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Investigating Epigenetic Effects of Prenatal Exposure to Toxic Metals in Newborns: Challenges and Benefits

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Abstract

Increasing evidence suggest that epigenetic alterations can greatly impact human health, and that epigenetic mechanisms (DNA methylation, histone modifications, and microRNAs) may be particularly relevant in responding to environmental toxicant exposure early in life. The epigenome plays a vital role in embryonic development, tissue differentiation and disease development by controlling gene expression. In this review we discuss what is currently known about epigenetic alterations in response to prenatal exposure to inorganic arsenic (iAs) and lead (Pb), focusing specifically on their effects on DNA methylation. We then describe how epigenetic alterations are being studied in newborns as potential biomarkers of *in utero* environmental toxicant exposure, and the benefits and challenges of this approach. In summary, the studies highlighted herein indicate how epigenetic mechanisms are impacted by early life exposure to iAs and Pb, and the research that is being done to move towards understanding the relationships between toxicant-induced epigenetic alterations and disease development. Although much remains unknown, several groups are working to understand the correlative and causal effects of early life toxic metal exposure on epigenetic changes and how these changes may result in later development of disease.

Keywords

Arsenic; lead; prenatal exposure; DNA methylation; epigenetics

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Introduction

Environmental contaminants including toxic metals are widespread and often disproportionally affect certain populations within the US. Toxic metal exposures have been associated with a number of diseases, including cardiovascular, neurological and autoimmune diseases as well as cancer [1]. The molecular mechanisms underlying toxic metal-induced diseases are complex and in many cases can be linked to oxidative stress and altered expression of genes in key cellular pathways [2]. Recent reports have demonstrated epigenetic alterations with toxic metal exposure [3–4, reviewed in 5].

Current research efforts have focused on increasing understanding of the importance of the epigenome and the role it plays in cellular homeostasis. Studies are also beginning to shed light on the notion that epigenetic alterations resulting from early-life exposures may play a major role in mediating the links between disease and the environment. This review focuses on toxic metal-induced dysregulation of DNA methylation, with particular attention given to imprinted genes. Herein, we highlight the effects of exposure to inorganic arsenic (iAs) and lead (Pb) on DNA methylation. We discuss how epigenetic alterations are being studied in newborns for development as biomarkers of *in utero* environmental toxicant exposure, and the benefits and challenges of this approach. Lastly, we provide a brief summary of unresolved questions regarding how arsenic and lead exposures alter epigenetic profiles in early life and the larger implications of these changes.

Developmental Origins of Disease and Epigenetics

The developmental origins of disease hypothesis postulates that altered maternal nutrition and/or endocrine status during prenatal development result in persistent changes in development, physiology, and metabolism, predisposing that individual to cardiovascular, metabolic and endocrine related diseases in adult life [6]. This is thought to reflect heightened vulnerability to environmental influences during specific periods of development. Human and animal studies support that prenatal growth is substantially influenced by the *in utero* environment.

Fetal programming is a phenomenon whereby a stimulus or insult during critical periods can have persistent effects on the structure, physiology and metabolism of that individual later in life [6]. Environmental exposures during early development can have lasting effects and epigenetic modifications play a vital role in the response to *in utero* environmental factors such as toxic exposures and nutritional factors. These exposures result in changes in regulatory and growth-related gene expression that are important components of fetal programming.

Epigenetics refers to modifications that occur “above the genome” that provide somatically heritable, stable regulatory information outside of the DNA sequence [7]. DNA methylation involves the addition of a methyl group to the 5-carbon position of the cytosine pyrimidine ring (5-methycytosine, [5-mC]) via DNA methyltransferases (DNMTs). Cytosines followed by guanines are the targets of DNA methylation, and most CpG dinucleotides are methylated throughout the genome. The exception to this is CpG islands, which are stretches of sequence that are densely populated with unmethylated CpGs. These CpG islands, and the

sequence immediately adjacent to CpG islands (CpG shores [8]); can exhibit altered methylation resulting from environmental influences and in disease. Some CpG islands are methylated differentially on the two chromosomal copies inherited from each parent; these are associated with genomically imprinted genes, discussed below. DNA methylation plays an important role in the regulation of transcription by either attracting or inhibiting the binding of transcriptional modulators. Changes in DNA methylation patterns are the most common alterations in cancers [9]. Complete reprogramming of DNA methylation occurs during gametogenesis and just after fertilization (when cell- and tissue-specific DNA methylation patterns are established) and these patterns can be modified during puberty and during the aging process [9]. DNA methylation patterns are likely most susceptible to environmental influences during the processes of methylation reprogramming and during maintenance methylation as cells prepare for division during which methylation profiles need to be faithfully recapitulated on the nascent DNA of the daughter cell. DNA synthetic rates are high during prenatal development and this may represent one of the most critical windows of vulnerability to environmental influences to the epigenome.

Toxic metal exposure and epigenetics

Arsenic

Arsenic (As) and Lead (Pb) are ranked number 1 and 2 on the Agency for Toxic Substances and Disease Registry (ATSDR) 2011 Substance Priority List [10]. This list ranks substances based on their toxicities and potential for human exposure at locations on the National Priorities List (NPL). Arsenic occurs naturally in the environment as an element of the earth's crust. When combined with other elements such as oxygen, chlorine and sulfur, As becomes an inorganic compound (iAs). Higher than average iAs is found in certain occupational settings, hazardous waste sites, and areas with high levels of naturally occurring iAs (soil, rocks and water). Drinking water contaminated with iAs is the major source of iAs exposure worldwide. The World Health Organization (WHO) recommends that iAs in drinking water not exceed 10 parts per billion, but it is estimated that more than 100 million people worldwide are exposed to levels of iAs in drinking water that are considered harmful to human health [11]. Although iAs exposure has been extensively studied and linked to numerous chronic conditions including cardiovascular disease, diabetes mellitus, neurological effects and cancers of the skin, lung, liver, and urinary bladder [12], the precise molecular mechanisms connecting exposure to disease are not well understood.

Evidence suggests that there are long-term health consequences of prenatal iAs exposure and that this may occur through epigenetic mechanisms. Arsenic exposure and effects on DNA methylation have been studied *in vitro*, *in vivo*, and within human populations, as reviewed in [13], yet the biological consequences of the observed changes have not been established. In addition, a causal relationship between iAs exposure, DNA methylation changes, and oncogenesis has not been established. The long-term health consequences associated with prenatal iAs exposure support that iAs may exert its effects through epigenetic mechanisms as it is not a mutagen. The effects of chronic iAs exposure and development of disease are more extensively described by Bailey and Fry [13].

Lead (Pb)

Pb is a naturally occurring metal and is a ubiquitous non-degradable toxic pollutant of the environment through natural and anthropogenic sources. Pb has both acute and chronic effects on human health. The most common sources of Pb exposure are inhalation of Pb-contaminated dust, ingestion of Pb-tainted food and/or water, and direct contact with Pb-polluted soil. As a result of the government-mandated removal of Pb from paints and gasoline in the US, it is less of a contamination hazard yet remains a threat to human health as it can still be found in many products including batteries, ceramics, toys, and plumbing pipes.

Within the US there remain populations at higher risk of Pb exposure based on age of housing and occupation. Children comprise the highest risk group because they are most vulnerable to Pb for several years following birth during brain and neurological development. They are also more sensitive to Pb poisoning because they have thinner skin which more easily absorbs Pb. Young children frequently place items in their mouth, which increases exposure if the items contain Pb or are contaminated with Pb from other sources [14]. The ability of Pb to freely cross the placental barrier and to be mobilized from maternal bone stores during pregnancy place the developing infant at risk of Pb exposure, where even low level exposure may be harmful [15]. Epidemiological studies provide compelling evidence that blood Pb levels below the current CDC action level (5 μg/dL) have detrimental effects on the developing brain. Pb exposure-related health outcomes have been most often studied in the field of neurological development and disease. Even at very low levels, prenatal Pb exposure results in poorer cognitive performance in boys [16]. The mechanisms by which prenatal Pb exposure compromises human development and leads to late-onset disease are not fully understood. There are few studies to date on the epigenetic effects of prenatal Pb exposure in humans. Pilsner et al. published the first human study in 2009 showing that cumulative measures of Pb in maternal bone were associated with changes in DNA methylation levels in the umbilical cord blood leukocytes of the offspring [17].

Toxic metals are widespread environmental contaminants and in recent years there has been increased interest in understanding the molecular factors involved in the etiology of metal-induced diseases (detailed review in [18]). There are several studies that show epigenetic alterations following environmental toxicant exposure, implicating epigenetic mechanisms as a potential link between exposure and later life disease.

DNA methylation and Genomic Imprinting

Approximately 90 genes in humans have been identified thus far that are subject to a unique form of gene regulation referred to as genomic imprinting, whereby only one of the two inherited parental alleles is functional. The other allele is permanently silenced in a parental origin-dependent manner by epigenetic mechanisms, including DNA methylation that is differentially established in sperm and egg. Methylation patterns in these regulatory regions of imprinted genes may be particularly susceptible to environmental effects [19].

Appropriate expression of imprinted genes is critical for normal growth and development. These genes are often organized in clusters in imprinted domains and are coordinately

regulated by imprinting control regions. Therefore disruption of epigenetic regulatory mechanisms at these regions can alter the imprinting and/or expression of multiple imprinted genes [20]. The DNA methylation patterns at the regulatory regions of imprinted genes are mitotically heritable [21]. In early development, the epigenetic state of imprinted genes is labile and can be influenced by environmental factors. Accumulating evidence supports the notion that early life is a critical window of vulnerability where there may be increased susceptibility to epigenetic dsyregulation at imprinted regulatory regions.

Investigating past environmental exposures in newborns

Based on the developmental origins of disease hypothesis, a likely target by which early-life environmental exposures cause late-onset disease is through epigenetic mechanisms, particularly during reprogramming of the epigenome that takes place during embryonic development. The epigenome is highly vulnerable to environmental insults during this period of development [22]. Understanding epigenetic events in early life and how environmental exposures impact fetal outcomes is important for elucidating molecular mechanisms and how the epigenome can be modified or manipulated to prevent later undesirable outcomes. The study of epigenetics and perinatal health is becoming increasingly important as the body of evidence grows supporting the idea that diverse environmental exposures can alter epigenetic programming and trans-generational risk of disease. Perinatal exposures to arsenic, tobacco smoke, air pollutants, and endocrine disrupting chemicals have all been shown to alter epigenetic profiles (reviewed in [23]).

Evidence of exposure in the prenatal environment

To date there are several studies that seek to better understand how environmental exposures *in utero* effect the epigenetic profile of the offspring. Individuals exposed to severe caloric restriction *in utero* have a higher incidence of several chronic adult diseases including type 2 diabetes, coronary heart disease, neurological disorders, obesity, and certain cancers [24]. One of the most profound findings of the Dutch Famine study is that the changes in methylation were still detectable six decades after caloric restriction occurred. This demonstrates the long term effects of the exposure, and also that the methylation change is stable over long periods of time, supporting the idea that historical exposure information is stably archived by the genome [25]. Studies investigating maternal nutrition have found that folic acid supplementation before or during pregnancy is associated with altered DNA methylation at two differentially methylated regions regulating imprinted Insulin-like Growth Factor 2 (*IGF2*) with males showing more prominent effects than females [26]. Additional work by our group has shown that maternal influences, including smoking status, body mass index, depressed mood and antibiotic use during pregnancy can result in altered DNA methylation profiles at imprinted gene regulatory regions in newborns. High prenatal iAs exposure and DNA methylation at LINE-1 repetitive elements are positively associated in both maternal and fetal leukocytes [27]. In addition, we have previously published on prenatal iAs exposure and effects on the epigenome in umbilical cord blood (UCB) [28]. Prenatal Pb exposure has been studied in the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENTS) cohort, in which maternal patella Pb is associated

with UCB LINE-1 methylation while maternal tibia Pb is negatively associated with UCB genomic DNA methylation of Alu repeats [17].

Benefits and Challenges in measuring exposures in newborns

Benefits

A better understanding of the epigenetic targets and mechanisms involved is required if we hope to prevent adult onset disease directly related to suboptimal conditions in the intrauterine environment. Studying epigenetic alterations in newborns from samples collected at the time of parturition provides the benefit of identifying changes that resulted from what was experienced *in utero.* It may also be possible to detect changes that were heritably transmitted through the gametes of the prior generation from these types of analyses. A benefit of measuring iAs and Pb exposure in cord blood is that these cells may be exposed to higher maternal blood levels of toxic metals. If calcium intake is not sufficient during pregnancy, maternal bone stores of toxic metals are often released into the blood stream due to the bone resorption process, thus exposing the infant to increased levels of toxic metals. Studying epigenetic profiles in newborns is an ideal study design to analyze tissues naiive to the *ex utero* environment and can provide information about the collective effects of the *in utero* environment on the infant's epigenome.

Challenges

There are considerable challenges associated with studying the effects of prenatal iAs and Pb exposure and epigenetic mechanisms in newborns. Non-cancer cohort studies investigating epigenetics in preclinical populations with specific environmental exposures have the unique challenge of relying on surrogate tissues (buccal swabs, cord blood, placentas, etc.), which may have variable epigenetic correlation with target tissues. Measuring iAs and Pb exposure in surrogate tissues presents challenges in terms of explaining the biological relevance of these effects without having access to target tissues (i.e. neurological tissues, liver, or kidney). *In vitro* studies have relied on cancer cell lines vs. normal target cells for studying epigenetic mechanisms of iAs and Pb exposure. An additional challenge faced in environmental exposure studies is the understanding of the relationship between DNA methylation, histone modifications, and miRNAs for specific gene targets and how alterations in these mechanisms together contribute to disease outcome. Rarely are all three epigenetic mechanisms studied concurrently and rarely have functional endpoints been assessed in relationship to the epigenetic modifications. Lastly, we do not expect to see large differences in methylation from *in utero* exposures; large differences would likely often not be compatible with viability [29], thus analysis requires technologies that are capable of detecting small differences in DNA methylation.

Conclusions and Future Directions

The studies highlighted in this review indicate that the epigenome is impacted by early life exposure to iAs and Pb. A causal relationship between toxicant-induced epigenetic alterations and disease development has not been established. More work is needed to understand causal effects of early life toxic metal exposure on epigenetic changes, and how

these changes result in onset/progression of various disease states. Much remains to be learned about the combined effects of toxicants on the epigenome.

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Important unanswered questions

- What is normal epigenetic variability in newborns, during puberty and with aging?
- At what point do methylation changes become an adversity?
- What is the interaction between the genome and epigenome in response to toxic metals on a genome-wide scale?
- How do iAs and Pb effect cellular plasticity?
- What proteins/enzymes respond to iAs and Pb exposure to drive epigenetic change?