Epigenetics and environmental chemicals

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Purpose of review

Epigenetics investigates heritable changes in gene expression occurring without changes in DNA sequence. Several epigenetic mechanisms, including DNA methylation, histone modifications, and microRNA expression, can change genome function under exogenous influence. Here, we review current evidence indicating that epigenetic alterations mediate toxicity from environmental chemicals.

Recent findings

In-vitro, animal, and human investigations have identified several classes of environmental chemicals that modify epigenetic marks, including metals (cadmium, arsenic, nickel, chromium, and methylmercury), peroxisome proliferators (trichloroethylene, dichloroacetic acid, and TCA), air pollutants (particulate matter, black carbon, and benzene), and endocrine-disrupting/reproductive toxicants (diethylstilbestrol, bisphenol A, persistent organic pollutants, and dioxin). Most studies conducted so far have been centered on DNA methylation, whereas only a few investigations have studied environmental chemicals in relation to histone modifications and microRNA.

Summary

For several exposures, it has been proved that chemicals can alter epigenetic marks, and that the same or similar epigenetic alterations can be found in patients with the disease of concern or in diseased tissues. Future prospective investigations are needed to determine whether exposed individuals develop epigenetic alterations over time and, in turn, which such alterations increase the risk of disease. Also, further research is needed to determine whether environmental epigenetic changes are transmitted transgenerationally.

Keywords

DNA methylation, environment, epigenetics, histone modification, microRNA

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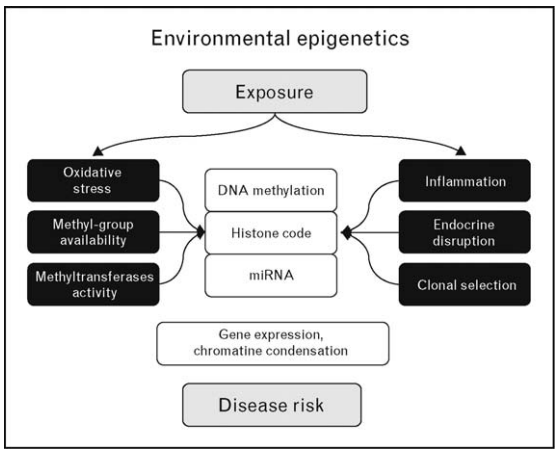
Introduction

Identifying the effects of environmental exposures on human health is a major objective of life sciences and biomedical research. In environmental health, the recognition that exposures could produce DNA mutations represented a major landmark for risk assessment and prevention [1]. Consequently, chemical agents have been categorized according to their capability to alter the DNA sequence. Such information has been fundamental to determine environmental risks and shape current regulatory efforts for exposure reduction [2]. Recent evidence suggests that the molecular influence of the environment may extend well beyond the interaction with the DNA sequence [3,4]. Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence [5]. Epigenetic mechanisms are flexible genomic parameters that can change genome function under exogenous influence. Here, we review current evidence indicating that epigenetic mechanisms can mediate the toxicity of environmental chemicals.

Overview of epigenetic mechanisms

The current field of epigenetics includes a number of mechanisms, including DNA methylation, histone modification, and microRNAs (miRNAs) [6,7] (Fig. 1). DNA methylation is a covalent modification, heritable by somatic cells after cell division. 5-Methyl-cytosine (5MeC) represents 2–5% of all cytosines in mammalian genomes and is found primarily on CpG dinucleotides [8]. DNA methylation is involved in regulating many cellular processes, including chromatin structure and remodeling, X-chromosome inactivation, genomic imprinting, chromosome stability, and gene transcription [9,10]. Generally, gene promoter hypermethylation is associated with decreased expression of the gene [11]. However, more than 90% of all genomic 5MeCs are not directly related to gene function, as they lie on CpG

Figure 1 Potential mechanisms linking environmental exposures to epigenetic effects



Environmental chemicals may modify multiple biological processes that affect epigenetic mechanisms, including DNA methylation, histone codes, and miRNA expression. These changes may, in turn, modify chromatin organization and condensation, gene expression, and affect disease risk. miRNA, microRNA.

dinucleotides located in transposable repetitive elements, also referred to as transposons [12]. Alu and long interspersed nuclear element-1 (LINE-1) are the most common and well characterized transposable sequences, and measurements of Alu and LINE-1 methylation have been used to estimate global genomic DNA methylation content [12]. Global hypomethylation, as well as hypomethylation of transposable repetitive elements, have been associated with reduced chromosomal stability and altered genome function [13,14].

Histones are globular proteins that undergo posttranslational modifications that alter their interaction with the DNA and other nuclear proteins [15]. H3 and H4 histones have long tails protruding from the nucleosome, which can be covalently modified by acetylation, methylation, ubiquitination, phosphorylation, sumoylation, citrullination, and ADP-ribosylation, and thus influence chromatin structure and gene expression (Fig. 2).

miRNAs are single-stranded RNAs of approximately 21–23 nucleotides in length that are transcribed from DNA but not translated into proteins (noncoding RNAs). Mature miRNAs are partially complementary to one or more mRNA molecules. The main function of miRNA is to downregulate gene expression by interfering with mRNA functions (Fig. 3) [16,17].

Epigenetics and the environment

Some environmental factors have been linked to aberrant changes in epigenetic pathways both in experimental and epidemiological studies. In addition, epigenetic mechanisms may mediate specific mechanisms of toxicity and responses to certain chemicals. Whereas mechanisms of action of some of these agents are understood, for others the mode of action remains to be completely elucidated [18]. Because these epigenetic changes are small, potentially cumulative, and they may develop over time, it may be difficult to establish the cause–effect relationships among environmental factors, epigenetic changes, and diseases. Below, we review the effects of individual environmental toxicants on epigenetic mechanisms, as derived from in-vitro, animal, or human studies, which we summarize in Table 1 (effects on DNA methylation) [19–37], Table 2 (effects on histones modifications) [38–43], and Table 3 (effects on miRNA) [18,44].

Metals

Several studies [45–47] have established an association between DNA methylation and environmental metals, including nickel, cadmium, lead, and particularly arsenic. Metal-induced oxidative stress may represent a unifying process to account for these findings across different metals [48]. Metals are known to increase production of reactive oxygen species (ROS) in a catalytic fashion via redox cycling [49,50]. Oxidative DNA damage can interfere with the ability of methyltransferases to interact with DNA [51], thus resulting in a generalized altered methylation of cytosine residues at CpG sites [52].

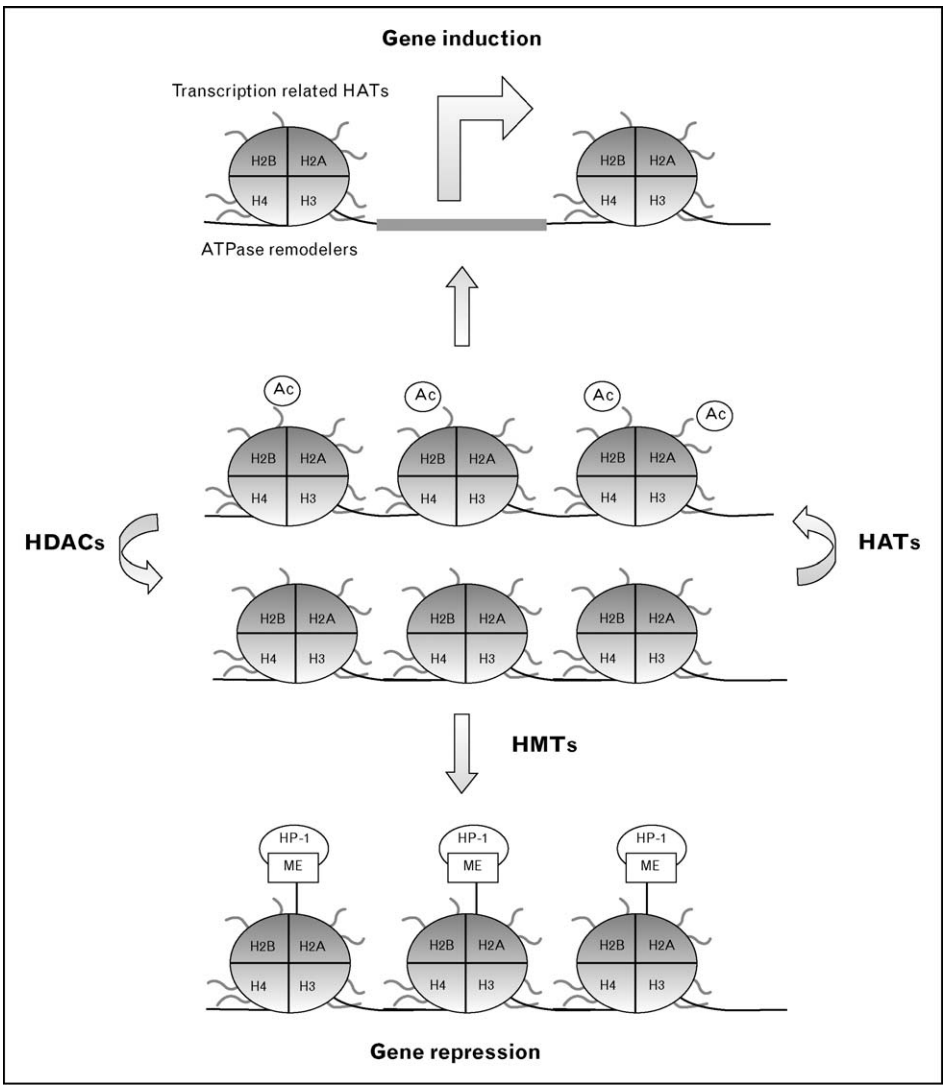
Cadmium

Cadmium is an established carcinogen that has very low mutagenicity [53]. Many possible mechanisms of cadmium carcinogenesis have been suggested and, among them, induction of ROS and alteration of DNA methylation seem to play a predominant biological role [54]. Takiguchi *et al.* [26] showed that cadmium reduces genome methylation, inhibiting DNA methyltransferases in a noncompetitive manner. This finding is suggestive of interference in enzyme–DNA interaction, possibly through an interaction of cadmium with the methyltransferase DNA-binding domain [26]. Cadmium can also inhibit DNA methylation in proto-oncogenes inducing oncogene expression and resulting in cell proliferation [54,26].

Arsenic

Arsenic is an established carcinogen that lacks carcinogenicity in animal models. Inorganic arsenic is enzymatically methylated for detoxication, using up *S*-adenosyl methionine (SAM) in the process. The observation that DNA methyltransferases also require SAM as their methyl donor suggested a role for DNA methylation in arsenic carcinogenesis and other arsenic-related effects [19]. In rat-liver epithelial cell lines treated with chronic low arsenic doses, Zhao *et al.* [19] showed malignant transformation associated with

Figure 2 Example of possible mechanisms of gene regulation by histone modifications



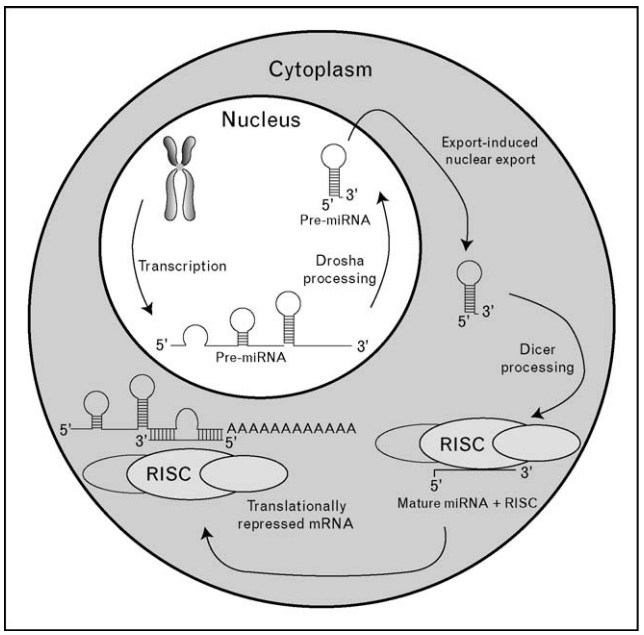
Modifications under HMTs, HATs, and HDACs control gene expression. HMTs add MEs that may interact with HP1. HATs and HDACs determine the addition and removal of acetyl groups (Ac). Modifications may generate a structure that contains bromo-domains and chromo-domains allowing the recruitment of ATP-dependent chromatin remodeling factors to open the chromatin. Effects of histone modifications on gene expression are dependent on the specific position of the chemical modification on the histone subunit. HATs, histone acetyltransferases; HDACs, histone deacetylases; HMTs, histone methylases; HP1, histone protein 1; MEs, methyl groups.

depressed SAM levels, global DNA hypomethylation, and decreased DNA methyltransferase activity. Following these findings, several studies [20–22,55] have shown that arsenic is associated with gene-specific hypermethylation as well as global DNA hypomethylation [56–58]. An unexpected finding was recently reported *in vivo*, as a global dose-dependent hypermethylation of blood DNA was observed in Bangladeshi adults with chronic arsenic exposure [23]. This effect was modified by folate, suggesting that arsenic-induced increases in DNA methylation were dependent on methyl availability [23]. The same group, however, reported that lower blood DNA methylation was a risk factor for arsenic-induced skin lesions in a related Bangladeshi population [24].

In a human study [22] from India, significant DNA hypermethylation of p53 and p16 promoter regions was observed in blood DNA of individuals exposed to toxic levels of arsenic compared with controls. In this study, hypermethylation showed a dose-response relationship with arsenic measured in drinking water.

Arsenic toxicity has been recently related to changes in miRNA expression. Marsit *et al.* [18] showed alterations in miRNA profiles of human lymphoblastoid cells grown

Figure 3 MicroRNA processing and activity



miRNAs are initially transcribed by RNA polymerase II and expressed as a part of pri-miRNAs. The miRNA portion of the pri-miRNA transcript likely forms a hairpin with signals for dsRNA-specific nuclease cleavage. The dsRNA-specific ribonuclease Drosha digests the pri-miRNA in the nucleus to release hairpin that is called premiRNA (approximately 70 nucleotides). Exportin-5 exports premiRNAs from the nucleus to the cytoplasm, in which Dicer cleaves the premiRNA approximately 19 bp from the Drosha cut giving a mature miRNA. Each mature miRNA is complementary to a part of one or more mRNAs. The annealing of the miRNA to the mRNA(s) inhibits protein translation. bp, base pair; dsRNA, double-stranded RNA; miRNA, microRNA; pri-miRNAs, primary miRNAs; RISC, RNA-induced silencing complex.

under sodium arsenite treatment. Interestingly, arsenic altered expression of specific miRNAs that were involved in one-carbon metabolism [18].

Nickel

The mechanisms underlying nickel health-related effects, including carcinogenicity and cardiorespiratory disease, are still largely unknown. It has been proposed that nickel may replace magnesium in DNA interactions, enhance chromatin condensation, and trigger de-novo DNA methylation [27]. In Chinese hamster G12 cells transfected with *Escherichia coli gtp* gene, Lee *et al.* [27] demonstrated nickel-induced hypermethylation leading to the inactivation of the expression of the transfected gene.

Several studies [38] have shown that nickel affects histone modifications. Exposure to soluble $NiCl_2$ has been shown to reduce histone acetylation, increase demethylation of H3K9, and increase monoubiquitination of H2A and H2B *in vitro*. Broday *et al.* [39] studied nickel effects, at nontoxic levels, on yeast and mammalian cells and found a decrease in histone H4 acetylation, affecting only lysine 12 in mammalian cells and all of the four H4 lysines in yeasts.

Nickel ion exposure has been shown to increase global H3K9 monomethylation and dimethylation, both of which have been associated with increased DNA methylation and long-term gene silencing. Nickel ions also interfere with the removal of histone methylation *in vivo* and directly decrease the activity of a Fe(II)-2-oxoglutarate-dependent histone H2K9 demethylase in nuclear extract *in vitro* [40]. In human lung cells exposed to soluble nickel compounds, three major changes in histone modifications have been observed: loss of acetylation of H2A, H2B, H3, and H4; increased H3K9 dimethylation; and increased ubiquitinylation of H2A and H2B [28,38–42].

It has been proposed that the binding of Ni^{2+} is able to promote a secondary structure with organized side-chain orientation on the N-terminal tail of histone H4. Acetylation of lysine 12 and 16 in yeast exposed to nickel was more robustly affected than lysine 5 and 8. Nickel binding to histidine 18 in histone H4 may be accountable for this effect, acting as an anchoring binding site for metal ions [59].

Chromium

Kondo *et al.* [29] investigated p16 methylation using a methylation-specific PCR method in 30 lung cancer cases associated with chromate exposure and 38 nonchromate lung cancers. A variety of genetic changes in lung cancers from chromate-exposed individuals are known, but the epigenetic effects of chromium are still poorly understood. Kondo *et al.* [29] showed that chromate exposure influenced p16 hypermethylation measured in lung cancer tissues compared with tissues from nonchromate lung cancer. Chromium has been shown to reduce in-vitro H3 phosphorylation and trimethylation, as well as various acetylation marks in H3 and H4 [60].

Methylmercury

Methylmercury is an environmental contaminant and a potential neurotoxic agent that may be present at high levels in seafood. Perinatal exposure to methylmercury causes persistent changes in learning and motivational behavior in mice. Developmental exposure to low levels of methylmercury induces epigenetic suppression of brain-derived neurotrophic factor (*BDNF*) gene expression in the hippocampus and predisposes mice to depression [30].

Trichloroethylene, dichloroacetic acid, and TCA

Trichloroethylene (TCE), dichloroacetic acid (DCA), and TCA are environmental contaminants that are peroxisome proliferators and carcinogenic in mouse liver. Decreased methylation in the promoter regions of the *c-jun* and *c-myc*

Table 1 Effects of environmental chemicals on DNA methylation

Exposure	↑/↓*	Genes	Type	Tissue	References
Arsenic	↓	Global	Rat	Liver	Zhao *et al.* [19]
	↑	*p53*	*In vitro*	A549 cells	Mass and Wang [20]
	↑↓	Multiple genes	*In vitro*	Human kidney cells	Zhong and Mass [21]
	↑	*p16*, *p53*	Human	PBL	Chanda *et al.* [22]
	↑	Global	Human	PBL	Pilsner *et al.* [23,24]
	↑	*p16*	Human	PBL	Zhang *et al.* [25]
Cadmium	↓	Global	*In vitro*	Rat liver cells	Takiguchi *et al.* [26]
Nickel	↑	*ATF-1*, *HIF-1*, *Rb*	*In vitro*	G12 cell line	Lee *et al.* [27]
	↑	*p16*	Mouse	Histiocytomas	Govindarajan *et al.* [28]
Chromium	↑	*p16*	Human	Lung	Kondo *et al.* [29]
Methylmercury	↑	*BDNF*	Mouse	Hippocampus	Onishchenko *et al.* [30]
TCE, DCA, TCA	↑	*c-jun*, *c-myc*	Mouse	Liver	Tao *et al.* [31]
Air pollution	↓	Global (*Alu*, *LINE-1*)	Human	Buffy coat	Tarantini *et al.* [32]
	↓	*iNOS*			
Benzene	↓	Global (*Alu*, *LINE-1*)	Human	Blood	Bollati *et al.* [33]
	↑	*p15*			
	↓	*MAGE*			
Vinclozolin	↑	Gene-specific	Ray	Testis	Anway *et al.* [34]
DES	↓	Global	Mouse	Uterus	Li *et al.* [35]
BPA	↓	Agouti gene, *CabpIAP*	Mouse	Embryo	Dolinoy *et al.* [36]
POPs	↓	*Alu*, *LINE*	Human	Blood	Rusiecki *et al.* [37]

ATF-1, activating transcription factor 1; *BDNF*, brain-derived neurotrophic factor; BPA, bisphenol A; DCA, dichloroacetic acid; DES, diethylstilbestrol; *HIF-1*, hypoxia-inducible factor-1; *LINE-1*, long interspersed nuclear element-1; *MAGE*, melanoma antigen-1; PBL, peripheral blood leukocyte; *Rb*, retinoblastoma; TCE, trichloroethylene.
* Increase (↑) or decrease (↓) in DNA methylation.

Table 2 Effects of environmental chemicals on histones

Exposure	↑/↓*	Modification	Type	Tissue	References
Nickel	↓	Acetylation	*In vitro*	Liver, brain	Ke *et al.* [38]
	↑	H3K9 dimethylation			
	↑	H2A and H2B monoubiquitination			
	↓	H4K12 acetylation	*In vitro*	Yeast cells	Broday *et al.* [39]
	↓	H4K4 acetylation	*In vitro*	Mammalian cells	
	↑	H3K9 monomethylation and dimethylation	*In vitro*	G12 cell line	Chen *et al.* [40]
	↓	Acetylation of histone H2B	*In vitro*	HAE and NRK cell lines	Golebiowski and Kasprzak [41]
	↓↑	H2B ubiquitination	*In vitro*	HAEo and HPL1D cell lines	Karaczyn *et al.* [42]
Arsenic	↑	H3K9 dimethylation	*In vitro*	A549 cell line	Zhou *et al.* [43]
	↓	H3K27 trimethylation			
	↑	H3K4 trimethylation			

HAE, human airway epithelial; NRK, normal rat kidney.
* Increase (↑) or decrease (↓) in histone modification.

genes and increased levels of their mRNAs and proteins were found in livers of mice exposed to TCE, DCA, and TCA. Methionine supplement prevented both the decreased methylation and the increased levels of the mRNAs and proteins of the two proto-oncogenes [31]. This work supported the hypothesis that these carcinogens may act by depleting the availability of SAM, whereas methionine would prevent DNA hypomethylation by maintaining adequate SAM levels [31].

Air pollution

Exposure to air pollution, particularly to particulate matter, has been associated with increased morbidity and mortality from cardiorespiratory disease, as well as with lung cancer risk [61–65]. In a human study, we recently investigated the effects of particulate matter exposure on global (estimated through Alu and LINE-1 repeated elements) and gene-specific methylation in workers of a steel plant with well characterized exposure

Table 3 Effects of environmental chemicals on microRNA

Exposure	↑/↓*	Genes	Type	Tissue	References
RDX	↓	Tumor-suppressing miRNAs	Mouse	Liver, brain	Zhang and Pan [44]
	↑	Oncogenic miRNAs			
Arsenic	↓↑	Multiple miRNAs	*In vitro*	Lymphoblastoid cells	Marsit *et al.* [18]

miRNAs, microRNAs; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine.
* Increase (↑) or decrease (↓) in microRNA expression.

to particulate matter with aerodynamic diameters of less than 10 μm (PM_{10}). Inducible nitric oxide synthase (iNOS) promoter methylation was significantly lower in postexposure blood samples compared with baseline [32]. Long-term exposure to PM_{10} was negatively associated with methylation in both Alu and LINE-1. In a recent investigation, we showed that exposure to black carbon, a marker of traffic particles, was also associated with decreased DNA methylation in LINE-1 measured in 1097 blood DNA samples from the Normative Aging Study (NAS), a repeated measure investigation of elderly men in the Boston area. As global DNA hypomethylation has been found in patients with cancer [66] or cardiovascular disease [67], such changes may reproduce epigenetic processes related to disease development and represent mechanisms by which particulate air pollution affects human health [68].

In an animal study, Yauk *et al.* [69] showed that sperm DNA of mice exposed to steel plant air was hypermethylated compared with control animals, and this change persisted following removal from the environmental exposure. This finding calls for further research to determine whether air pollutants produce DNA methylation changes that are transmitted transgenerationally.

Benzene

In a recent study, we investigated whether DNA methylation changes are induced by low-benzene exposure in peripheral blood DNA of gasoline station attendants and traffic police officers. High-level exposure to benzene has been associated with increased risk of acute myelogenous leukemia (AML) [70], which is characterized by aberrant global hypomethylation and gene-specific hypermethylation/hypomethylation. In our study, airborne benzene exposure was associated with a significant reduction in global methylation measured in LINE-1 and Alu. Airborne benzene was also associated with hypermethylation in p15 and hypomethylation of the melanoma antigen-1 (*MAGE-1*) cancer-antigen gene [33]. These findings show that low-level benzene exposure may induce altered DNA methylation reproducing the aberrant epigenetic patterns found in malignant cells.

Hexahydro-1,3,5-trinitro-1,3,5-triazine

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a common environmental pollutant resulting from military and civil activities that has been associated with neurotoxicity, immunotoxicity, and increased risk of cancer [71]. Zhang and Pan [44] evaluated the role of RDX in modifying miRNA expression in mouse liver and brain, as measured by miRNA expression microarrays. Several miRNAs were found to be differentially expressed in exposed mice, with specific miRNA expression profiles in gene pathways related to cancer, toxicant-metabolizing enzymes, and neurotoxicity [44].

Endocrine-disrupting chemicals and reproductive toxicants

Developing organisms are extremely sensitive to perturbation by endocrine-disrupting chemicals with hormone-like activity. In mammal germ cells and preimplantation embryos, DNA methylation undergoes two distinct cycles of demethylation/remethylation in which methylation patterns are reprogrammed genome wide, and cells with a broad developmental potential are generated [10]. Available evidence from animal models indicates that exposure to xenobiotics during critical periods of mammalian development may induce persistent and heritable changes of epigenetic states.

Diethylstilbestrol

Diethylstilbestrol (DES) is a developmental stage-specific nongenotoxic carcinogen that was used in the past to prevent miscarriages in pregnant women [72]. In mice models, prenatal and neonatal DES exposure causes a wide range of gene expression changes. Exogenous estrogen treatment results in persistent expression of certain genes, including lactoferrin, epidermal growth factor, and proto-oncogenes such as *c-fos*, *c-jun*, and *c-myc* [73–76]. Xie *et al.* [77] have provided data supporting an indirect relationship between exogenous estrogen and methylation changes, showing that estrogen inhibited catechol-*O*-methyltransferase (COMT) gene transcription. COMT is a ubiquitous enzyme catalyzing the transfer of the methyl groups from SAM to one of the hydroxyl groups of catechols in presence of Mg^{2+}. Inhibition of this enzyme results in inhibition of the methylation process [77].

Bisphenol A

Bisphenol A (BPA) is a chemical with estrogenic properties that is present in many commonly used products including food and beverage containers, baby bottles, and dental composites. Dolinoy *et al.* [36] used a rat exposure model to show that maternal exposure to BPA modified methylation of the metastable loci A^{vy} and $Capb^{IAP}$. Interestingly, this effect on DNA methylation and the associated change in coat color of the exposed animals were prevented by maternal dietary supplementation, with a source of methyl group such as folic acid or with the phytoestrogen genistein [36].

Persistent organic pollutants

Rusiecki *et al.* [37] evaluated the relationship between plasma persistent organic pollutant (POP) concentrations and blood global DNA methylation estimated in Alu-repeated elements in 70 Greenlandic Inuit, a population presenting some of the highest reported levels of POPs worldwide. In this work, a significant inverse linear relationship was found for dichloro-diphenyl-trichloroethane (DDT), dichloro-diphenyl-dichloroethylene

(DDE), β-benzenehexachloride (β-BHC), oxychlordane, α-chlordane, mirex, several polychlorinated biphenyls (PCBs), and total of all POPs [37].

Dioxin

Dioxin toxicity is mediated by the aryl hydrocarbon receptor (AhR) [78–80] and appears to require altered transcription of target genes [79]. Moffat *et al.* [81] hypothesized that miRNAs might be responsible for this mRNA downregulation in dioxin/AhR-related pathways, but using two different miRNA array platforms and real time PCR, they just found a few changes of small entity in miRNA levels, thus indicating a limited role of miRNA in dioxin toxicity [81].

Potential roles of environmental epigenetic effects in determining transgenerational risks and fetal origins of diseases

In animal studies, several chemicals including alloxan [82], cyclophosphamide [83], orthoaminoasotoluol [84], benzopyrene [85], DES [86], and vinclozolin [34] have been reported to induce transgenerational phenotypic effects. Transgenerational transmission of chemically induced epigenetic changes has been suggested as a potential mechanism for these effects. Anway *et al.* [34] showed that gestational exposure of female rats to the endocrine disruptor vinclozolin at the time of gonadal sex determination caused a variety of abnormalities in the offspring that were then transmitted down the male line for at least three generations. The high incidence of the defects (approximately 90% of all males in all generations) and the absence of abnormalities when passed down the female line suggested gametic epigenetic inheritance. In this study, altered DNA methylation in two candidate genes was seen in sperm from vinclozolin-exposed males, and these abnormal methylation patterns were inherited. These results indicate that exposure of germ cells, possibly at a specific developmental stage, is necessary to produce heritable epigenetic changes. In addition, epigenetic mechanisms may underlie the effects of in-utero and early-life exposures on adult health, as in-utero/early-life exposures to epigenetically active chemicals may produce health effects later in life, even independently of environmental risk factors in adults [87]. As reported in the sections above, most of the studies on epigenetic effects of environmental chemicals have shown changes in DNA methylation, histone modifications, or miRNA in somatic cells of adult individuals. Whether epigenetic changes observed in somatic cells are correlated with germline epigenetic changes is uncertain. Environmentally induced epigenetic somatic alterations may be sufficient to cause anomalies in biological functions, but these changes are not heritable *per se* and may not be associated to any transgenerational risk.

Conclusion

In the last few years, several investigations have examined the relation between exposure to environmental chemicals and epigenetics and identified several toxicants that modify epigenetic marks. Most of the studies conducted so far have been centered on DNA methylation, whereas only a few recent investigations have studied the effects of environmental chemicals on histone modications and miRNA. Epigenetics holds substantial potential for developing biological markers to predict which exposures would put exposed individuals at risk and which individuals will be more susceptible to develop disease. In human studies, this will require the use of laboratory methods with enhanced precision and sensitivity, so that epigenetic changes can be detected as early as possible and well ahead of disease diagnosis. It is worth noting that several human studies have investigated the effects of environmental exposures on tissues such as blood that are easy to obtain, but that, as effects of environmental chemicals may be tissue or even cell specific, do not necessarily represent epigenetic patterns in the target tissues. For several exposures, however, it has been proved that chemicals can alter epigenetic marks, and that the same or similar epigenetic alterations can be found in patients with the disease of concern or in diseased tissues. At the current stage, the missing link is to determine whether environmentally induced epigenetic alterations are part of the causative pathways that lead to the disease development. Future prospective investigations are needed to determine whether exposed individuals develop epigenetic alterations over time and, in turn, whether such alterations increase the risk of disease.

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