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Alcohol effects on the epigenome in the germline: Role in the inheritance of alcohol-related pathology

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ABSTRACT

Excessive alcohol exposure has severe health consequences, and clinical and animal studies have demonstrated that disruptions in the epigenome of somatic cells, such as those in brain, are an important factor in the development of alcohol-related pathologies, such as alcohol-use disorders (AUDs) and fetal alcohol spectrum disorders (FASDs). It is also well known that alcohol-related health problems are passed down across generations in human populations, but the complete mechanisms for this phenomenon are currently unknown. Recent studies in animal models have suggested that epigenetic factors are also responsible for the transmission of alcohol-related pathologies across generations. Alcohol exposure has been shown to induce changes in the epigenome of sperm of exposed male animals, and these epimutations are inherited in the offspring. This paper reviews evidence for multigenerational and transgenerational epigenetic inheritance of alcohol-related pathology through the germline. We also review the literature on the epigenetic effects of alcohol exposure on somatic cells in brain, and its contribution to AUDs and FASDs. We note gaps in knowledge in this field, such as the lack of clinical studies in human populations and the lack of data on epigenetic inheritance via the female germline, and we suggest future research directions.

Introduction

Epigenetics is the milieu of mechanisms that modify gene expression without changing the DNA sequence itself. These mechanisms may involve methylation of DNA, modifications of histones, and small noncoding RNAs. Epigenetics allows for adaptive changes in gene expression that can be quickly implemented but can have long-lasting effects. Much research has focused on the effects of environmental impacts on epigenetic machinery, such as the effects of stress (Franklin et al., 2010; Yao et al., 2014), environmental toxins (Anway, Cupp, Uzumcu, & Skinner, 2005), and drugs of abuse, including alcohol (Finegersh, Rompala, Martin, & Homanics, 2015; Yohn, Bartolomei, & Blendy, 2015) and other drugs Walker, Cates, Heller, & Nestler, 2015. Recent evidence also suggests that epigenetic modifications can be carried through the germline and through multiple generations. This type of inheritance is termed “epigenetic inheritance” and stands as a novel mechanism for inheritance. This compelling phenomenon is the subject of much current research, although many questions remain unanswered as to the mechanisms for epigenetic inheritance and for transgenerational epigenetic inheritance. The focus of this paper is to review the latest research on the epigenetic effects of alcohol exposure on the germline and on epigenetic inheritance of alcohol-related pathology. This review also discusses the gaps in knowledge relating to the mechanisms for epigenetic inheritance of alcohol exposure-related pathologies.

Alcohol-related pathology

Alcohol-use disorders (AUDs) are a significant psychiatric problem in the United States and worldwide. AUDs are characterized by excessive drinking and abuse of alcohol that leads to adverse social or health consequences for the drinker (APA, 2013; Agrawal, Heath, & Lynskey, 2011). AUDs may involve alcohol dependence or addiction to alcohol, which involves neural adaptations to chronic alcohol use leading to aversive emotional and physical consequences when alcohol use is discontinued, thus maintaining the alcohol abuse to avoid negative consequences (Koob, 2013). AUDs are associated with increased risk for fatal accidents and many serious health problems including depression,

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stroke, sleep disorders, heart disease, liver disease, and cancer (Rehm, Samokhvalov, & Shield, 2013; Rehm et al., 2009; Shield, Gmel, Patra, & Rehm, 2012). It is estimated that as many as 17 million adults in the US may have AUDs (Kessler et al., 1997; Volpicelli, 2001).

When a pregnant woman abuses alcohol, her exposed children are at a high risk for developing fetal alcohol spectrum disorders (FASDs), which are developmental disorders which can be characterized by diverse symptoms, including delayed growth, craniofacial abnormalities, intellectual impairment, anxiety, depression, and social impairment (Bakoyiannis et al., 2014; Driscoll, Streissguth, & Riley, 1990; Kelly, Day, & Streissguth, 2000). The occurrence and severity of symptoms depend on the duration and developmental timing of alcohol exposure (Alfonso-Loeches & Guerri, 2011). The most severe form of the disorder is called fetal alcohol syndrome (FAS), which is characterized by facial dysmorphologies, impaired growth, and central nervous system dysfunction (Sokol, Delaney-Black, & Nordstrom, 2003). Children that do not meet the full criteria for FAS but show a spectrum of the disorder are included under the umbrella term FASD. Children with FASD suffer long-lasting intellectual, social, and emotional problems, which persist through adulthood. The risk for developing AUDs has been found to "run in families", due to a combination of social and genetic components, and studies suggest epigenetic factors also play a role in the development and inheritance of alcohol-related health problems. However, the epigenetic mechanisms for heritability of AUDs and familial alcohol-related health defects are far from understood and are discussed in this review.

Epigenetics

Epigenetics is the study of changes in gene expression, cell fate, and potentially heritability without any changes in DNA sequence. It can be thought of broadly as a bridge between genotype and phenotype (Goldberg, Allis, & Bernstein, 2007). Epigenetics is known to be regulated by three mechanisms, which interact in complex ways, including 1) DNA methylation, 2) histone modifications, and 3) noncoding RNAs. DNA methylation occurs on the cytosine residue of CpG dinucleotides and is performed by DNA methyltransferases (DNMTs) through the transfer of 5-methylcytosine from the methyl donor *S*-adenosylmethionine (SAM) to the CpG. In eukaryotes, DNA methylation status is passed down during DNA replication to the daughter strand DNA by the enzyme DNMT1, which performs the "maintenance" methylation activities, ensuring the methylation status of the parent cells is preserved in the daughter cells, while DNMT3a and DNMT3b are "*de novo*" methylation enzymes, which establish new methylation patterns during embryogenesis (Holliday & Pugh, 1975; Okano, Bell, Haber, & Li, 1999; Riggs, 2002), although recent evidence suggests there is some overlap in the function of maintenance and *de novo* enzymes (Jones & Liang, 2009). Methylation of DNA is functionally associated with gene silencing and is for the most part limited to so−called "CpG islands", which are areas rich in CpG dinucleotides and are typically located near the promoter regions of genes and the transcription start site. Methylated DNA at the promoter regions can inhibit transcription by recruiting gene-silencing repressive proteins to the methylated region, such as methyl-CpG-binding protein 2 (MeCP2) and methyl-binding-domain (MBD) proteins (Cedar & Bergman, 2009). In contrast, hypomethylated CpG islands and promoter regions are associated with transcriptionally active histone modifications (discussed further below) and increased accessibility of the DNA transcription factors and RNA polymerase II to the DNA (Zhou, Goren, & Bernstein, 2011). Methylation status is mitotically stable and relatively long lasting, and evidence suggests that methylation status can even be passed on from the germline to the offspring, as this review will discuss. Nonetheless, flexibility in methylation status is also possible, and DNA methylation can also be reversed passively by the loss of 5 mC during successive cycles of DNA replication, or by the recently discovered active demethylation of DNA, which can occur by oxidation of 5-methylcytosine by ten-eleven translocation (TET) family enzymes (Kohli & Zhang, 2013). Recent studies also suggest oxidized 5-methylcytosine, 5-hydroxymethylcytosine (5-hmC), may play an important role as an epigenetic mark, although the functional role of 5-hmC is still under investigation (Branco, Ficz, & Reik, 2012).

Alongside changes in DNA methylation, epigenetic machinery also includes a variety of changes in histones, both in post-translational histone modifications and in histone variants (for review see Bannister & Kouzarides, 2011; Kouzarides, 2007; Zhou, Goren, et al., 2011). In eukaryotes, proteins called histones package DNA into units called nucleosomes, which allow for protection and organization of the DNA. The cell has several enzymes that enact numerous covalent modifications on residues of histone tails (for example, methylation, acetylation, phosphorylation, ubiquitylation, and sumoylation), which have been theorized to serve as a "histone code" to allow for either increased transcription or silencing of genes (Strahl & Allis, 2000). Some examples of important modifications include methylation at histone 3 lysine 9 (H3K9me3) and lysine 27 (H3K27me3), which are associated with heterochromatin and gene silencing. In contrast histone 3 lysine 4 methylation (H3K4me2 and H3K4me3), acetylation, and the histone variant H2A.Z are marks associated with euchromatin, active promoters, and increased activation of gene expression (Zhou, Goren, et al., 2011). Histone-modifying enzymes include histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone demethylases, and kinases that catalyze modifications on the histones, which are then interpreted by chromatin-modifying protein complexes which are able to modify chromatin structure, allowing for decreased or increased accessibility of the transcription machinery to the DNA (Henikoff, 2008; Kouzarides, 2007; Nakao, 2001; Ng & Bird, 1999). While DNA methylation is more permanent, histone modifications are more dynamic and transient, but it is important to stress that histone modifications and changes in DNA methylation are not separate entities, and that they work together to orchestrate transcriptional response and possibly affect epigenetic inheritance (Nakao, 2001; Ng & Bird, 1999).

An additional, a more recently identified mechanism of epigenetic modification includes modulation by noncoding RNAs (ncRNAs), RNAs that are transcribed from DNA but are not translated into protein (for reviews see Berezikov, 2011; Larriba & del Mazo, 2016; and Yan, 2014). Small ncRNAs, which are less than 200 nucleotides (nts) in length, include microRNAs (miRNAs), short interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs), which have been shown to silence gene expression by targeting mRNA transcripts, sometimes via histone and DNA methylation mechanisms, leading to their downregulation (Holoch & Moazed, 2015). Long non-coding (lncRNAs) (>200 nt in length) also participate in epigenetic-mediated gene silencing. Especially, long non-coding intergenic RNA (lincRNAs) are transcribed from regions close to genes that will be silenced, and have been discovered to serve as sequence guides to attract protein complexes to either increase DNA methylation or repressive histone modifications. Examples of the work of lincRNAs include important roles in inactivation of the X chromosome (Ng, Pullirsch, Leeb, & Wutz, 2007) and in genomic imprinting (Gabory, Jammes, & Dandolo, 2010). As ncRNAs participate in important developmental events that affect the primordial germ cells and can also be affected by environmental influences, they could participate, in theory, as an