant exposures using deciduous teeth [24], to establish definitive links between developmental environmental exposures, deregulation of epigenetic processes, and disease.

The discovery of disease-related miRNA biomarkers ranging from cancer to type 2 diabetes has now moved scientific research from the bench to the public — where several miRNA therapies are currently at the stage of clinical drug trials for target-specific interventions [62]. Although most miRNA research examines intracellular miRNA composition in response to environmental exposure, current research should also explore the lesser known, extracellular miRNA composition of miRNA carriers such as exosomes, ribonucleoprotein complexes, and membrane vesicles to identify miRNA biomarkers that can be easily accessed through liquid biopsies [43]. Refining the current knowledge of miRNAs and lncRNAs (exploring the tissue, developmental stage, and sex specificity) and expanding the breadth of toxicological research in search of other ncRNA components such as piRNAs and circRNAs are critical for the advancement of disease and diagnosis in toxicoepigenetic research.

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Conflict of interest statement

Nothing declared.

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- of special interest
 - of outstanding interest
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