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Epigenetic regulation in alcoholic liver disease

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Abstract

Alcoholic liver disease (ALD) is characterized by steatosis or fat deposition in the liver and inflammation, which leads to cirrhosis and hepatocellular carcinoma. Induction of target genes without involving changes in DNA sequence seems to contribute greatly to liver injury. Chromatin modifications including alterations in histones and DNA, as well as post-transcriptional changes collectively referred to as epigenetic effects are altered by alcohol. Recent studies have pointed to a significant role for epigenetic mechanisms at the nucleosomal level influencing gene expression and disease outcome in ALD. Specifically, epigenetic alterations by alcohol include histone modifications such as changes in acetylation and phosphorylation, hypomethylation of DNA, and alterations in miRNAs. These modifications can be induced by alcohol-induced oxidative stress that results in altered recruitment of transcriptional machinery and abnormal gene expression. Delineating these mechanisms in initiation and progression of ALD is becoming a major area of interest. This review summarizes key epigenetic mechanisms that are dysregulated by alcohol in the liver. Alterations by alcohol in histone and DNA modifications, enzymes related to histone acetylation such

as histone acetyltransferases, histone deacetylases and sirtuins, and methylation enzymes such as DNA methyltransferases are discussed. Chromatin modifications and miRNA alterations that result in immune cell dysfunction contributing to inflammatory cytokine production in ALD is reviewed. Finally, the role of alcohol-mediated oxidative stress in epigenetic regulation in ALD is described. A better understanding of these mechanisms is crucial for designing novel epigenetic based therapies to ameliorate ALD.

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Key words: Alcohol; Epigenetics; Histones; Acetylation; DNA methylation; miRNA; Genes

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INTRODUCTION

Chronic alcohol consumption has a significant impact on human health and is identified as a major risk factor for development of liver disease. Alcoholic liver disease (ALD) is one of the leading causes of liver disease mortality worldwide. Alcohol-related liver injury includes a spectrum of pathological conditions in the liver including steatosis, steatohepatitis, cirrhosis and hepatocellular carcinoma. Of the molecular mechanisms studied, epigenetic mechanisms altered by alcohol appear to play a significant role in development and progression of disease. These mechanisms have been identified in parenchymal and non-parenchymal cells in the liver and contribute to induction of fatty liver, inflammation, as well as hepatocyte

apoptosis and necrosis. In the past decade, a number of studies have reported epigenetic alterations by alcohol in the liver including histone modification, DNA and histone methylation derived from the methyl group donating system, S-adenosyl methionine (SAME), miRNA as post-transcriptional modifiers, and chromatin remodeling enzymes responsible for epigenetic regulation such as histone acetyltransferases, histone deacetylases and DNA methyltransferases^[1,2]. Defining an epigenetic imprint in alcohol-induced liver injury will provide new insights into pathophysiological mechanisms and open avenues for potential novel epigenetics-based therapeutics.

The goal of this concise article is to review alcohol-mediated epigenetic alterations, in parenchymal and non-parenchymal cells of the liver. Specifically, alcohol-mediated alterations in epigenetic enzymes, miRNAs as epigenetic modifiers, chromatin modifications affecting immune cell function in alcoholic liver, and the role of oxidative stress in chromatin remodeling that regulates alcohol-mediated gene expression in the liver are described.

THE EPIGENETIC CODE

The classical view defines epigenetics as heritable changes that affect gene expression without altering the DNA sequence. Epigenetic regulation of gene expression is facilitated through different mechanisms such as DNA methylation, histone modifications, and RNA-associated silencing by small non-coding RNAs. All these mechanisms are crucial for normal development, differentiation and tissue-specific gene expression. These three systems interact and stabilize one another and can initiate and sustain epigenetic silencing, thus determining heritable changes in gene expression. Alterations in one or more of these systems leads to inappropriate target gene expression or silencing that results in epigenetic regulation of human diseases such as cancer, autoimmune diseases, and age-related as well as neurological disorders^[3,4]. Epigenetic abnormalities are diverse, tissue-specific and can occur due to various environmental factors such as toxins and drugs, including alcohol.

Epigenetic regulation of gene expression primarily works through modifying the secondary or tertiary structures of DNA (chromatin), which makes it more or less accessible to transcription. Chromatin is made up of repetitive structural units called nucleosomes. Nucleosomes are comprised of a histone octamer and the DNA that wraps around it. Histones are globular basic proteins that are subject to various covalent modifications that occur primarily on the N-terminal tail^[5]. The histone octamer contains two molecules of each of the histones H2A, H2B, H3 and H4, around which the DNA wraps. Histone H1 the “linker histone” along with “linker DNA” physically connects the adjacent nucleosome core particles. Covalent histone modifications appear to act sequentially or in combination to form a recognizable code that is identified by specific proteins to regulate distinct downstream

Table 1 Histone modifications, enzymes and genes

Modifications	Enzymes	Target gene transcription
Acetylation		
H3	GNAT	Activating
H4	MYST	Activating
H3 and H4	CBP/p300	Activating
Deacetylation		
H3 and H4	HDACs 1-11	Silencing
Methylation		
H3K4	Set1	Activating
H3K36	Set2	Activating
H3K79	DoT1L	Activating
H3K27	EZH2	Silencing
H3K9	SUV39H1	Silencing
H4K20	SUV4-20H1	Silencing
Phosphorylation		
H3S10	RSK2	Silencing
Methylated CpG	DNMT1, DNMT3a-3b	Silencing

events such as transcriptional activation or repression^[5]. Histones are subject to various post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitinylation and sumoylation, all having an impact on gene transcription^[5]. Histone acetylation is a transcription-activating modification that is achieved by addition of acetyl groups to lysine residues by enzymes called histone acetyltransferases (HATs). The major sites of acetylation in histone H3 are Lys4, Lys9, Lys14 and Lys28. Acetyl groups are removed by histone deacetylases (HDACs) and this is generally associated with loss of gene expression or silencing^[6]. Mammalian HDACs have been classified into four classes. Class I HDACs (1, 2, 3 and 8) are found predominantly in the nucleus, whereas Class II HDACs (4, 5, 6, 7, 9 and 10) shuttle between the nucleus and cytoplasm. Class I and Class II HDACs have tissue-specific expression profiles. HDACs 1, 2 and 3 are expressed in various immune cells^[7]. Class III HDACs (SIRT1-SIRT7) form a distinct class of NAD-dependent enzymes, can be inhibited by nicotinamide, and are important in DNA repair and anti-apoptotic functions^[8]. HDAC 11 possesses properties of Class I and II HDACs and is classified as Class IV. Table 1 illustrates the histone modifications and enzymes linked to the changes.

Histone methylation is catalyzed by histone methyltransferases at lysine and arginine residues on histone H3 and H4 and can be mono-, di- and tri-methylated. The major sites on histone H3 are Lys4, Lys9, Lys27, Lys36 and Lys79, whereas histone H4 is methylated on Lys20^[5]. The methyl group donor is S-adenosyl methionine. The effects of alcohol exposure on SAME-mediated epigenetic changes have been under investigation^[9]. Arginine methylation is transcription-activating, and lysine methylation can cause either transcriptional activation or repression, depending on the lysine residue methylated. For instance, H3K9 can be acetylated as well as methylated and have opposite transcriptional consequences; H3AcK9 causes transcriptional activation whereas H3K9me (mono-, di- and tri-) leads to transcriptional repression. Thus, a

balance in H3K9 acetylation and methylation may be important in determining chromatin architecture and gene silencing or activation^[5].

Histone phosphorylation is a transcription-activating modification achieved by kinases that catalyze the transfer of a phosphate group from ATP or GTP to the serine or threonine residue of histone H3. Besides phosphorylation, histone H1, H2A, H2B and H3 can be ubiquitinated at lysine residues that activate transcription. Sumoylation on the other hand occurs on lysine residues and is a transcriptionally repressive modification.

DNA methylation involves transfer of a methyl group to cytosine bases at the C5 position of CG dinucleotides, referred to as CpG dinucleotides, and may occur in clusters, known as CpG islands. By definition, CpG islands are genomic regions that are at least 200 base pairs long, with $\geq 50\%$ GC content and $\geq 60\%$ expected CpG ratio^[10]. The methyl donor is S-adenosylmethionine (SAMe) and the enzyme involved is DNA methyltransferase (DNMT). Two groups of mammalian DNMTs, one that *de novo* methylates DNA, and the other that maintains the methylation status, are classified as four different types: DNMT 1, 2, 3A and 3B. Although DNMT 3A and 3B are *de novo* methylation enzymes^[11], DNMT 1 maintains methylation status, whereas the function of DNMT 2 is not yet clear and it has weak methyltransferase activity. DNA methylation leads to transcriptional silencing due to chromatin condensation, increased recruitment of methylated CpG binding transcriptional repressor, and inhibition of DNA binding of transcriptional activators^[11]. The unmethylated CpG islands are associated with transcriptionally active promoters, and how CpG islands remain unmethylated is still unclear^[12].

Non-coding RNA (ncRNA) is another mechanism of epigenetic regulation and is driven by long or small ncRNAs. Long ncRNAs such as *Air*^[13], *Kcnq1ot1*^[14] and *H19*^[15] exert their epigenetic effect by genomic imprinting, which involves DNA methylation. On the other hand, small ncRNAs such as miRNA affect translational repression of mRNA, mRNA degradation, DNA methylation, and chromatin modification^[16]. miRNAs are short [~ 22 nucleotides(nt)] ncRNAs that regulate gene expression by binding to their cognate binding sites at the 3'-end of the target mRNA, and inhibiting their translation. miRNAs are transcribed from miRNA genes mostly by RNA polymerase II into long primary miRNA (pri-miRNA) transcripts that often contain thousands of nucleotides and form hairpins (stem loops). The pri-miRNA is processed in the nucleus by Drosha-DGCR8 complex to produce 70-80-nt precursor miRNA (pre-miRNA)^[16]. Pre-miRNAs are transported from the nucleus to the cytoplasm by exportin-5 and Ran-GTP complex, where they are further processed into ~ 22 -nt long miRNA/miRNA duplex by Dicer, a RNase type III enzyme. One of the miRNA strands bind to the cognate binding site on the target mRNA with a ~ 2 -nt mismatch, and it is binding of the RNA silencing complex at the 3' UTR of the target mRNA that represses its translation and results in gene silencing^[16].

The crosstalk between various epigenetic mechanisms

described above can determine downstream chromatin remodeling and gene expression. Mechanisms such as histone acetylation and methylation, DNA methylation and ncRNA-mediated modifications, are acquired throughout life, and persist, and influence the ability to deal with environmental factors such as nutritional factors, toxicants and lifestyle-related factors including tobacco smoke, alcohol, chemical carcinogens, infectious agents and UV radiation, thus influencing the clinical outcomes of health and disease^[17].

EPIGENETIC DYSREGULATION IN ALD

The emerging role of epigenetic regulation of gene expression by alcohol and its effect on organ injury comes from studies in the past decade^[1,2]. Here, we review mechanisms related to histone modifications and DNA methylation induced by alcohol exposure in liver cells, which contribute to ALD.

Histone modifications: acetylation, phosphorylation and methylation

Multiple lines of evidence from *in vitro* and *in vivo* studies have established that alcohol induces epigenetic modifications in various organs including the gastrointestinal system, brain and liver^[2]. In the liver, alcohol alters histone acetylation, methylation as well as phosphorylation. Selective acetylation of histone H3 at lys 9 (H3AcK9) has been observed in primary rat hepatocytes exposed to alcohol *in vitro*^[18]. On the other hand, other lysine residues H3 lys14, lys18 and lys23 were not acetylated. In the liver, H3 acetylation was modulated by alcohol *via* increased HAT activity and HDAC inhibition^[19].

The status of histone acetylation depends on the activity of HAT and HDAC^[20]. In some instances, the balance of HAT/HDAC ratio determines the acetylation of histone residues and influences gene expression^[21]. Alcohol exposure appears to alter HAT and HDAC activity in hepatocytes^[19]. *In vitro* alcohol exposure of hepatic cells impairs HDAC6 function, which directly affects microtubule dynamics^[22]. The mRNA expression of class III HDAC, sirtuin 1 (SIRT1) is reduced in alcohol-exposed hepatocytes^[23]. Furthermore, an essential role for SIRT1 in mediating effects of alcohol on SREBP-1 and hepatic lipid metabolism in alcoholic fatty liver has been reported^[24]. These results indicate that SIRT1 can be developed as a therapeutic target in ALD. Overall, alcohol seems to influence HATs and HDACs in hepatocytes. Studies are needed to determine the precise role of these altered enzyme activities in the context of ALD *in vivo*.

Similar to acetylation, phosphorylation of histones is crucial to chromatin modifications, and activates gene transcription downstream of cell signaling events^[25]. Acute alcohol exposure modulates H3 phosphorylation at serine 10 and serine 28 in rat hepatocytes, and this is dependent on p38 mitogen-activated protein kinase activity but not extracellular signal-regulated kinase and C-Jun N-terminal

kinase^[26]. Recent studies also have indicated that *in vivo* acute alcohol exposure induces H3 serine-10 and serine-28 phosphorylation which was transiently increased at 1 h, but decreased at 4 h after alcohol administration^[27]. On the other hand, persistent H3K9 acetylation in the liver was observed at 4 h after acute alcohol exposure *in vivo*^[27]. A relationship between acetylation and phosphorylation in context with gene activation has been examined^[25]. For instance, cytokine-induced gene expression mediated by nuclear IKK α leading to nuclear factor (NF)- κ B activation involves coupling of H3 phosphorylation at serine 10 and acetylation at lysine 14^[28,29]. On the other hand, retinoic acid receptor- β and c-jun gene regulation is linked to phosphorylation of histone H3 and not acetylation, which indicates that these two epigenetic changes can occur independently^[30-32]. Whether alcohol induces histone phosphorylation and acetylation synergistically or as independent pathways to regulate target gene expression in ALD remains to be investigated.

Specific methylated lysine residues on histones function as stable epigenetic marks that direct particular biological functions, ranging from transcriptional regulation to heterochromatin assembly^[33]. Histone methyltransferases catalyze transfer of a methyl residue predominantly to H3 and H4 histones that impart biological functions such as transcription or epigenetic silencing^[33]. Shukla *et al*^[34] have shown differential methylation of H3 and H4 in rat hepatocytes exposed to alcohol *in vitro*. These studies have indicated that methylation at H3meLys9 is decreased and is associated with downregulation of genes such as Lsdh, whereas increased methylation of H3meLys4 is associated with upregulation of alcohol dehydrogenases (ADH1)^[34]. Whether acute or chronic alcohol-mediated histone methylation serves as a stable genomic imprint that determines the transcriptional state of a gene contributing to disease is still unknown.

DNA methylation in ALD

Methylation of cytosine at C5 in the CpG dinucleotides silences transcription, whereas absence of methylation activates transcription. Although 80% of CpG dinucleotides are methylated, unmethylated CpG residues in promoter regions of constitutively active genes are referred to as CpG islands^[35]. The predominant methyl donor, SAMe, which is important in DNA methylation, is depleted in alcoholic livers, which results in hyperhomocysteinemia that is commonly observed in patients with ALD^[36]. Decreased SAMe in alcoholic livers also seems to affect DNA methylation. Rats fed an intragastric alcohol diet for 9 wk exhibited decreased methionine, SAMe, glutathione and loss of DNA methylation by 40%^[37]. This DNA hypomethylation can lead to alteration in gene expression and chromatin structure, results in increased DNA damage and strand breaks^[37,38], which predisposes cells to malignant degeneration^[39]. An association between alcohol intake and hypomethylation of the O⁶-methylguanine DNA methyltransferase gene has been noted in context with hepatocellular carcinoma^[40]. Studies also have shown that alcohol-metabolizing enzyme, ADH1,

is regulated by epigenetic mechanisms in hepatoma cells involving DNA hypomethylation^[41]. In addition to direct effects of alcohol on DNA hypomethylation, direct effects of acetaldehyde on DNA methyltransferase^[42] and methionine synthase^[43,44] have been reported. Whether alcohol directly alters DNA methyltransferase activity and methionine synthase is yet unknown. Chronic alcohol consumption induces global DNA hypomethylation^[45], whereas hypermethylation of DNA is observed in human peripheral blood cells after alcohol consumption in humans^[46,47]. Alcohol-associated hypomethylation thus far is linked to decrease in SAMe, the methyl donor.

An interplay between histone acetylation and DNA methylation is involved in the process of gene transcription and/or silencing in diseased states^[17]. For instance, hypermethylation of CpG islands in target gene promoters triggers deacetylation of local histones, whereas lower levels of histone acetylation seem to sensitize DNA to methylation. Although there is an intimate communication between these two epigenetic phenomena, it is still not clear whether there is any hierarchical order of these events. In chronic alcohol exposed livers, whether an interconnection exists between hyperacetylation of H3K9, loss of methylation of H3K9, and increased methylation of H3K4, along with global hypomethylation of DNA is unknown. Studies undertaken to identify the interplay of epigenetic events will provide new insights into mechanisms of ALD.

miRNAs AS EPIGENETIC MODULATORS IN ALD

The role of miRNAs as epigenetic modulators is apparent from its regulation of gene expression at the post-transcriptional level. miRNAs elicit degradation of target mRNA or hinder translational mechanisms such as initiation, elongation, degradation or segregation of mRNA into P bodies for translational inhibition^[48-50]. The importance of miRNAs in liver diseases such as viral hepatitis, alcoholic fatty liver disease and non-alcoholic fatty liver disease (NAFLD), fibrosis, and hepatocellular carcinoma has recently been recognized^[51]. Studies have shown that alcohol exposure regulates miRNAs that control post-transcriptional events and influence gene expression that are important in ALD^[52]. miRNAs have been linked to lipid metabolism and inflammation in steatohepatitis^[53-56]. Dolganiuc *et al*^[52] have shown that alcohol feeding increases and decreases ~1% of known miRNA, whereas methionine/choline-deficient diet upregulates 3% and reduces 1% of known miRNAs. Common to ALD and NAFLD, miR-705 and miR-122 are increased with both diets. However, miR-182, miR-183 and miR-199a-3p are increased in NAFLD and decreased in ALD mouse models. The functional relevance of miRNAs in ALD and NAFLD remains to be determined. Using hepatocyte cultures, recent studies have shown that ~11 miRNAs are altered in hepatic lipid droplets^[57]. MiR-181d is the most efficacious inhibitor and reduces lipid droplets by 60%, which confirms its role in cellular synthesis of triglycerides

and cholesterol esters^[57]. More recent studies have shown that chronic alcohol induces miRNA-155 in liver macrophages^[58]. Chronic alcohol exposure also alters miRNAs that affect intestinal permeability^[59]. Alcohol intake results in induction of miR-212, which in turn downregulates tight junction protein zonula occludens 1^[59]. miRNA-212 is also higher in colon biopsy samples of patients with ALD, which confirms the pathophysiological significance of miRNA-212 in altering intestinal permeability during ALD. The role of miRNAs as epigenetic modulators of gene expression and silencing is becoming evident in ALD. An improved understanding of miRNAs and subsequent post-transcriptional regulatory mechanisms will be of importance in advancing their application as diagnostic or prognostic markers, as well as therapeutic targets in ALD.

EPIGENETICS AND INFLAMMATION IN ALD

The first report on aberrations in the epigenetic code in an inflammatory disease condition such as rheumatoid arthritis was reported in 1990^[60]. Anti-inflammatory effects of HDAC inhibitors used in a number of experimental models of inflammatory diseases have confirmed a role for epigenetics in immune function^[61-63]. Epigenetic regulatory mechanisms are central to the immune response, allowing an appropriate gene expression pattern in immune cells^[64,65]. Environmentally regulated or endogenously mediated epigenetic alterations contribute to environment-host interactions in various forms of inflammatory diseases^[66].

Innate immune responses and macrophage function play a central role in ALD^[67,68]. Epigenetic modifications that influence inflammatory responses have been reported in macrophages^[69] during lipopolysaccharide (LPS) tolerance. This phenomenon is dependent on histone acetylation and H3K4 methylation, as well as chromatin remodeling enzymes such as SW1/SNF^[70]. Acute alcohol exposure decreases LPS induced proinflammatory responses in human monocytes^[71,72]. Whether acute alcohol mediates histone modifications and recruitment of nucleosome remodeling enzymes at the promoters of the cytokine genes is yet to be determined.

The transcription factor NF- κ B is a master regulator of proinflammatory responses in macrophages and monocytes^[73,74]. It is the organization of the chromatin structure that controls the kinetics of NF- κ B recruitment to target gene promoters that represents a focal point in mediation of transcription cooperativity (Figure 1). Acute and chronic alcohol exposure modulates NF- κ B DNA binding that regulates expression of proinflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6^[68,75]. A tailored immune response that is regulated by NF- κ B *via* interaction with HATs and HDACs elicits epigenetic modifications^[76]. For instance, HDAC-1 is known to form a complex with NF- κ Bp50 homodimers to repress gene transcription^[77]. On the other hand, inflammatory stimuli such as LPS can induce phospho-

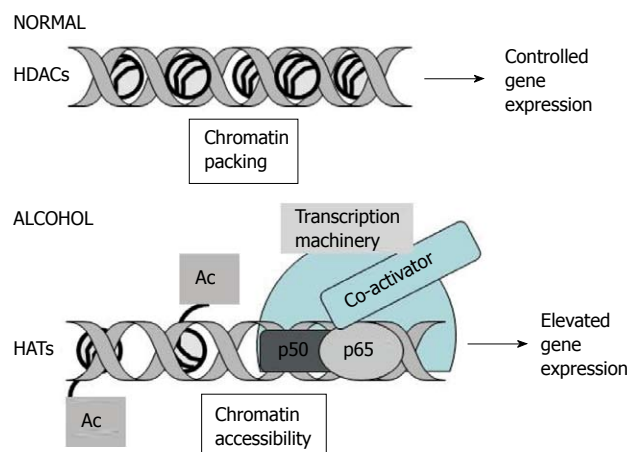


Figure 1 Epigenetic regulation of gene expression via histone modification. Under normal conditions, activation of histone deacetylases (HDACs) inhibits acetylation (Ac) of histones, which results in chromatin packaging, restriction of DNA accessibility for transcription, and controlled target gene expression. During alcohol exposure, histone modifications mediated by histone acetyltransferases (HATs) influence elevated transcription of target genes, due to chromatin accessibility, which leads to increased DNA occupancy of transcription factors such as nuclear factor- κ B (p50/p65), co-activators and transcriptional complexes, and elevated target gene expression.

acetylation of histone H3 (K9/S10) at a subset of cytokine and chemokine gene promoters for increased NF- κ B recruitment^[29,78,79]. Reductions in H3 Lys9 methylation, along with increased H3/H4 acetylation are strongly correlated with RNA polymerase II recruitment, which results in transcription of NF- κ B-inducible inflammatory genes^[79]. Whether alcohol exposure affects epigenetic mechanisms to prolong or reduce DNA binding of NF- κ B to the cytokine gene promoters in monocytes and macrophages is not yet known. Another environmental agent, cigarette smoke induces post-translational modification of phospho-deacetylases, HDAC2 and SIRT1, acetylation of p65-NF- κ B and phosphoacetylation of histone H3 that leads to chromatin rearrangement and sustained proinflammatory gene transcription^[80,81]. It is likely that chronic alcohol exposure modulates HDAC2 and SIRT1 in immune cells to regulate epigenetic events that promote prolonged proinflammatory responses in ALD.

In macrophages stimulated with viruses or bacteria, miRNA-155 is the only increased miRNA induced by both stimulants^[82,83]. miRNA-155 regulates TNF- α production positively by enhancing its translation^[84]. Bala *et al.*^[58] recently reported that chronic alcohol induces miRNA-155 in an NF- κ B-dependent manner that increases mRNA stability and TNF- α expression in ALD. miR-146 is predominantly expressed in T regulatory and Th1 cells^[85] and is upregulated in toll-like-receptor-stimulated macrophages in an NF- κ B-dependent manner. miR-146 targets IRAK-1 and TRAF6 genes and is unaffected by chronic alcohol exposure in liver immune cells^[58]. Overall, these findings suggest that miRNAs are capable of regulating alcohol-induced innate immune cell function and thus determining cellular memory *via* miRNA-mediated epigenetic modulation.

ALCOHOL, OXIDATIVE STRESS AND CHROMATIN REMODELING

Oxidative stress regulates chromatin remodeling by alteration of histone acetylation and deacetylation events *via* HAT/HDAC activity^[86,87]. Acute and chronic alcohol exposure increases reactive oxygen species (ROS) production and lowers antioxidant levels that enhance oxidative stress in the liver^[88]. Metabolism of alcohol through alcohol dehydrogenase and microsomal cytochrome P450 2E1 leads to enhanced production of ROS in the liver^[88]. Acetylation of histone H3 by alcohol in rat hepatocytes is mediated by ROS^[89]. Inhibition of NADPH-oxidase-mediated ROS results in decreased H3AcK9, whereas ROS inducers directly increase alcohol-induced acetylation of H3K9, along with induction of ADH1 mRNA expression^[89]. A redox-sensitive class III HDAC molecule, SIRT1, is also decreased in alcohol-exposed rat hepatocytes and livers of alcohol-fed rats^[24]. Whether alcohol-induced ROS^[90] play an important role in modulation of SIRT1 to regulate steatosis remains to be determined. In addition to alcohol, acetaldehyde and acetate, which are products of alcohol metabolism, cause acetylation of H3K9. Pyrazole, an inhibitor of alcohol dehydrogenase and methyl cyanamide, an inhibitor of aldehyde dehydrogenase, both reduce H3K9 acetylation, which indicates that alcohol and its metabolites can trigger acetylation of histone residues. Similar to *in vitro* observations, *in vivo* acute alcohol exposure in rats also shows that H3K9 acetylation is significantly increased in the liver^[91]. Studies have shown that H3K9 acetylation in hepatocytes due to alcohol exposure correlates with transcriptional increase in alcohol dehydrogenase (ADH1)^[19]. It is likely that alcohol-induced acetylation is required for activation of alcohol-metabolizing enzymes, which induce oxidative stress. This, in turn, induces acetylation that creates an amplifying “autocrine loop” between alcohol metabolism and epigenetic events. Future studies to determine the precise role of alcohol-mediated oxidative stress in chromatin modifications in hepatocytes and liver macrophages will identify new pathophysiological mechanisms and epigenetic targets of gene expression in ALD.

Stress-induced heat shock transcription factor (HSF)1 plays an important role as a transcriptional repressor of proinflammatory cytokine genes^[92,93]. Recent studies have suggested that HSF1 serves as a master regulator of global acetylation in normal cells, whereas, in stressed cells, HSF1 interacts with HDAC1 and HDAC2, which induce histone deacetylation and chromatin remodeling^[94]. Studies from our laboratory have shown that alcohol exposure induces HSF1 DNA binding activity in monocytes and macrophages^[95]. Whether alcohol-induced HSF1 induces chromatin reorganization that affects proinflammatory cytokine production is being investigated. Another key stress-induced molecule, heat shock protein (hsp)90, is characterized as an epigenetic “gatekeeper” that interfaces with the environment and may finally determine whether certain epigenetic markers succeed in downstream pheno-

typic expression^[96-98]. Chronic alcohol exposure upregulates hsp90 expression in liver macrophages^[95]. It is likely that hsp90 facilitates an alcohol-mediated “epigenetic code” in the liver. Studies to delineate epigenetic effects of alcohol-induced hsp90 on gene transcription in liver macrophages and hepatocytes are awaited.

Hydroxyl radicals generated by oxidative stress interfere with the ability of DNA to function as a substrate for DNMT, which results in global hypomethylation^[99,100]. Recent studies have provided evidence for the role of endoplasmic reticulum stress pathways and epigenetic gene regulation^[101]. It is likely that alcohol-mediated oxidative stress regulates epigenetic markers in ALD.

CONCLUSION

The interplay of epigenetic mechanisms and their influence on gene transcription in ALD is evolving. Epigenetic alterations associated with acute and chronic alcohol exposure of hepatocytes and immune cells in relation to ALD is discussed in this review. Studies thus far have shown that alcohol exposure, probably *via* oxidative stress, exhibits differential regulation of acetylation, phosphorylation and methylation of histones that regulate chromatin remodeling and gene expression. The effects of alcohol on DNA methylation in hepatocytes and miRNA regulation have been elucidated. An integrative approach of the various mechanisms that lead to genomic imprinting during alcohol exposure will identify novel pathways in the alcoholic liver and support epigenetic therapeutic interventions.

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