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DNA modifications in models of alcohol use disorders

Christopher T. Tulisiak [a], R. Adron Harris [a, b], Igor Ponomarev [a, b, *]

[a] Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, 2500 Speedway, A4800, Austin, TX 78712, USA
[b] The College of Pharmacy, The University of Texas at Austin, 2409 University Avenue, A1900, Austin, TX 78712, USA

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ABSTRACT

Chronic alcohol use and abuse result in widespread changes to gene expression, some of which contribute to the development of alcohol-use disorders (AUD). Gene expression is controlled, in part, by a group of regulatory systems often referred to as epigenetic factors, which includes, among other mechanisms, chemical marks made on the histone proteins around which genomic DNA is wound to form chromatin, and on nucleotides of the DNA itself. In particular, alcohol has been shown to perturb the epigenetic machinery, leading to changes in gene expression and cellular functions characteristic of AUD and, ultimately, to altered behavior. DNA modifications in particular are seeing increasing research in the context of alcohol use and abuse. To date, studies of DNA modifications in AUD have primarily looked at global methylation profiles in human brain and blood, gene-specific methylation profiles in animal models, methylation changes associated with prenatal ethanol exposure, and the potential therapeutic abilities of DNA methyltransferase inhibitors. Future studies may be aimed at identifying changes to more recently discovered DNA modifications, utilizing new methods to discriminate methylation profiles between cell types, thus clarifying how alcohol influences the methylomes of cell-type populations and how this may affect downstream processes. These studies and more in-depth probing of DNA methylation will be key to determining whether DNA-level epigenetic regulation plays a causative role in AUD and can thus be targeted for treatment of the disorder.

1. Introduction

The transcription of a gene is controlled, in part, by its availability to the binding of transcription factors, which usually only have access to regulatory regions and promoters if the DNA is in the euchromatin state, unwound from histone proteins, with unmodified, unbound nucleotides. The state of the chromatin (DNA wound around histones and compacted) is controlled by epigenetic modifications – a complex of molecular machinery involved in regulation of gene expression at the individual gene and gene network levels. Crucially, epigenetic factors are capable of dynamically regulating gene expression within a cell, which, despite each cell in an organism having the same genotype, results in multiple cell types during development and, at the organism level, allows for expression of varied phenotypes. Epigenetic modifications include chemical residues or "marks" which may be added to or removed from amino acids of histone protein tails or DNA nucleotides. Addition of these marks by enzymatic "writers," such as addition of methyl groups by methyltransferases, or their removal by "erasers," such as removal of acetyl groups by deacetylases, enables dynamic regulation of the chromatin state, providing access to the DNA for transcription factors or for "readers," such as methyl-binding domain (MBD) proteins, which bind certain marks to produce a downstream effect. Expression of these regulatory elements can be influenced by the environment, including exposure to ethanol (ethyl alcohol) and stress, and, through their effects on gene transcription, can lead to behavioral changes in an individual. In this way, epigenetic regulation

Abbreviations: Alcohol use disorder, AUD; substance use disorder, SUD; fetal alcohol spectrum disorder, FASD; cytosine-phosphate-guanine, CpG; cytosine-phosphate-[non guanine base], CpH; DNA methyltransferase, DNMT; ten-eleven translocator, TET; 5-methylcytosine, 5mC; 5-hydroxymethylcytosine, 5hmC; 5-formylcytosine, 5fC; 5-carboxylcytosine, 5caC; methyl-binding domain, MBD; S-adenosyl-methionine, SAM; base excision repair, BER; activation-induced deaminase, AID; thymine DNA glycosylase, TDG; single-nucleotide polymorphism, SNP; 5-azacytidine, 5-aza.

* Corresponding author. Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, 2500 Speedway, A4800, Austin, TX 78712, USA.
E-mail address: ponomarev@utexas.edu (I. Ponomarev).

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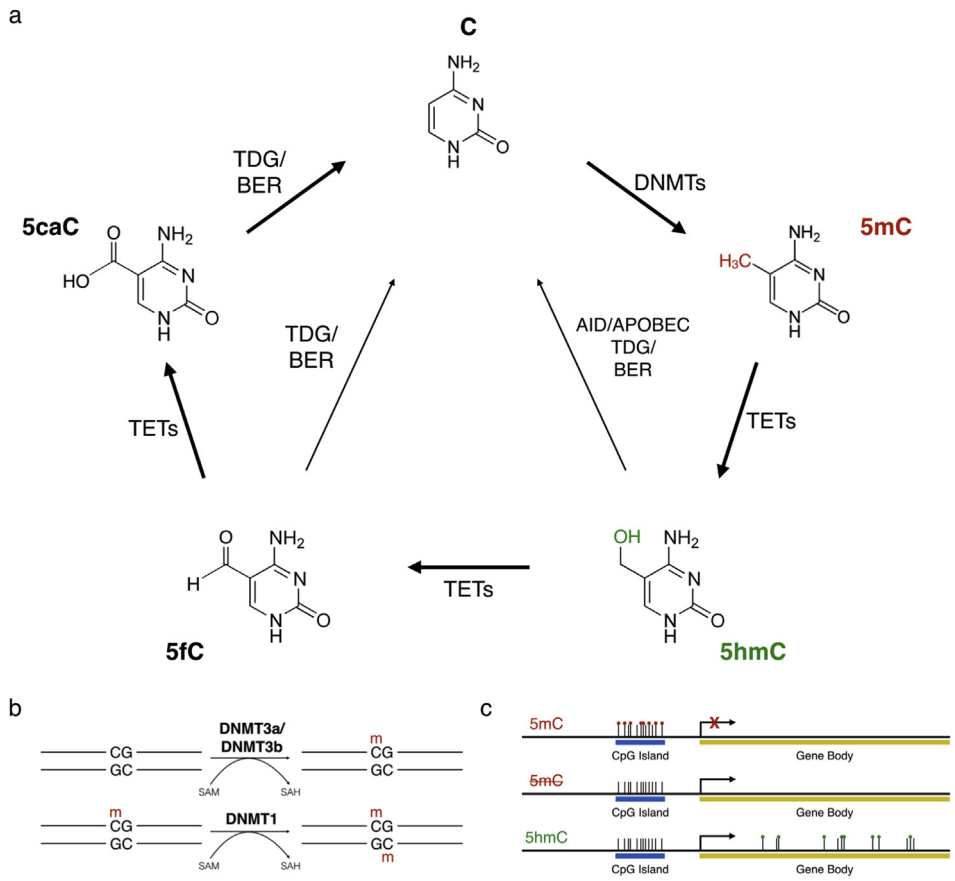

Fig. 1. DNA modifications and their effects on gene expression. a) Cytosines (C) may be methylated at the 5-carbon by DNA methyltransferase proteins (DNMTs) to produce 5-methylcytosine (5mC, red). 5mC can then be converted to 5-hydroxymethylcytosine (5hmC, green) by Ten-Eleven Translocator proteins (TETs). Further oxidization of 5hmC by TETs yields 5-formylcytosine (5fC), which can be converted to 5-carboxylcytosine (5caC) by TETs. Conversion of 5mC by TETs is the first stage in the active DNA demethylation pathway. 5hmC may be converted to cytosine demethylated through activation-induced deaminase (AID)/apolipoprotein B mRNA editing enzyme complex (APOBEC) to create 5-hydroxymethyluracil, followed by creation of an abasic site by thymine DNA glycosylase, which is then repaired to C by base excision repair. 5fC and 5caC can bypass the AID/APOBEC pathway. b) Top: *de novo* CpG methylation. DNMT3a and DNMT3b, the *de novo* methyltransferases, add a methyl group from donor S-adenosyl methionine (SAM) to methylate one cytosine of a complementary pair of unmethylated CpG dinucleotides. Bottom: maintenance CpG methylation. DNMT1, the maintenance methyltransferase, methylates the unmethylated cytosine of a hemi-methylated complementary pair of CpG dinucleotides to produce a complementary pair of CpGs methylated at the cytosines on both strands. c) 5mC is frequently found in clusters of CpG dinucleotides, called CpG islands, which are frequently found, in turn, in gene promoter regions. 5mC in promoter CpG islands (top) is typically a repressive mark and acts by blocking gene transcription as indicated by a red cross over the black arrow. Absence of 5mC in CpG islands (center) and presence of 5hmC in gene bodies (bottom) are often associated with transcriptional activation (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mediates the complex relationship between an individual's genotype and environment, resulting in changes in gene expression and downstream phenotypes.

Epigenetic systems have garnered growing attention in the study of disease states, since alterations to chromatin states are capable of creating contexts of widespread, aberrant gene expression that are capable of significantly contributing to development and expression of various complex pathologies. Though understudied in the context of models of alcohol use disorders (AUDs) until the past decade, recent evidence demonstrates that exposure to alcohol is associated with a wide range of epigenetic modifications, which may underlie alcohol-related behaviors. The focus of this review is on DNA-level epigenetic modifications, with emphasis on DNA methylation. We aim to provide a relevant background and an overview of DNA modification research in the context of AUD, including alcohol's effects in brain and peripheral tissues in both human and animal models, the effects of alcohol on the methylome of the developing brain, and the evidence supporting DNA modifications as a potential therapeutic target for treatment of AUD. Challenges and future directions of this research are also discussed.

2. DNA modifications

2.1. DNMT and 5mC

Epigenetic DNA modifications are made by the addition of chemical groups, such as a methyl group, to DNA bases. The cytosine of 5'-cytosine-phosphate-guanine-3' (CpG) dinucleotides is the most common epigenetically modified nucleotide, with the most frequent chemical mark on these cytosines being the addition of a methyl group to carbon 5 of the cytosine ring by DNA methyltransferase (DNMT) enzymes to form 5-methylcytosine (5mC) (Fig. 1a). CpG dinucleotides are frequently found clustered together to form CpG islands, which can often be found in regulatory regions, such as gene promoters. DNMT proteins catalyze the addition of a methyl group from the cell's primary methyl group donor, *S*-adenosyl-methionine (SAM; Fig. 1b), which itself is synthesized from adenosine triphosphate (ATP) and methionine by methionine adenosyltransferase as part of the folate and methionine cycles (Hamid, Wani, & Kaur, 2009; Mentch & Locasale, 2016). It was shown long ago that diets deficient in methyl donors, such as folates, choline, and some B-complex vitamins that work as co-