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Review article

Epigenetic mechanisms in alcohol- and adversity-induced developmental origins of neurobehavioral functioning

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ABSTRACT

The long-term effects of developmental alcohol and stress exposure are well documented in both humans and non-human animal models. Damage to the brain and attendant life-long impairments in cognition and increased risk for psychiatric disorders are debilitating consequences of developmental exposure to alcohol and/or psychological stress. Here we discuss evidence for a role of epigenetic mechanisms in mediating these consequences. While we highlight some of the common ways in which stress or alcohol impact the epigenome, we point out that little is understood of the epigenome's response to experiencing both stress and alcohol exposure, though stress is a contributing factor as to why women drink during pregnancy. Advancing our understanding of this relationship is of critical concern not just for the health and well-being of individuals directly exposed to these teratogens, but for generations to come.

1. Introduction

Adverse experiences during fetal development and early postnatal life can significantly affect the physiological and behavioral trajectory of offspring. Prenatal alcohol and toxic stress exposure are two of the most common agents resulting in disturbed development of offspring and often act concomitantly (CDC, 2017; DiPietro, 2012; Driscoll et al., 1990; Watson et al., 1999). Many women turn to substance use as a way to cope with stress during pregnancy (Hanna et al., 1994; Skagerström et al., 2011; Yali and Lobel, 1999). Understanding the biological basis of the significant physical and behavioral consequences of developmental alcohol and/or stress exposure is important for developing care guidelines for pregnant women and parents as well as for developing therapeutic interventions for affected offspring. Epigenetic modifications to chromatin represent mechanistic pathways through which early teratogen exposure can affect brain and behavioral development. Widely studied in developmental biology, epigenetics refer to events that alter gene activity without directly impacting the DNA sequence. For this review, epigenetics includes DNA methylation, histone acetylation and methylation, posttranscriptional regulation of gene expression via microRNAs, and multigenerational (referring to intergenerational and/or transgenerational) effects. These events are important during development to direct cell proliferation, differentiation, and neural patterning (Guibert and Weber, 2013; Monk et al., 1987), as well as in the postnatal and adult brains, where *de novo* epigenetic modifications represent a path through which environmental influences can affect gene activity (Jones and Takai, 2001).

Changes to chromatin structure can occur at many levels. DNA methylation, the addition of methyl groups to cytosines in DNA, is one of the most studied epigenetic modifications in terms of developmental alcohol or stress exposure. Methylation often represses gene transcription (Jones and Takai, 2001) (as depicted in Fig. 1), though this effect is dependent on cytosine location in the genome (Guibert and Weber, 2013). Methyl groups are added to DNA via DNA methyltransferases (DNMT1, DNMT3A, DNMT3B, and DNMT3L), which are differentially expressed throughout development (Okano et al., 1999). DNMT1 is typically associated with maintenance of methyl marks carried through replication or “cell memory,” while DNMT3A and 3B are essential for *de novo* methylation (Okano et al., 1999). DNMT3L has been less well-studied, but has been shown to act through suppression of inherited maternal methylation marks and stimulation of DNMT3A activity (Bourc'his et al., 2001; Hata et al., 2002). Active demethylation of DNA can occur through hydroxymethylation, with the formation of 5-hydroxymethylcytosine (5-hmC) catalyzed by the ten-eleven translocation methylcytosine dioxygenase (TET) family of enzymes (Guibert and Weber, 2013). It should be noted that the majority of studies discussed in this review do not distinguish between 5mC and 5hmC. Methylation patterns are highly dynamic across development, and methylation is a critical part of stage-dependent gene regulation (Guibert and Weber, 2013; Monk et al., 2016; Monk et al., 1987). Thus, disruption of

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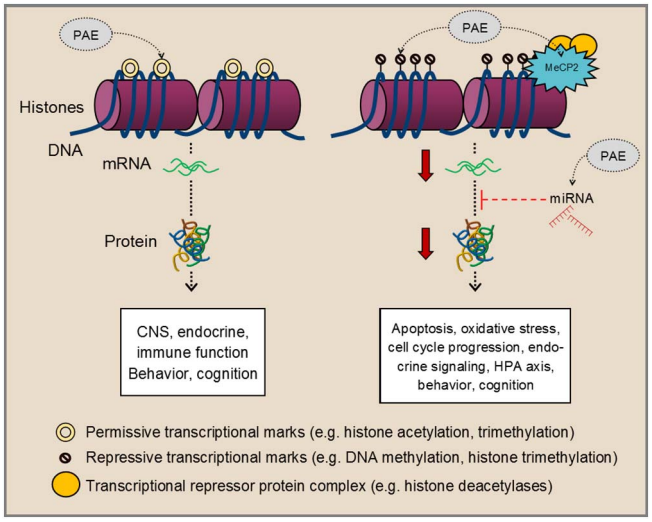

Fig. 1. Overview of epigenetic modifications induced by prenatal alcohol exposure (PAE). The presence of permissive transcriptional marks, such as histone acetylation or trimethylation, results in transcription of DNA into mRNA and translation of mRNA into protein. Under control conditions, normal CNS, endocrine, and immune function take place. PAE can remove these pro-transcriptional marks or increase the presence of repressive transcriptional marks, such as DNA methylation. Methyl groups either directly disrupt the ability of transcription factors to bind to DNA or recruit other transcriptional repressor proteins (i.e. MeCP2), reducing gene expression. miRNAs act post-transcriptionally to prevent mRNA from being translated into protein. This is associated with increased cell death and oxidative stress, altered cell cycle progression, disrupted endocrine and hypothalamic–pituitary–adrenal (HPA) axis signaling, and behavioral and cognitive deficits.

methylation patterns during gestation or postnatally by teratogens would have lasting ramifications on ongoing developmental processes. In addition, environmentally-driven alterations to methylation status can remain stable across the lifespan and even be perpetuated across generations (Laird, 2003; Meaney and Szyf, 2005).

Other chromatin modifications can alter gene activity, including histone acetylation and trimethylation (Fig. 1). Histone acetylation loosens chromatin to make DNA more accessible to transcription factors (Grunstein, 1997; Struhl, 1998). In terms of histone methylation, downstream effects on gene regulation depend largely on the specific amino acid modified. For example, trimethylation of Histone 3 lysine 4 (H3K4me3) is associated with activated transcriptional activity, while trimethylation of other lysine residues is associated with transcriptional repression. Beyond modifications to individual amino acids, chromatin accessibility is controlled through complex combinations of modifications to histone tails (Jenuwein and Allis, 2001). Specialized protein domains recognize each combination and are directed to alter chromatin organization. Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs), are additional pathways through which prenatal alcohol exposure (PAE) and stress can alter protein synthesis (Fig. 1). Mature miRNAs are fragments of RNA cleaved from primary miRNA (pri-miRNA) by the enzyme Dicer (He and Hannon, 2004). These mature miRNAs silence gene expression either by preventing translation of mRNA into protein or by speeding up degradation of the mRNA. Other ncRNAs have important biological roles as well, including RNA splicing and DNA replication (Mercer et al., 2009).

This review discusses the growing body of evidence that both PAE and developmental stress exposure affect an individual's cognitive development and risk for psychopathology in part through changes to the epigenome. The primary focus of this review is on animal models of PAE or gestational or postnatal stress as most epigenetic research has utilized these models. Where appropriate, the translational relevance of animal data is discussed. Areas where the effects of developmental alcohol and stress intersect will be discussed as well. These areas of overlap include discussion of epigenetic modification of genes involved in neural development and hypothalamic–pituitary–adrenal (HPA) axis regulation, molecules involved in the regulation of epigenetic marks, and miRNA expression. In addition, we discuss important areas for further research, including multigenerational effects of alcohol and stress as a way in which teratogen exposure could impact the health and well-being of several generations, and possible pharmacological, nutritional, and behavioral interventions to rescue negative outcomes of developmental alcohol or stress exposure, specifically in relation to their effect on the epigenome.

2. Prenatal alcohol exposure

One of the most widely used teratogenic substances is alcohol, and the National Institute on Alcohol Abuse and Alcoholism estimates that 1 in 8 women drink alcohol while pregnant (2015). PAE leads to an array of adverse physical, cognitive, and behavioral outcomes, with Fetal Alcohol Spectrums Disorders (FASD) recognized as the leading preventable cause of developmental disability in the United States (CDC, 2017). Heavy alcohol exposure results in characteristic craniofacial and eye malformations, which are present alongside low birth weight and growth retardation and classified as Fetal Alcohol Syndrome (FAS). PAE also alters neurobehavioral outcomes, with patients exhibiting memory (Mattson et al., 1999; Rasmussen and Bisanz, 2011; Rasmussen et al., 2010), executive functioning (Bertrand and Interventions for Children with Fetal Alcohol Spectrum Disorders Research Consortium, 2009; Connor et al., 2000), and social functioning deficits (Irner et al., 2012; Stevens et al., 2012), as well as increased impulsivity (Franklin et al., 2008) and risk of incarceration (Streissguth et al., 2004).

A myriad of contributing factors dictate the type and severity of alcohol-induced damage, including the dose and pattern of alcohol exposure, the developmental stage of the embryo or fetus, and individual differences in genetics and metabolism. The role of epigenetic modifications in risk and resilience to the teratogenic effects of PAE is still largely unknown, with emerging data consistent with the notion that PAE produces epigenetic changes that serve as a mediator of alcohol-induced damage. Epigenetic modifications also represent an avenue through which alcohol exposure could have a lasting negative impact on an individual throughout the lifespan.

Animal models of FASD have played a critical role in both classifying alcohol-related birth defects and understanding mechanisms of alcohol teratogenesis. The use of animal models allows for variables such as timing, dose, and method of exposure to be manipulated while controlling other environmental factors. The vast majority of studies use rodent models and administer alcohol either to the pregnant dam (modeling exposure during the first two trimesters of human pregnancy) or directly to the pups early in the postnatal period (modeling third trimester-equivalent exposure). Common routes of administration for alcohol include i.p. injection (of the dam prenatally or, less commonly, to the pups postnatally), intragastric gavage (either of the dam in prenatal models or of the pups in postnatal models), voluntary drinking (prenatal only), liquid diet (prenatal only), or placement of the dam and/or pups into a vapor chamber. The experimental question often leads to the use of a specific species or exposure paradigm; the models used for each study discussed in this review will be described as necessary. More detailed explanations of rodent models of FASD can be found elsewhere (Boschen and Klintsova, 2017; Gil-Mohapel et al., 2010). One strength of animal models is the ability to investigate the time course of epigenetic modifications in different organ types and brain regions following alcohol exposure during development.

Changes to the epigenome plausibly explain many of the deficits observed following PAE, including altered gene expression patterns, cognitive and behavioral impairments, and increased risk of mental dysfunction later in life; however, the longevity of epigenetic marks following PAE and their causal relation to behavioral outcome has not been fully explored. The following three sections discuss evidence from prenatal, postnatal, and *in vitro* FASD models of alcohol's ability to