DNA methylation changes induced by prenatal toxic metal exposure: An overview of epidemiological evidence

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Abstract

Accumulating evidence suggests that exposure to unfavorable conditions early in life can substantially contribute to the risk of chronic disorders later in life ('developmental programming' phenomenon). The mechanistic basis for this phenomenon remains poorly understood so far, although epigenetic mechanisms such as DNA methylation, histone modifications and microRNA-mediated gene regulation apparently play a crucial role. The key role of epigenetic modifications triggered by unfavorable environmental cues during sensitive developmental periods in linking adverse early-life events to later-life health outcomes is evident from a large body of studies, including methylome-wide association studies and research of candidate genes. Toxic metals (TMs), such as heavy metals, including lead, chromium, cadmium, arsenic, mercury, etc., are among environmental contaminants currently most significantly impacting human health status. Since TMs can cross the placental barrier and accumulate in fetal tissues, exposure to high doses of these xenobiotics early in development is considered to be among important factors contributing to the developmental programming of adult-life diseases in modern societies. In this mini-review, we summarize epidemiological findings indicating that prenatal TM exposure can induce epigenetic dysregulation, thereby potentially affecting adult health outcomes.

Key words: toxic metals; prenatal exposure; epigenetics; DNA methylation; developmental programming; chronic disease

Introduction

Currently, there is a trend toward a rise in the prevalence of chronic non-communicable disorders (NCDs) worldwide. This is because the world's population is rapidly aging, and burden of age-related disorders constitutes a major public health problem globally [1]. Adult-life lifestyle factors (sedentary behavior, westernized dietary patterns, etc.) obviously play a major role in the etiology of NCDs. However, accumulating evidence suggests that exposure to adverse environmental factors early in life can also substantially contribute to the pathogenesis of NCDs. To explain this phenomenon, the Developmental Origins of Health and Disease (DOHaD) concept has been postulated [2]. According to this concept, adverse environmental cues early in development may reprogram an individual for immediate adaptation to pre- and/or neonatal conditions. Such an adaptive strategy, however, can result in an increased risk of pathological conditions during adulthood, including type 2 diabetes (T2D), cardiovascular (CVD) and neurodegenerative disorders, as well as cancers [2]. The mechanistic basis for the phenomenon of 'developmental programming' of adult-life NCDs remains poorly understood so far, although epigenetic mechanisms such as DNA methylation,

histone modifications and microRNA-mediated gene regulation apparently play a crucial role. In mammals, epigenome (a complex profile of epigenetic marks across the entire genome) has been found to be especially susceptible to adverse environmental conditions during gametogenesis and embryogenesis [3]. This is because epigenome undergoes the most significant modification during these stages of development. Once established, epigenetic profiles are stable and are transmitted into subsequent generations of somatic cells and, in some cases, into germ cells [4]. On the stage of maturation of germ cells, a wave of the genome-wide DNA demethylation takes place followed by remethylation before fertilization. One more wave of DNA demethylation takes place after fertilization in early human embryogenesis, and previously erased methylation patterns are re-established after embryo implantation [3]. This process of demethylation and remethylation during a post-fertilization period is believed to play a central role in the removal of epigenetic marks acquired by the previous generation.

In humans, the window of plasticity is known to extend from pre-conception until early childhood ('First 1000 days' concept) [5]. Epigenetic modifications triggered by unfavorable environmental cues during this sensitive developmental period may persist

through many cell divisions and cause various pathological conditions later in life [5]. The key role of epigenetic modifications in linking adverse early-life events to later-life health outcomes is evident from a large body of methylome-wide association studies (MWAS) and studies of candidate genes that were performed during the last years [6].

In the past, famine-caused starvation and infections were the environmental factors most strongly affecting processes involved in early-life epigenetic (re)programming. Currently, due to industrial, traffic and agrochemical pollutions, exposure to xenobiotics (chemical substances foreign to biological systems, including humans) has more and more come to the fore. Toxic metals (TMs), such as heavy metals, are among environmental contaminants essentially influencing the health status of people [7, 8].

Exposure to TMs early in development is considered to be among important factors contributing to the developmental programming of adult-life diseases in modern societies [9]. The effects of TMs on epigenetic processes have been demonstrated in a large body of experimental *in vitro* and *in vivo* studies (for reviews, see refs. [10, 11]). They were found to be able to directly affect gene expression by binding particular metal response elements to target gene promoters [12]. More specifically, nickel can bind to chromatin, thereby causing its condensation, and chromium may crosslink chromatin-associated proteins to DNA [13]. Moreover, cadmium was shown to be able to promote global DNA hypermethylation by either stimulating activity of DNA methyltransferase (DNMT) or inducing its gene expression [14], and arsenite may deplete the main cellular methyl donor, S-adenosyl methionine, thereby causing DNA hypomethylation [15]. In addition, TMs may affect epigenetic pathways indirectly by inducing oxidative stress or disrupting cell metabolism [11].

The purpose of this mini-review was to summarize findings from epidemiological studies, indicating that prenatal TM exposure can induce epigenetic dysregulation at the DNA methylation level, thereby potentially affecting adult health outcomes. Since effects of developmental exposure to TMs on epigenetic mechanisms other than DNA methylation (such as histone modifications and regulation by non-coding RNAs) have not yet been comprehensively investigated in epidemiological studies, we will not discuss them here.

Toxic Metals and Human Health

Environmental pollution by TMs such as lead (Pb), chromium (Cr), cadmium (Cd), arsenic (As) and mercury (Hg) is currently a matter of high concern because of their adverse effects on human health, which is increasingly becoming a challenge for health-care systems globally [16]. These inorganic pollutants enter the environment and contaminate the soil and groundwater owing to rapidly growing metal and agricultural industries and also pesticide and fertilizer production [7]. Although all TMs are naturally occurring elements, long-term intake of large amounts of these metals and their compounds can result in health hazards. Mechanistically, these deleterious effects can be likely mediated by inducing oxidative/nitrosative stresses following the TM exposure. These stresses may result in damage to vital biological macromolecules, such as lipids, proteins and DNA, as well as in disrupting essential signaling pathways, which in turn can contribute to the pathogenesis of human disorders [16]. Indeed, binding of TMs to DNA and nuclear proteins has been shown to be a primary cause of oxidative deterioration of vital biomolecules. Moreover, TMs may lead to disrupted integrity of important cellular processes due to displacement of certain essential metals from their respective sites

[16]. The heavy metal–induced toxicity may lead to a decrease of energy levels and impaired functioning of the kidney, liver, brain, lungs and other vital organs [17]. As a result, these processes can initiate physical, muscular and neurological degenerative changes, thereby promoting the onset of diseases such as muscular dystrophy, Parkinson's disease, Alzheimer's disease, multiple sclerosis and cancer [7, 17].

From a DOHaD perspective, an important point is that TMs can cross the placental barrier, adversely affect placental functions and accumulate in fetal tissues. The placenta is known to act as a barrier that protects the growing fetus from external threats, including the potentially harmful chemical agents. The placental barrier is, however, not fully impenetrable. Indeed, relatively high concentrations of TMs have been detected in placental tissues, as well as in amniotic fluid and umbilical cord blood [18]. Evidence is also obtained that *in utero* exposures to lead and mercury pose a threat to health, particularly to the developing brain, while prenatal exposures to lead and cadmium are associated with both reduced birth size and birth weight [19]. Metal-specific placental transfer and impairment of placental functions were proposed to explain observed epidemiological associations between the prenatal exposure to TMs and the subsequent risk for developmental, endocrine and neurological disorders [18, 19].

There is compelling evidence that prenatal exposure to high doses of TMs is associated with adverse health outcomes in adulthood [7, 16, 17]. Recent findings indicate that these effects can be mediated by epigenetic modifications persisting throughout the life of affected offspring [20]. In the subsequent section, evidence from epidemiological studies is provided that epigenetic mechanisms may contribute to the link between prenatal exposure to TMs and adult health outcomes.

Epigenetics as a Mediator between Prenatal Heavy Metal Exposures and Adult Health
Cadmium

Cadmium is a toxic, non-essential metal causing adverse health outcomes such as renal toxicity, cardiovascular disease and cancer [21]. These health outcomes have been well established in adults, although some research findings indicate that developmental exposures to cadmium may result in an increased risk of developing chronic diseases later in life. Several research findings suggest that long-lasting adverse health consequences of prenatal cadmium exposure may be mediated by epigenetic mechanisms. Sex-specific association between *in utero* exposure to cadmium and adult cord blood DNA methylation levels has been reported [22]. In girls, such methylation changes have been observed primarily in genes responsible for organ development and also for the bone morphology and mineralization, whereas in boys, these changes have been shown mainly in genes related to cell death due to apoptosis (for more details, see Table 1). In another MWAS conducted in leukocytes from mother–baby pairs exposed to cadmium during pregnancy, distortions in DNA methylation levels were observed primarily in genes implicated in processes of apoptosis and transcriptional regulation [23]. Evidence was also provided that cadmium-triggered developmental toxicity can be associated with disrupted expression patterns of certain imprinted placental genes that exhibit monoallelic expressions based on the parent of origin. More specifically, exposure to higher cadmium concentrations *in utero* was related to higher levels of expression of the distal-less homeobox 5 (*DLX5*) gene and lower levels of expression of the imprinted maternally expressed transcript (*H19*) and necdin, MAGE family member (*NDN*) gene [24]. Maternal cadmium

Table 1: summary of epigenetic modifications caused by prenatal exposure to heavy metals

TM	Country	Age at detection	Tissue/cells	Gene/element	Epigenetic outcome	Ref.
Cadmium	Bangladesh ($n = 127$)	Mother– child pairs; children at 4.5 years of age	Mothers: blood Children: urine	MWAS	Differential DNA methylation	[22]
	USA ($n = 17$)	Mother–newborn pairs	Venous maternal blood; newborn cord blood	MWAS	Differential DNA methylation	[23]
	USA: New Hampshire, ($n = 326$); Rhode Island ($n = 211$)	At birth	Placenta	DLX5 H19 NDN	High expression Low expression Low expression	[24]
	USA ($n = 319$)	Mother–newborn pairs	Venous maternal blood; newborn cord blood	PEG3	Lowered DMR methylation in female offspring	[25]
Arsenic	Bangladesh ($n = 127$)	At birth	Mononuclear cells from cord blood	MWAS	Differential DNA methylation	[27]
	USA ($n = 134$)	At birth	White cord blood cells	MWAS	Differential DNA methylation	[28]
	Bangladesh ($n = 113$)	Mother–newborn pairs	Maternal and cord blood samples	LINE-1 p16	Higher methylation levels	[29]
	Bangladesh ($n = 101$)	Mother–newborn pairs	Cord blood	Alu, LINE-1, LUMA	Positive associations of methylation in males and inverse in females	[31]
	Taiwan ($n = 299$)	Mother–newborn pairs	Cord blood	MWAS	~60% of identified CpGs positively associated with arsenic exposure	[32]
	Thailand ($n = 32$)	At birth	Cord blood	CXL1 DUSP1 IER2 EGR-1 JUNB MIRN21 OSM PTGS2 SFRS5 SOC3 RNF149	Altered expression	[33]
	Belgium ($n = 183$)	Mother–newborn pairs	Cord blood	sFLT1	Higher expression in girls	[34]
	Mexico ($n = 38$)	Mother–newborn pairs	Cord blood leukocytes	16 genes	Changes in DNA methylation and messenger RNA expression	[35]
	Thailand ($n = 71$)	Mother–newborn pairs	Cord blood lymphocytes	p53	Increased promoter methylation	[36]
	Bangladesh ($n = 551$)	Mother– child pairs; 9-year-old children	Maternal and child urine	IGFBP3	Differentially methylated regions	[37]
Lead	Mexico ($n = 103$)	Neonate	Cord blood	Alu, LINE-1	Decreased methylation	[39]
	Mexico ($n = 48$)	Mother– child pairs	Cord blood	5-hmC	Differentially hydroxymethylated regions	[40]
	Mexico ($n = 89$)	Mother–child pairs	Umbilical cord blood leukocytes	15 gene pathways	Differentially methylated CpG sites	[41]
	USA ($n = 268$)	At birth ($n = 268$); mid-childhood ($n = 240$)	Cord blood Venous blood	DNHD1 CLEC11A	Differentially methylated regions	[42]
Mercury	USA ($n = 321$)	At birth ($n = 321$); early childhood ($n = 75$); mid-childhood ($n = 291$)	Cord blood Venous blood Venous blood	MWAS	Differentially Methylated regions	[44]
	USA ($n = 138$)	Mother–newborn pairs	Cord blood	MWAS	Lowered regional DNA methylation; associated changes of expression at PON1 gene	[45]

(continued)

Table 1: (Continued)

TM	Country	Age at detection	Tissue/cells	Gene/element	Epigenetic outcome	Ref.
	USA ($n = 306$)	At birth ($n = 306$); early childhood ($n = 68$); mid-childhood ($n = 260$)	Cord blood Venous blood Venous blood	MWAS	Lowered 5-hmC genomic content	[46]
	USA ($n = 141$)	At birth	Cord blood	ANGPT2 PRPF18 FOXD2 TCEANC2	Differentially methylated regions	[47]
	USA ($n = 192$)	At birth	Placental samples	EMID2	Hypomethylated loci	[48]
	Japan ($n = 67$)	At birth	Cord tissue	HDHD1	Increased body methylation	[49]
	Seychelles ($n = 406$)	Children at 7 years of age	Saliva	GRIN2B NR3C1	Increased methylation at CpG sites	[50]

exposure during early pregnancy was also associated with low birth weight and significantly lowered methylation level in the differentially methylated region (DMR) of the imprinted paternally expressed gene 3 (*PEG3*) in female offspring [25].

Arsenic

Arsenic is a potent toxicant and carcinogen. More than 100 million persons are currently exposed to high concentrations of this environmental pollutant, mostly via drinking water and industrial emissions [26]. Epidemiological studies conducted in regions with higher levels of arsenic in drinking water, such as several areas in Bangladesh and Chile, are indicative of an association between prenatal exposure to arsenic and risk for chronic disorders in adult life [26].

Epigenetic effects of prenatal exposure to arsenic are most thoroughly investigated among all TMs so far. Maternal arsenic levels early in gestation were associated with genome-wide methylation levels in cord blood of neonates; these effects were found to be more pronounced in boys than in girls [27]. Much stronger associations have been shown for arsenic exposure during early relative to late gestation. In the pathway analysis performed, overrepresentation of cancer-associated genes in boys, but not in girls, has been revealed. A linear dose-dependent relationship between *in utero* arsenic exposure levels and methylation at individual CpG loci in white cord blood cells was reported [28]. In both maternal and fetal leukocytes, exposure to high arsenic levels was positively associated with *LINE-1* methylation levels and, to a lesser degree, with methylation of CpG sites within a promoter region of the tumor suppressor gene *p16* [29]. Effects of prenatal arsenic exposure on DNA methylation levels in cord blood of newborns were found to be exposure-dependent; indeed, arsenic levels were shown to be associated with altered DNA methylation across the genome. This association, however, was largely attenuated after adjusting for leukocyte distribution [30]. Prenatal arsenic exposure may be associated with global methylation profiles in a sex-specific manner. Such differences between sexes were observed, e.g. in the study by Pilsner *et al.* [31] where impacts of *in utero* exposure to arsenic on global DNA methylation patterns were assessed either by genome-wide assays [methyl-incorporation and LUminometric Methylation Assay (LUMA)] or by repetitive element assays (*Alu* and *LINE-1*). In this study, associations between prenatal arsenic exposure and methylation levels evaluated by LUMA and also methylation of *Alu* and *LINE-1* repetitive elements were found to be negative among female newborns but positive among male newborns [31]. Significant associations

between intrauterine arsenic exposure and DNA methylation levels have been reported in a Taiwanese population [32]. Among all identified CpGs, about 60% have been shown to be positively related to arsenic exposure. Following functional annotation analysis of these *5′-C-phosphate-G-3′*, 17 pathways were identified including those associated with risk for T2D and CVD later in life. Consistent associations have been also observed in other independent cohorts [32].

The genome-wide impact of prenatal arsenic exposure on the newborn gene expression profiles was evaluated by microarray in cord blood cells [33]. In comparing transcripts from 21 newborns whose mothers were exposed to arsenic during pregnancy versus 11 newborns whose mothers were unexposed, about 450 differentially expressed genes were identified. In a network analysis of arsenic-modulated transcripts, activation of molecular networks related to stress, apoptosis, metal exposure and inflammation was demonstrated. Arsenic concentrations in maternal blood positively correlated to the expression of the soluble fms-like tyrosine kinase-1 (*sFLT1*) gene, playing an important role in the inhibition of placental angiogenesis and associated growth retardation, as well as several genes associated with DNA methylation and oxidative stress [34]. Moreover, genes that demonstrated most pronounced differences in DNA methylation and expression levels in newborn cord blood after prenatal exposure to arsenic were found to be enriched for binding sites of the early growth response (EGR) and CCCTC-binding transcription factors [35]. Evidence is also obtained that *in utero* exposure to arsenic can affect DNA methylation at the *p53* promoter region, thereby potentially promoting carcinogenesis [36].

In discussing the potential role of epigenetic mechanisms in mediating developmental programming effects of TMs, an important point is that prenatal arsenic exposure was shown to be able to cause persisting epigenetic modifications in affected offspring. In Bangladesh, prenatal maternal exposure to arsenic has been positively associated with concentrations of insulin-like growth factor-binding protein 3 (IGFBP3) in 9-year-old children [37]. It has been suggested that these associations can be epigenetically mediated. Specifically, altered methylation of 12 CpG sites mediated the association between prenatal arsenic exposure and IGFBP3 levels in these children [37].

Lead

Lead is a TM known to strongly affect human beings. Fetuses and young children were found to be especially susceptible to its neurotoxic effects because this metal is characterized by high gastrointestinal absorption and is able to penetrate the blood–brain

barrier [38]. Exposure to lead *in utero* has been repeatedly reported to be related to a hematopoietic toxicity and also to impaired neurological development and severe cognitive deficits in adulthood. Several studies conducted mainly in Mexican population have demonstrated a role of epigenetic mechanisms in mediating these associations [39–42]. In evaluating the impact of prenatal lead exposure on global *DNA* methylation levels (assessed by *Alu* and *LINE-1* repetitive elements) in cord blood leukocytes, an inverse dose-response relationship between lead concentrations in maternal patella and *LINE-1* methylation levels has been observed, whereas lead levels in maternal tibia have been found to be inversely related to *Alu* methylation levels [39]. An association between intrauterine exposure to lead and genome-wide changes in 5-hydroxymethylcytosine (5-hmC) profiles in CpG islands in cord blood cells was also reported [40]. Moreover, trimester-specific prenatal lead exposure–associated DMRs was found in umbilical cord blood leukocytes in 15 gene pathways related to a nodal signaling and neurological system processes and also involved in immunoglobulin binding, transmembrane receptor activity, detection of chemical stimulus, sensory perception and smell, and olfactory receptor activity [41]. In the United States, strong inverse associations with prenatal lead exposure were observed for methylation levels of CpGs in genes encoding Dynein heavy Chain Domain 1 (DNHD1), known to be highly expressed in human brain, as well as in genes encoding C-Type Lectin Domain Family 11, Member A (CLEC11A), a growth factor for primitive hematopoietic progenitor cells [42]. These epigenetic alterations have been found to be more pronounced in female than in male neonates. More recently, however, no association between prenatal lead exposure and cord blood methylation was observed [43]. Such differences in results could be likely attributed to differences in protocol, cohort characteristics, exposure levels, timing of exposure measurement, methods used for statistical analysis and so on.

Mercury

Prenatal exposure to another TM, mercury, was repeatedly shown to be associated with severe neurodevelopmental outcomes and with a lower cognitive performance in childhood [44]. Several studies showed that this association is mediated by epigenetic mechanisms. Prenatal mercury exposure has been associated with differential DNA methylation, mostly in CpG islands, in umbilical cord blood [45]. According to the authors, these changes were suggestive of altered immune profiles in exposed infants. Mercury-induced changes in methylation levels of Paraoxonase 1 (*PON1*) gene, which predict lower cognitive test scores, were shown to be able to persist into early childhood [44]. In addition, mercury exposure *in utero* has been related to lower global 5-methylcytosine content and respective increase in the ratio of 5-methylcytosine to 5-hmc in cord blood. These associations have been shown to persist into early but not into middle childhood [46]. Prenatal concentrations of mercury and methylated form of mercury (methylmercury) were both associated with changes in DNA methylation at several important genes, including Transcription Elongation Factor A N-Terminal and Central Domain Containing 2 (*TCEANC2*) gene, in newborns [47]. Associations between concentrations of infant toenail mercury, disturbed placental DNA methylation, e.g. in *EMID2* gene, and high-risk neurodevelopmental profiles in newborns have been also reported [48]. Association between mercury concentration in cord serum and DNA methylation in cord tissues was found to be sex-specific, at least for several genes. For example, in a recent study by Nishizawa-Jotaki *et al.* [49], methylated locus located in the intron of haloacid dehalogenase-like hydrolase domain-containing protein 1 (*HDHD1*) gene, encoding a binding site for the zinc finger protein CCCTC-binding factor, was shown to be positively correlated with mercury concentrations in cord serum in male, but not in female newborns. In another recent study, prenatal exposure to higher levels of methylmercury from maternal fish consumption resulted in epigenetic changes in offspring which have persisted at least until 7 years of age [50]. These changes were observed for certain nervous system–associated genes, including those encoding glutamate receptor subunit NR2B (*GRIN2B*) and glucocorticoid receptor (*NR3C1*). More specifically, exposure to higher levels of methylmercury *in utero* was associated with elevated methylation in particular CpG sites (located mostly in transcription factor binding sites) of these genes [50].

Conclusions and Perspectives

In conclusion, research evidence provided in this Perspective paper seems to be sufficient to conclude that prenatal exposure to environmental xenobiotics such as TMs can be an important factor contributing to developmental epigenetic programming of health status and disease profiles in modern human populations. Studies aimed at the identification of epigenetic pathways involved in these processes seem all the more important given the fact that current living conditions are very different from those constituted environmental context of human evolutionary history. In present-day environmental conditions, exposure to man-made environmental pollutants such as high-dose TMs is undoubtedly a factor significantly influencing genetically determined pathways of epigenetic regulation and thereby causing various pathological outcomes. Exposure to TMs *in utero* can be especially hazardous. This is because an organism is most sensitive to stressful events throughout prenatal and early postnatal periods [3]. Moreover, developmentally induced epigenetic modifications can persist long even after a transient environmental signal has disappeared, thereby enhancing the risk for future disorders, sometimes even in subsequent generations (due to the mechanism of transgenerational epigenetic inheritance) [51–55].

Currently, an exploration of the role of epigenetic processes in mediating long-term health effects of developmental exposure to TMs is in the focus of various research activities [56–58]. Multiple genes have been identified to date, which are involved in these associations. While significant progress has been made over recent years in this research field, many issues still remain to be addressed. Most evidence for epigenetic effects of prenatal TM exposures was obtained from newborn cord blood samples and, more rarely, from placental tissues [24, 48]. Long-term persistence of such effects beyond birth was reported only in few studies, and evidence that epigenetic changes represent a mechanism linking developmental toxic exposure to adverse health outcomes later in life is also limited to few studies that performed causal mediation analysis. The causality cannot be definitely inferred from epidemiological studies; therefore, further experimental studies are needed to elucidate the mechanistic basis for these effects.

In discussing mechanisms underlying effects of developmental exposures to TMs on DNA methylation, it is usually assumed that these effects can be mediated by a direct action of environmental chemicals on the enzyme families such as DNMT and ten-eleven translocation protein (enzyme families responsible for the DNA methylation and demethylation, respectively) [57]. Moreover, prenatal exposures to TMs can affect the availability of S-adenosylmethionine (the substrate required for DNA methylation). In addition, according to the transcription factor

occupancy theory, it is suggested that dysregulation of transcription factors by toxic environmental exposures can affect site-specific patterns of DNA methylation, because the presence or absence of transcription factors on DNA allows or denies access to the DNA methylation machinery. More specifically, binding of certain transcription factors could inhibit DNMT from accessing DNA for methylation of particular genes, thereby causing gene-specific hypomethylation, while a lack of transcription factor binding might allow DNMT access to certain genomic locations, thereby leading to gene-specific hypermethylation [57].

One potential challenge is that specific epigenetic patterns are characteristic for certain cell types and also for particular cellular signaling pathways. Therefore, the focus of further research activity probably should have been shifted from the research of single candidate genes to investigating candidate gene pathways known to be epigenetically labile and react in a specific manner in response to specific environmental triggers. Characterization of these gene pathways by performing studies integrating all the potentially hazardous TM exposures *in utero*, epigenetic modifications induced by these exposures and resulting adult health outcomes would provide an opportunity to further elucidate epigenetic mechanisms mediating these associations. An important issue in studying long-term epigenetic changes is that such changes can significantly vary in sign and magnitude in various adult body tissues [59]. This is an essential challenge, given the difficulty of obtaining biological samples in human research. Today, only samples from peripheral blood or buccal swabs were used in such studies. A study of postmortem samples could provide such an opportunity [60]. However, to our knowledge, such studies have not been carried out to date. Thus, performing studies in animal models is the only reasonable alternative now in further elucidating such inter-tissue differences. Such an approach, in turn, raises the question on the specificity of these pathways across species and, more specifically, on similarities or differences between such pathways in experimental animals and humans. In addition, the process of developmental programming can be largely influenced by interplays between environmental factors operating in fetal and early postnatal life [61, 62]. Furthermore, since epigenome remains sufficiently labile and capable of responding to environmental factors during adulthood as well (although to a lesser extent than during early development) [3], the potential role of stressful events in adult life also needs to be taken into account. Given the complexity of potentially interacting factors influencing these processes, choosing the right study design is an important issue. In particular, cross-sectional approach, which is most commonly used in epigenetic epidemiology now, allows only limited inferences about causal relationships between early-life risk factors and later health outcomes. Therefore, longitudinal cohort design will be likely the approach of choice in future epigenetic epidemiological studies, including those aimed at appraising long-term health outcomes of early-life exposures to TMs.

The importance of these studies lies in the fact that, since developmental epigenetic modifications are potentially reversible [3], a better understanding of mechanisms contributed to effects of adverse *in utero* exposures may provide an opportunity for developing novel therapeutic approaches aimed at correction of developmentally disrupted epigenetic profiles. One promising direction in this area is the development of new drug classes (so-called "epigenetic drugs") [63]. Such drugs, include, among others, pharmacological agents targeted at enzymes involved in epigenetic regulation, such as inhibitors of DNMTs and histone deacetylases [64], and these pharmaceuticals are under active investigation and clinical implementation now.

Data Availability

Data might be available under request to corresponding author.

Funding

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