Neurotoxicology and Teratology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Review article

Epigenetic mechanisms in developmental neurotoxicity

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A B S T R A C T

The constant interplay between environment (including both exogenous and endogenous factors) and epigenome (defined as the combination of chromatin, its covalent modifications and noncoding RNAs) triggers epigenetic events that, by modulating gene expression, capture information about changes in the environment. In this mini review, we will focus on the neurodevelopmental implications of exposure to adverse prenatal milieu with emphasis on mechanistic and functional aspects.

Several neurotoxic insults have been shown to affect epigenetics with negative consequences on the development of the nervous system; among them are methylmercury, lead, arsenic and cadmium, as well as excess of glucocorticoids. Further investigations on the individual susceptibility to epigenetic changes are needed to propose and validate such modifications as possible biomarkers for early identification of neurological/neurodevelopmental disorders and for predicting/monitoring response to treatment.

1. Introduction

Prenatal adverse events, including maternal stress and exposures to neurotoxic agents, can affect brain development leading to increased risk for neurological disorders later in life (Harris and Seckl, 2011; Hashimoto-Torii et al., 2014; Tran and Miyake, 2017). Growing evidence indicates that the long-term effects of prenatal insults are related to disruption of the epigenome and these effects seem to underlie the developmental origins of vulnerability to nervous system disorders (Harris and Seckl, 2011; Kundakovic and Jaric, 2017; Perera and Herbstman, 2011; Tran and Miyake, 2017).

During early development the epigenome is particularly susceptible to environmental exposures as extensive epigenetic reprogramming and programming events take place to establish cell- and tissue-specific gene expression (Perera and Herbstman, 2011). In mammals, the best characterized epigenetic reprogramming is the genomic imprinting occurring just after fertilization resulting in monoallelic gene expression and X-chromosome inactivation. It involves the almost complete erasure of DNA methylation in both maternal and paternal genome and leads to the establishment of a differential DNA methylation and gene expression pattern (Perera and Herbstman, 2011).

Later during development epigenetic programming events take place and are crucial for later lineage commitment (Hemberger et al., 2009). For example, during neurodevelopment epigenetic programming processes regulate neural stem cells (NSCs) commitment toward neuronal or glial fate, by inducing the expression of specific factors promoting neuronal or glial genes transcription (Hirabayashi and Gotoh, 2010). Therefore, the epigenome is a main target through which environmental insults can disrupt gene expression patterns associated with normal neurodevelopment, leading to long-term consequences on brain structure and function.

There are three main epigenetic mechanisms regulating gene expression: DNA methylation, posttranslational covalent modifications of histone proteins, and non-coding RNA (ncRNA)-mediated modulation of gene expression (Bollati and Baccarelli, 2010; Perera and Herbstman, 2011). DNA methylation is the most commonly studied epigenetic process and involves mainly cytosine methylation of CpG dinucleotides. CpG rich regions, known as “CpG islands”, are located in proximity to critical *cis* elements within promoters and are typically unmethylated (Bollati and Baccarelli, 2010; Perera and Herbstman, 2011). Methylation of CpG dinucleotides is often associated with inhibition of transcription as it interferes with transcription binding proteins. Moreover, methylated DNA represents a recognition site for methyl-DNA binding proteins (MBP) that, in turn, recruit other chromatin remodeling enzymes and create a silenced chromatin state (Perera and Herbstman, 2011).

Gene expression is also regulated by histone modifications (Bannister and Kouzarides, 2011). Histones are alkaline proteins that organize the DNA in structural units, named nucleosomes, and contribute to gene transcription regulation by modulating chromatin compaction and accessibility (Campos and Reinberg, 2009). Indeed, the terminal tails of histones protrude from the nucleosome and undergo

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https://doi.org/10.1016/j.ntt.2017.12.002
Received 25 July 2017; Received in revised form 28 November 2017; Accepted 4 December 2017

covalent posttranslational modifications (PTMs) altering their interaction with DNA. The main histone PTMs are acetylation, phosphorylation, methylation or ubiquitination, that are operated by histone acetyltransferases, histone deacetylases, histone methylases and histone demethylases (Bannister and Kouzarides, 2011).

Another important epigenetic mechanism involves ncRNAs, defined as functional RNA molecules that are not translated into proteins (Senut et al., 2012). Epigenetic-related ncRNAs include miRNAs, siRNAs, piRNAs and snoRNAs, which influence gene expression at both transcriptional and posttranscriptional level (Esteller, 2011).

2. Prenatal insults affecting neural development

It is well established that *in utero* exposure to adverse prenatal environments, including maternal stress, toxins, drugs and infections, can disrupt normal neurodevelopment with long-term adverse effects on the nervous system structure and function (Kundakovic and Jaric, 2017). Epigenetic events have been shown to be essential for normal brain development, as they provide a critical contribution to the modulation of neural genes expression during specific developmental time windows (Banik et al., 2017; Feng et al., 2007). Consequently, perturbation of the epigenetic machinery leads to abnormal fetal development and contributes to the etiopathogenesis of nervous system disorders (Banik et al., 2017), including neuropsychiatric disorders, such as depression, autism and schizophrenia (Kundakovic and Jaric, 2017; Tran and Miyake, 2017). Several neurotoxic insults, including chemicals and pharmaceuticals, have been reported to alter epigenetics, with negative consequences on the development of the nervous system (Banik et al., 2017). Among them are heavy metals, including methylmercury, lead, arsenic and cadmium, as well as excess of glucocorticoids (GCs), such as the GCs analog Dexamethasone (Dex).

2.1. Heavy metals

Experimental and epidemiological studies have shown that neurodevelopmental disorders can be associated with prenatal exposure to environmental pollutants, including heavy metals (Tran and Miyake, 2017). This is related to physiological mechanisms that are unique to the developing brain and that make it particularly vulnerable to toxicants (Ek et al., 2012; Saunders et al., 2012, 2000). Consequently, even exposure to low level of neurotoxicants may cause long- lasting impairments in fetal brain development.

2.1.1. Methylmercury

Methylmercury (MeHg) is a widespread environmental contaminant with established neurotoxic effects, especially during development. Inorganic mercuric salts enter the environment naturally from volcanoes, oceanic sediments, crust degassing and forest fires, or anthropogenically through mining, industrial processes and waste incineration (Clarkson, 2002; Clarkson et al., 2003; Hintelmann, 2010). Mercury is converted to MeHg by methylation activity of sulphate-reducing bacteria in marine ecosystem and anaerobic bacteria in the soil. Human exposure mainly derives from consumption of aquatic food like fish, shellfish and sea mammals (Clarkson, 2002; Clarkson et al., 2003; Hintelmann, 2010). About 95% of MeHg ingested *via* food is absorbed in the gastrointestinal tract and easily crosses the BBB through neutral amino acid transport system (Aschner and Aschner, 1990; Kerper et al., 1992). MeHg developmental neurotoxicity was recognized following the Minamata catastrophe that occurred between the 1950s and early 1960s. In this time range, children with neurological disorders were born from symptoms-free women who were exposed to MeHg *via* contaminated fish, indicating for the first time that the developing brain is more vulnerable to MeHg-induced neurotoxicity than the adult brain (Harada, 1995).

Growing evidence points to the association between early developmental exposure to low doses of MeHg and long-term behavioral deficits (Grandjean et al., 1998; Johansson et al., 2007; Onishchenko et al., 2008, 2007). A study by Onishchenko et al. has shown that exposure of pregnant mice to 0.5 mg/kg/day MeHg from gestational day 7 until postnatal day 7, induces long-lasting alterations in learning capabilities and depression-like behavior in young and adult male mice (Onishchenko et al., 2007).

According to the neurotrophic hypothesis of depression, a deficiency in neurotrophic factors contributes to the pathogenesis of depression (Castrén et al., 2007; Nestler et al., 2002). In this regard, one of the most investigated factors is brain-derived neurotrophic factor (BDNF), which is expressed at lower level in mouse models of depression, as well as in depressed patients (Chen et al., 2017; Shimizu et al., 2003). Moreover, alterations in BDNF gene expression have been shown to be associated with epigenetic changes in its chromatin structure (Castrén et al., 2007; Chen et al., 2017; Onishchenko et al., 2008).

In agreement, Onishchenko et al. showed that perinatal exposure to MeHg is associated with depression-like behavior and epigenetic changes at the promoter of the BDNF gene (Onishchenko et al., 2008). In particular, MeHg exposure induces a long-lasting repressive state in the chromatin structure at the BDNF promoter region, as shown by DNA hypermethylation, increased histone H3-K27 tri-methylation and decreased H3 acetylation at promoter IV, strongly indicating that Me-Hg-induced effects are mediated by epigenetic events (Onishchenko et al., 2008).

Several studies suggest the association between depressive disorder and hippocampal neurogenesis. In support to this hypothesis, Bose et al. found that there is a clear trend to a decrease in the number of dividing hippocampal neural progenitor cells derived from MeHg-exposed mice exhibiting depression-like behavior (Bose et al., 2012). Moreover, the same exposed mice show a reduction in the total number of neurons in the dentate gyrus (DG) which could be restored by treatment with the antidepressant fluoxetine (Bose et al., 2012).

Additional evidence from the same study point to long-lasting effects of low levels of MeHg on neurogenesis (Bose et al., 2012). In fact, it has been demonstrated that nanomolar doses MeHg (2.5–10 nM) induce a heritable decrease in proliferation of rat NSCs by mechanisms involving the upregulation of two senescence genes, namely *p16* and *p21* (Bose et al., 2012). This data indicate that MeHg induces premature senescence, as confirmed by the heritable downregulation of B lymphoma Mo-MLV insertion region 1 homolog (Bmi1) gene expression and the upregulation of the high mobility group A1 (Hmga1) (Bose et al., 2012). These findings are in line with a previous study showing that, in human umbilical cord blood–derived multipotent stem cells, the inhibition of DNA (cytosine-5-)-methyltransferase (DNMT) upregulates the expression of *p16* and *p21*, leading to G1-phase cell cycle arrest, decreased cell proliferation rate, and induction of cellular senescence (So et al., 2011). Notably, the persistence of MeHg-induced effects in "daughter cells" never directly exposed to MeHg, has been shown to be mediated by epigenetic changes involving a global DNA hypomethylation, which is associated with a decreased expression of DNMT 3b mRNA expression, an enzyme involved in *de novo* methylation (Okano et al., 1999).

In conclusion, both *in vitro* and *in vivo* studies provide evidence that maternal exposure to MeHg induces long-lasting functional alterations, which are associated to epigenetic mechanisms.

2.1.2. Lead

Lead is a ubiquitous persistent toxic pollutant which has been mined for thousands of years and its neurotoxicity has been known since Roman times. Although lead has been removed from paints and gasoline, it remains a serious problem as it can still be found in a number of products used daily, including batteries, lead-glazed ceramics, toys, food and water (Olympio et al., 2009). Human exposure to inorganic and organic lead mainly occurs through inhalation of air contaminated with lead dust, ingestion of contaminated food/water, or *via* direct contact with lead-polluted soil through the skin (Olympio et al., 2009;