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# Alcohol and epigenetic regulation: Do the products of alcohol metabolism drive epigenetic control of gene expression in alcohol-related disorders?

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## Scope

Epigenetic regulation, the persistent change in the gene regulatory state following a transient environmental perturbation, has become increasingly prominent in accounting for the consequences of exposure to addictive substances, including alcohol (ethanol). The purpose of this Virtual Issue is to draw attention to some of the recent advances in our understanding of how consumption of alcohol impacts the epigenetic landscape and causes such persistent changes in the regulation of gene expression and cellular function that affect behavior or disease susceptibility well after the alcohol challenge has occurred. Articles that recently appeared in ACER are placed in the context of the broader relevant literature and emerging opportunities. A key question we highlight relates to the role of ethanol metabolism in altering the spatiotemporal epigenetic landscape.

## Background

The term ‘epigenetics’ has been used to describe a range of molecular events, including DNA methylation, histone covalent modification, such as methylation, acetylation, and phosphorylation, other persistent protein and nucleic acid modifications, alternative splicing, RNA editing, and noncoding RNA mediated regulation of gene expression. Ethanol intake has been shown to lead to changes in several of these components, which can impact almost all levels of regulation of gene expression and protein function (for recent reviews, see Lunde et al, 2016; Kamat et al, 2016; Berkel & Pandey, 2017, Mahnke et al, 2017 and references therein). While numerous studies have documented the effects of ethanol on the epigenetic state, a holistic understanding of how the panoply of ethanol-induced epigenetic changes contribute to an integrated cellular/tissue response is lacking. In particular, the role of ethanol metabolism in driving and facilitating these epigenetic changes is yet to be fully understood.

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**Author Contributions**

RV and JBH worked together to conceptualize, organize, and write this commentary.

**Conflict of Interest**

Both authors declare no conflict of interest.

Ethanol metabolism occurs predominantly in the liver through two oxidative pathways, mediated by alcohol dehydrogenase (ADH) and by cytochrome P450 2E1 (CYP2E1). Both these pathways result in the formation of acetaldehyde, which is then effectively oxidized to acetate, largely by the mitochondrial aldehyde dehydrogenase (ALDH2). Given the high affinity of ALDH2 for acetaldehyde, accumulation of this highly reactive intermediate is generally in the low micromolar range. This serves as an effective mechanism to help contain the potential DNA damage and protein modification that can result from accumulation of these (or other) aldehyde species. In addition, ethanol oxidation through CYP2E1 can cause significant formation of reactive oxygen species (ROS), which may contribute to oxidative stress. However, in the normal liver this is largely contained by oxidative stress defenses that rely on the availability of reduced glutathione (GSH), the maintenance of which is closely interlinked with the activity of the methionine cycle. The methionine cycle is also the source of S-adenosyl methionine (SAM), the universal methyl donor for all methylation reactions. There is ample evidence that the activity of the methionine cycle is suppressed by chronic ethanol intake (Kharbanda, 2009). Acetate, the terminal product ethanol oxidation, is mostly released from the liver through transport into the circulation. During ethanol oxidation steady state concentrations of acetate can reach millimolar levels (Schug et al, 2016). Interestingly, although the liver has a relatively low capacity to activate acetate to acetyl CoA, acetate can serve as an effective substrate for other tissues, including heart, skeletal muscle and brain, where it is taken up and activated by acetyl CoA synthetase. Thus, acetate serves as a potentially important, but undervalued mediator of ethanol's systemic actions, including through its capacity to affect the epigenetic landscape.

Ethanol effects on epigenetic changes have been studied prominently in the context of DNA methylation, and histone methylation and acetylation, with a strong emphasis on the changes in the enzymatic machinery mediating these molecular events (Bekdash et al, 2014; Lopez-Moreno, 2015; Hagerty et al, 2016; Pinzon et al, 2017; Gavin et al, 2016). However, these epigenetic modifications are dependent not only on enzymes for transferring, removing and further modifying these groups, i.e., methyl transferases and demethylases, and histone acetyl transferases and deacetylases, but also on metabolic activities that produce the donors of methyl and acetyl groups, i.e. SAM and acetyl CoA (Van der Knaap and Verrijzer, 2016). There is extensive evidence of ethanol affecting the expression levels and activities of enzymes involved in the formation of methylation or acetylation substrates (Zakhari, 2013; Kharbanda, 2009; Schug et al, 2016). How does ethanol metabolism change the availability of these substrates and how do such differences in substrate supply affect the epigenetic landscape? This two-part question will be addressed in the following sections.

## Ethanol effects on methyl donor availability and its impact on protein and DNA methylation

It has long been recognized that ethanol-mediated changes in the expression and enzymatic activity of methionine synthase can limit the supply of SAM, the major methyl donor for epigenetic methylation reactions. This results in a decrease in the ratio of SAM/S-adenosyl homocysteine (SAH), in particular in the liver, imposing a substrate limitation for

methylation reactions on both DNA and histones. It is not entirely clear to what extent these ethanol-mediated changes depend on ethanol metabolism and can be attributed to effects of its downstream metabolites or the associated ROS production. Chronic ethanol consumption can also impair the methionine cycle through a restricted folate supply, which plays a role in recharging the methionine cycle. In addition, ethanol-mediated reduction in the cellular level of SAM can be accompanied by reduced expression of DNA methyltransferases and increased expression of demethylases and, taken together, these effects would be expected to lead to a reduced overall methylation. However, tissue-specific differences have been reported in effects of alcohol consumption on the DNA methylation profile, e.g., between liver and brain (Auta et al, 2017) and even in postmortem brain tissue obtained from patients with alcohol use disorders DNA CpG islands showed both hyper- and hypomethylation (Hagerty, 2016). Moreover, a recent study on mice (Gavin et al, 2016) reported strain differences in the effects of ethanol on gene-specific promoter methylation that correlated with drinking behavior. Also, the mechanism by which ethanol treatment decreases the activity of methionine synthase is not well characterized and may be tissue-specific (Waly et al, 2011), further complemented by tissue-specific expression and regulation of methionine adenosyl transferases. Furthermore, a key question is how the activity of methionine cycle enzymes themselves responds to changes in SAM availability or the SAM/SAH ratio through feedback regulation (Martinov et al, 2010; Reed et al, 2015). Chronic alcohol consumption had opposite effects on the SAM/SAH ratio and the “methylation index” in liver and brain (Auta et al, 2017) indicating that the tissue-specific nature of this feedback regulation of the methionine cycle may be a determinant of the DNA methylation profile. Interestingly, interfering at the level of substrate availability in the methionine cycle by providing excess betaine can restore SAM/SAH ratio in liver and recover DNA methylation and gene expression changes induced by ethanol exposure, and this supplementation also counteracts the ethanol effects on organ injury (Barak et al, 1993; Medici et al, 2014). The betaine-homocysteine methyltransferase (BHMT) pathway of methionine recovery is active predominantly in the liver. Yet, a recent study in avian embryos found evidence that moderate levels of betaine supplementation also recovered ethanol-induced cardiac development defects, correlated with an increase in DNA methylation index (Karunamuni et al, 2017). Thus, although a restriction in the supply of SAM may contribute to ethanol-induced changes in global DNA and histone methylation patterns, local determinants of the efficacy of methylation and demethylation reactions are fine-tuning the specific outcomes.

## Ethanol effects on acetyl CoA availability and its impact on protein acetylation

Ethanol metabolism provides multiple connections with the regulation of histone acetylation state, both directly, through an increased supply of acetate, the terminal product of alcohol oxidation and a precursor of cytosolic and nuclear acetyl CoA, and by its impact on the NAD redox state which regulates the activity of the Class III subfamily of histone deacetylases, known as sirtuins. Histone acetylation is critically dependent on an adequate supply of acetyl CoA, the substrate for Histone Acetyltransferases (HATs) and the overall rate of histone acetylation can be limited by availability of acetyl CoA (Zakhari, 2013; Schug et al, 2016). Acetyl CoA is also the acetyl donor for the wide array of protein lysine

acetylation reactions that are now recognized as a broadly distributed regulatory device in cells and tissues, the incidence of which is greatly enhanced by alcohol treatment (Shepard et al, 2010). Under normal conditions, catabolic processes such as glycolysis and fatty acid beta-oxidation provide the major source of acetyl CoA. Both of these processes generate acetyl CoA in mitochondria, from where it can be released into the cytosol in the form of citrate. This metabolic intermediate is then hydrolyzed by the enzyme ATP-citrate lyase (ACLy) to regenerate acetyl CoA available to cytosolic and nuclear reactions (Zhao et al, 2016). Inactivation of ACLy in tumor cells severely restricts histone acetylation and impairs tumor growth (Zaidi et, 2012). The other major source of acetyl CoA in the cytosol and nucleus is the *de novo* synthesis from acetate, mediated by the cytosolic Acetyl CoA Synthetase-2 (ACSS2). Under normal physiological conditions circulating acetate levels are low and acetate availability is largely restricted to its formation by deacetylases in the cytosol, with ACSS2 serving as a salvage pathway. However, oxidative metabolism of ethanol generates abundant acetate, much of which is released from the liver into the circulation to be metabolized in non-hepatic tissues, thereby increasing acetyl donor availability for epigenetic modifications throughout the organism (Schug et al, 2016).

## Relating overall substrate supply of epigenetic substrates to genomic locus-specific effects of ethanol metabolism

How important is the supply of substrates (SAM or acetyl CoA) in the ethanol-mediated regulation of epigenetic modifications? While the metabolic changes in production of methyl and acetyl donors can inform on the overall capacity for these changes, these do not appear to lead to uniform reductions in methylation or increases in acetylation at all genomic loci. Multiple additional elements can determine the outcome in epigenetic control of gene expression. For instance, Shukla et al (2015a) demonstrated that treatment of hepatocytes with acetate and ethanol has a markedly different impact on histone acetylation profiles, influenced by a different oxidative stress and MAPK signaling environment. Oxidative stress markers have also been identified as ethanol-dependent modifiers of the response to carcinogens in an animal model of squamous cell carcinoma (Urvalek et al, 2015). Alcohol drinking patterns can further influence the epigenetic profile (Shukla et al, 2015b; Lopez-Moreno, 2015). Importantly, chromatin conformation and DNA accessibility play crucial roles in regulating some of the locus-specific effects, including tissue-specific epigenetic changes (Auta et al, 2017; Tulisiak et al, 2017). However, locus-specific epigenetic changes also occur across the expressed genes within any tissue, suggesting additional layers of regulation beyond chromatin and DNA accessibility (e.g., Hagerty et al, 2016; Gavin et al, 2016).

Several questions remain unanswered. Are certain genomic loci more susceptible to epigenetic modifications when substrate levels are limiting, e.g., upon depletion of SAM (Van der Wijst et al, 2015), or are no longer limiting, e.g., with a large increase in acetyl-coA availability? If so, what characterizes these genomic loci? Emerging data point to regulation of genomic accessibility by protein complexes that phase-separate chromatin into distinct regions (Strom et al., 2017), as well as the structural organization of the genome into topologically associated domains that regulate transcription at specific genomic loci (Razin

et al., 2017). Understanding the effects of ethanol exposure on these mechanisms can help relate overall changes in substrates and enzymes for epigenetic modifications to the genomic locus-specific epigenetic changes.

## Time scale and epigenetic memory

Strikingly, epigenetic changes can be stable over a wide range of time scales ranging from minutes to decades, with even transgenerational effects (Mead and Sarkar, 2014; Basavarajappa and Subbanna, 2016; Lunde et al, 2016; Berkel and Pandey, 2017). Importantly, these ethanol-induced epigenetic changes account for altered regulation of cell function that persists well after ethanol or its metabolites have disappeared. Hence, the recent literature strongly supports the concept that epigenetic effects of even a transient ethanol exposure on tissues, organs and organisms can be sustained across these wide-ranging time scales, with the potential to drive persistent gene regulatory changes underlying fetal alcohol syndrome, cancer, and metabolic disorders. Available literature points to the dynamic nature of epigenetic changes, which are sensitive to activity levels of various enzymes and substrates, as discussed above, and yet, only a subset of these ethanol-induced changes appears to remain for long periods in certain tissues. How is this epigenetic memory maintained? What governs the stability of epigenetic changes in a locus-specific manner? What is the dependency of these epigenetic changes on the quantity, duration and pattern of alcohol use? The possible disease consequences of such stable and long-term changes have been studied in some detail in the context of fetal alcohol spectrum disorders (Mead and Sarkar, 2014; Lunde et al, 2016). In addition, recent perspectives suggest that ethanol-induced persistent epigenetic changes may be linked to the development of cancer later in life. However, there remains an almost complete lack of understanding of the molecular basis for the locus-specific persistence of these changes and of their dependency on the alcohol use profile and dose dependency. In the majority of these conditions, establishing a cause and effect relationship remains problematic and it is difficult to distinguish between damaging and beneficial or innocuous changes (Schug et al, 2016; Zakhari and Hoek, 2015). Thus, much remains to be discovered before a comprehensive understanding can be achieved of the molecular, biochemical, and biophysical underpinnings of the ethanol-susceptible, likely tissue/cell type-specific, genomic loci that show persistent epigenetic changes. Emerging tools such as CRISPR are starting to enable genomic locus-specific modification of the epigenome (Willyard, 2017), promising hitherto unavailable fine-grain interventions to reverse the ethanol-induced epigenetic changes. Many opportunities lie ahead to explore and intervene in the epigenetic basis of the multiple actions of alcohol and their systemic consequences.

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