Review

JHEP|Reports

Epigenetics of alcohol-related liver diseases

Nawras W. Habash, Tejasav S. Sehrawat, Vijay H. Shah,* Sheng Cao*

Summary

Alcohol-related liver disease (ARLD) is a primary cause of chronic liver disease in the United States. Despite advances in the diagnosis and management of ARLD, it remains a major public health problem associated with significant morbidity and mortality, emphasising the need to adopt novel approaches to the study of ARLD and its complications. Epigenetic changes are increasingly being recognised as contributing to the pathogenesis of multiple disease states. Harnessing the power of innovative technologies for the study of epigenetics (*e.g.*, next-generation sequencing, DNA methylation assays, histone modification profiling and computational techniques like machine learning) has resulted in a seismic shift in our understanding of the pathophysiology of ARLD. Knowledge of these techniques and advances is of paramount importance for the practicing hepatologist and researchers alike. Accordingly, in this review article we will summarise the current knowledge about alcohol-induced epigenetic alterations in the context of ARLD, including but not limited to, DNA hyper/hypo methylation, histone modifications, changes in non-coding RNA, 3D chromatin architecture and enhancer-promoter interactions. Additionally, we will discuss the state-of-the-art techniques used in the study of ARLD (*e.g.* single-cell sequencing). We will also highlight the epigenetic regulation of chemokines and their proinflammatory role in the context of ARLD. Lastly, we will examine the clinical applications of epigenetics in the diagnosis and management of ARLD.

Keywords: Alcohol liver disease; DNA methylation; Epigenetics; Histones; Acetylation; Chromatin architecture; Computational biology; Single cell epigenome; Epidrugs

Received 19 November 2021; received in revised form 14 February 2022; accepted 22 February 2022; available online 10 March 2022

Introduction

Alcohol consumption is a leading cause of preventable death and is responsible for roughly 3.3 million deaths annually (5.9% of all deaths).[1] Alcohol-related liver disease (ARLD) is one of the most prevalent forms of liver disease in the world. ARLD represents a spectrum of disorders that encompasses alcohol-related fatty liver, alcohol-related hepatitis (AH)/steatohepatitis (ASH), alcohol-related cirrhosis and hepatocellular carcinoma (HCC). The natural history and pathophysiology of ARLD are complicated. The vast majority of chronic alcohol users develop alcohol-related fatty liver, but only a minority progress to alcohol-related cirrhosis or HCC.[2] Genetic and epigenetic factors, at least in part, determine disease onset and progression. For example, genome-wide association studies revealed multiple genes that were linked to the risk and severity of ARLD (*e.g. PNPLA3, TM6SF2, MBOAT7, HSD17B13*).[3–14]

The management of ARLD is determined by the extent of the disease. Abstinence, nutritional support, and screening for associated complications (*e.g.*, HCC) represent the foundation of ARLD management.[2,15–17] Agents like the tumour necrosis factor (TNF)-α inhibitors infliximab and etanercept have been used to treat AH based on their anti-inflammatory properties, but results have been disappointing.[18,19] Currently no agents are available that truly alter the outcome of advanced ARLD. Accordingly, liver transplantation is the only long-term management solution. Notably, ARLD accounted for 28% of all patients on the liver transplant waiting list in the US between 2006 and 2014.[20]

The scarcity of available organs, the risk of relapse following transplantation, and the 'self-inflicted' and 'moral failing' view of ARLD raise numerous ethical questions, with the main question being 'Is it fair to give patients with ARLD such a limited resource?'. Studies have shown that the majority of patients transplanted for ARLD have good outcomes with relatively low rates of relapse when proper selection criteria are applied (*e.g.* abstinence for >6 months, presence of appropriate social support…etc).[21,22]

Given the significant burden associated with ARLD and limited treatment options, viewing ARLD through the lens of epigenetics is of paramount importance, particularly in the era of individualised and precision medicine. Understanding the intricate mechanisms that orchestrate the maintenance and reprogramming of the genetic code of the constituent cells of the liver in health and disease provides an unrivalled opportunity to prevent, better diagnose (*e.g.* liquid biopsies),[23–26] and

Gastroenterology Research Unit, Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN USA

* Corresponding authors. Address: Division of Gastroenterology and Hepatology, Mayo Clinic, 200 First St SW, Rochester, MN 55905, USA. Tel. 507-255-6028, fax: 507-255-6318.
E-mail addresses: shah.vijay@mayo.edu (V.H. Shah), cao.sheng@mayo.edu (S. Cao).

potentially reverse the deleterious effects of alcohol (*e.g.* epidrugs).[27,28]

Herein, we will cover the basic epigenetic mechanisms while highlighting relevant examples from the realm of ARLD when applicable. Additionally, we will discuss the chromatic structure and enhancer-promoter (E-P) interactions and their role in ARLD. We will conclude by summarising the clinical applications of epigenetics in the field of ARLD.

Key points

- The definition of epigenetics has evolved over time. Currently it refers to "the study of molecules and mechanisms that can perpetuate alternative gene activity states in the context of the same DNA sequence"
- The epigenetic machinery controls gene expression by modulating chromatin confirmation, high order chromatin 3D structure, and the interaction between genes and the transcriptional apparatus.
- Epigenetic dysregulation is central to the pathophysiology of ARLD.
- Advances in the study of epigenetics and single-cell epigenome technique and their implications in health and disease have paved the way for novel diagnostic modalities (liquid biopsy) and therapeutic options (epidrugs).
- Selective bromodomain inhibitors are a novel class of molecules with therapeutic potential in a wide range of liver diseases.

Epigenetics: the writers, the readers, and the erasers

The definition of epigenetics has evolved over time, in keeping with our deepening understanding of cell fate, pluripotency, and plasticity. Originally, the term *epigenetics* referred to the process by which the genotype brings the phenotype into being. Currently, it refers to 'the study of molecules and mechanisms that can perpetuate alternative gene activity states in the context of the same DNA sequence.'[29] The major epigenetic mechanisms include DNA methylation, post-translational modification of histones (methylation and acetylation of lysine and arginine, phosphorylation of serine and threonine, ubiquitination, and SUMOylation of lysine), ADP-ribosylation, histone replacement, and non-coding RNAs. Traditionally, epigenetic modifiers are classified into 3 groups: the writers, the readers, and the erasers. An in-depth description of the epigenetic machinery is outside the scope of this article. We refer the reader to the following recent publications for more details.[29–33] We also encourage the reader to refer to Fig. 1.

DNA methylation, histone modifications and non-coding RNA in ARLD

DNA methylation

DNA methylation is one of the better-described epigenetic modifications. Methylation occurs mostly at the 5th carbon of cytosine within CpG dinucleotide-rich islands that predominantly occupy the 5′ promoter region of genes. S-Adenosyl-L-methionine is the methyl group donor. The deposition, removal, and maintenance of methyl groups is a dynamic process that is mediated by a group of enzymes of 2 broad types: DNA

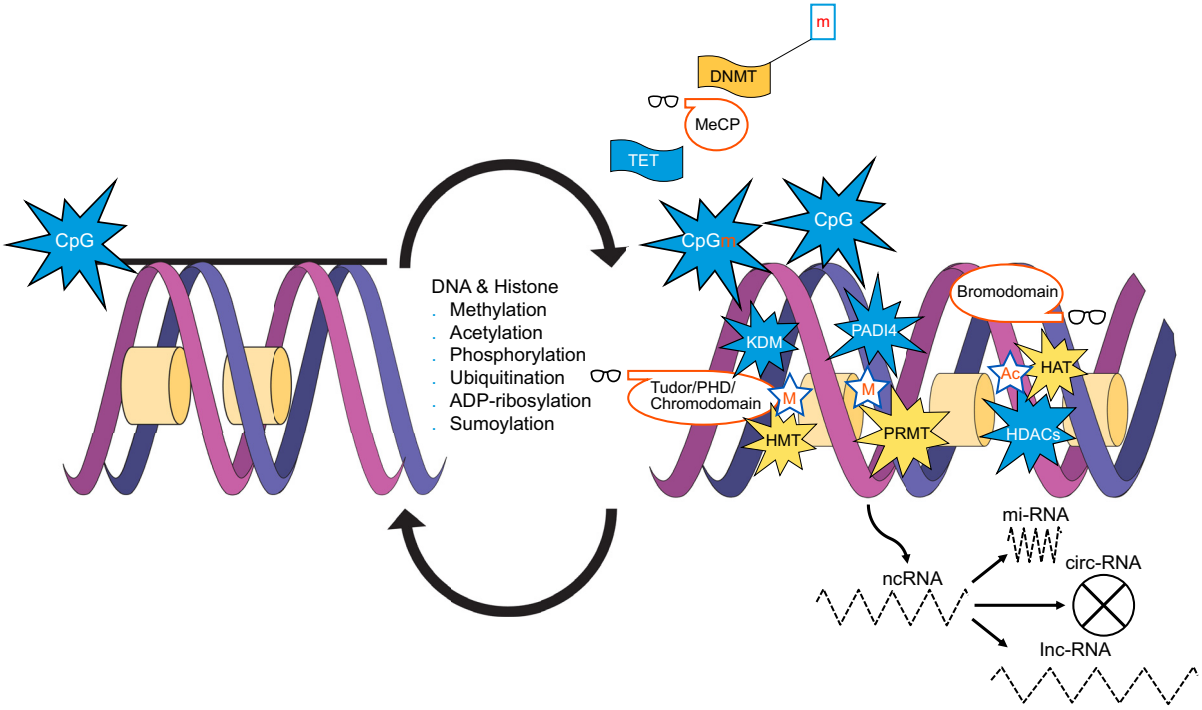


Fig. 1. The epigenetic modifiers: writers, readers, and erasers. DNA methylation is mediated by DNMT (writer). Methyl groups (M) are attached to CpG dinucleotide islands, reversed by TET (eraser). MECP2 (reader) recognises methyl groups and represses expression of associated genes. Histone methylation is mediated by HMT (writer). KDM and PADI4 (erasers) remove methyl groups from lysine and arginine, respectively. Tudor/PHD/chromodomain (readers) recognise methylated histones. Histone acetylation is mediated by HAT (writer) and reversed by HDAC (eraser). Acetyl groups are recognised by bromodomains (readers). Non-coding RNAs interact with the different epigenic modifiers and modulate their function. circRNA, circular RNA; DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; KDM, lysine demethylase; lncRNA, long non-coding RNA; MECP2, methyl-CpG binding protein 2; miRNA, microRNA; ncRNA, non-coding RNA; PADI4, peptidyl arginine deiminase 4; PHD, plant homeodomain; TET, ten eleven translocation.

methyltransferases (DNMTs) and DNA demethylases.[33] Methylated CpG islands act as binding sites for repressive proteins with a methyl-binding domain (*e.g.* methyl-CpG binding protein 2 [MECP2]).[34] DNA methylation can be altered in response to alcohol consumption via different mechanisms, namely, direct inhibition of DNMTs, distortion of the 1-carbon metabolism cycle via changes in the intracellular redox balance, or limited dietary intake of folic acid (methyl donor).[35] Differential methylation of specific CpG dinucleotides in patients with ARLD and non-alcohol-related fatty liver disease (NAFLD) has been well described.[36,37]

Alcohol-related steatosis and steatohepatitis and DNA methylation

Increased levels of DNA methylation were shown to be present in patients with alcohol-related disease by Bonsch *et al.* more than a decade ago.[38] In the subsequent years, numerous studies have linked DNA methylation and the development of ASH.[39–43] More recently, a study uncovered a new axis consisting of FKBP5-YAP-TEAD1-CXCL1 connecting ethanol consumption and the development of steatohepatitis.[44] FK506-binding protein 5 (FKBP5) is a cochaperone protein that is involved in stress-related disorders.[45] FKBP5 expression was shown to be upregulated in patients with ARLD and in ethanol-fed mice. When compared with *Fkbp5* knockout mice, wild-type mice had higher expression of FKBP5 after eating ethanol-containing chow. Ethanol feeding also led to neutrophilic infiltration of the liver, which is a histologic hallmark of AH.[46–48] Intriguingly, this effect was attenuated in *Fkbp5* knockout mice. In patients with ARLD, the promoter of *FKBP5* was hypomethylated. By interacting with Yes-associated protein (YAP) and TEA domain transcription factor 1 (TEAD1), FKBP5 increases the expression of the inflammatory C-X-C motif chemokine ligand (CXCL)1. This axis consisting of FKBP5-YAP-TEAD1-CXCL1 may provide new targets for future treatments.[44]

Liver fibrosis, ARLD and DNA methylation

Fibrosis is a common end pathway of many liver disease processes including ARLD.[43,49–51] In a healthy liver, hepatic stellate cells (HSCs) are quiescent perisinusoidal cells, but in response to hepatic damage, HSCs undergo transdifferentiation into extracellular matrix-depositing myofibroblasts.[50,51] HSC transdifferentiation is mediated in part by DNA methylation.[50,52]

In support of this finding, treatment of HSCs with the DNMT inhibitor azacitidine prevented HSC transdifferentiation in murine models.[53] Peroxisome proliferator activated receptor-γ (*PPARG*) is one of many genes that is differentially methylated during the process of HSC transdifferentiation. MECP2 promotes the methylation and repression of *PPARG* and inhibition of MECP2 was associated with reduced fibrosis in murine models.[54,55] Likewise, the histone methyltransferase G9a activity, alongside DNMT1, was linked to the fibrogenic activation of HSCs in patients with chronic liver injury including those with ARLD.[56] Intriguingly, the use of the novel dual G9a/DNMT1 inhibitor CM272 reduced the burden of fibrosis in mouse models.[56]

In patients with either alcohol or non-alcohol-related liver disease, the same differential methylation pattern at the *PPARG* promoter was detectable in the pool of cell-free DNA. Moreover, the levels correlated with the degree of hepatic fibrosis.[57] Interestingly, the negative effects of toxins, including alcohol, can influence subsequent generations, a phenomenon known as transgenerational epigenetic inheritance.[58,59] Against this background, the offspring of rats with carbon tetrachloride–induced hepatic fibrosis demonstrated upregulation of PPARγ as an adaptive protective mechanism. These effects are believed to be mediated through DNA methylation and histone acetylation in the paternal sperm.[60]

Post-translational histone modification

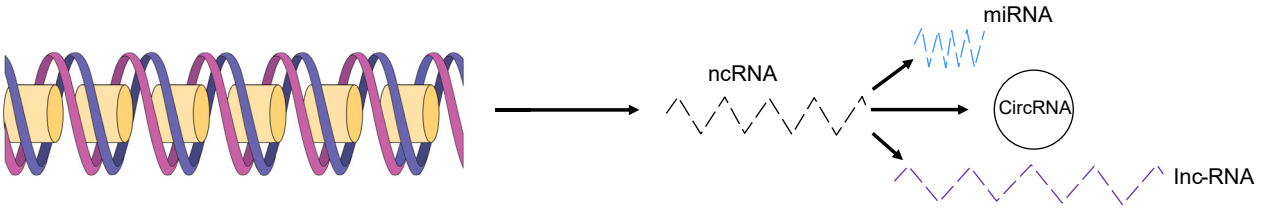
Histone methylation and acetylation have been studied extensively as markers of chromatin expression states.[61–63] Post-translational modification of histones affects DNA expression via 2 pathways: i) by changing the charge of histone proteins via acetylation, thus loosening the binding to nucleosomal DNA and leading to increased expression; and ii) by post-translational modifications acting as homing signals for proteins that can alter DNA expression.

Histone acetylation

Histone acetylation and deacetylation are catalysed by the enzymes histone acetyltransferase and histone deacetylase (HDAC), respectively.[32,64] Unlike histone methylation, histone acetylation is linked to transcriptional activation by promoting a less-taut 3D configuration of DNA or by acting as a signal for reader proteins (*e.g.*, bromodomain [BRD]-containing proteins).[32]

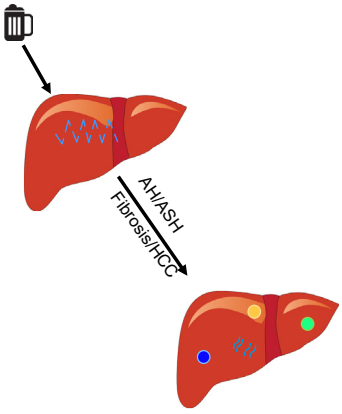
Ethanol is known to affect hepatocyte nuclear histone acetylation status in a time-and concentration-dependent manner.[65–67] For example, the expression of class I alcohol dehydrogenase (ADH1) – a key enzyme in the metabolism of alcohol – was upregulated in response to alcohol-containing chow in rats. This upregulation was associated with increased histone acetylation of the promoter region and coding region of the *ADH1* gene.[66,68] Excessive alcohol use can lead to hepatic steatosis. Interestingly, binge alcohol treatment affected metabolic pathways controlling lipogenesis and fatty acid β-oxidation by deregulation of various HDACs.[69] Similarly, increased histone acetylation of the promoter region of *PNPLA3* (patatin like phospholipase domain containing 3) was demonstrated in response to alcohol treatment in mouse models.[70] Sterol regulatory element-binding proteins (SREBPs) play a central role in cholesterol and lipid metabolism and dysregulation of SREBPs is associated with hepatic steatosis.[71] Interestingly, SREBP-1 activity is augmented in response to alcohol treatment via increased histone acetylation.[72] This effect was abolished following treatment with resveratrol, a potent sirtuin (SIRT)1 agonist.[72] Along the same line, overexpression of SIRT2 mediated deacetylation of CCAAT/enhancer binding protein-β, which prevented alcohol-induced liver injury.[73] Likewise, repression of carnitine palmitoyltransferase-1 gene expression via the action of HDAC1 explains the mechanism that underpins the ethanol-mediated decrease in carnitine palmitoyltransferase-1 expression and alcohol-related steatosis. This effect was ameliorated following treatment with the HDAC1 inhibitor tributyrin.[74]

Alcohol promotes inflammation.[75,76] In ARLD, alcohol erodes gut endothelial integrity, which leads to increased translocation of lipopolysaccharide (LPS) into the portal circulation.[48] Ethanol and its end product acetate directly affect macrophages' response to LPS.[77,78] Macrophages cultured in methanol-containing medium, exhibited enhanced expression of interleukin (IL)-6, IL-8, and TNF-α after LPS stimulation.[77,78] Promoter sites of proinflammatory genes in alcohol-treated macrophages exhibited increased acetylation which can be attributed to



Small ncRNA (<200 bp) | Long ncRNA (>200 bp) | Circular ncNRA (CiRNA) | Enhancer RNA (eRNA)

- Small interfering RNA's (siRNAs)
- Micro-RNAs (miRNAs)
- Piwi-interacting RNAs (piRNAs)
- Small nucleolar RNAs (snoRNAs)
- Small nucleolar RNAs (snoRNAs)
- Small Cajal body RNAs (scRNA)



miRNA

Upregulated		Downregulated	
MiRNA	Function	MiRNA	Function
miR-21	HCC activation/fibrosis	miR-26a	Hepatoprotective
miR-27a	Macrophage polarization (M2 phenotype)	miR-29	Antifibrotic
miR-34a	Steatosis, inflammation, fibrosis	miR-122	Lipid metabolis, HIF-1 a regulation
mir155	Kupffer cell activation	miR-125b	ECM production/fibrosis
miR-182	Inflammation/ chemokine release	miR-126	Antifibrotic
miR-200a	Hepatocyte apoptosis	miR-181b-3p	Antiinflammatory
miR-214 127	Hepatocyte oxidative stress	miR-199 128	Antiinflammatory
miR-217 99	Steatosis, inflammation	miR-223	Antiinflammatory
miR-291b	Inflammation	miR-378	Antifibrotic

Fig. 2. Role of ncRNA in alcohol-related liver disease. NcRNAs are a group of RNA molecules of various length that are not translated into protein. The role of miRNA in the pathogenesis of alcohol-related liver disease has been reviewed extensively over the years.[14,35,157–161] Dysregulation of miRNAs in alcohol-related liver disease is linked to the development of steatosis (yellow circle), inflammation via recruitment of inflammatory cells (blue circle), fibrosis (blue lines) and HCC (green circle). The associated table shows examples of dysregulated miRNAs in alcohol-related liver disease and their role in its pathogenesis. References (miR-21,[162–164] miR-26a,[165] miR-27a,[166,167] miR-29,[168] miR-34a,[169–171] miR-122,[95,172,173] mir155,[93,174–177] miR-125b,[178] miR-182,[179,180] miR-126,[181] miR-200a,[182] miR-181b-3p,[183] miR-214,[184] miR-199,[185] miR-217,[94] miR-223,[186] miR-291b,[187] miR-378[188]). EMT, epithelial-mesenchymal transition; HCC, hepatocellular carcinoma; ncRNA, non-coding RNA.

reduced HDAC activity. This effect was prevented by inhibiting the metabolism of ethanol into acetate.[77] Curiously, the use of the SIRT1 inhibitor sirtinol augmented TNF-α release from LPS-treated macrophages.[78]

Histone methylation

Histone methylation and demethylation at lysine and arginine residues of histones H3 and H4 are mediated by histone methyltransferase and lysine demethylase, respectively. Lysine and arginine can be monomethylated or dimethylated, and lysine can be trimethylated. The influence of lysine methylation on DNA expression is complex and depends on the lysine residue methylated.[79] Generally, methylation events occurring at some locations (*e.g.*, H3K4, H3K36, and H3K79) lead to transcriptional activation, whereas methylation at H3K9, H3K27, and H4K20 is linked to transcriptional repression.[80] In the context of ARLD, multiple studies showed altered histone methylation in rat hepatocytes after ethanol treatment, with increased H3K4 dimethylation and decreased H3K9 dimethylation in one study.[81] Notably, the changes observed in the histone methylation status were dependent on the mode of alcohol exposure, namely, acute binge model *vs.* chronic model.[82] Other studies investigated the role of histone methylation in AH and fibrosis. For example, LPS leads to increased methylation of the *TNF* promoter region. This effect was abrogated by S-adenosyl L-methionine treatment.[83] Regarding fibrosis, a direct effect of ethanol was seen on HSCs. When cultured in ethanol-containing media, primary rat HSCs demonstrated increased expression of extracellular matrix-associated genes, including type I/III collagen, elastin, and tissue inhibitor of metalloproteinases.[37] MLL1 (*KMT2A*) and H3k4 methylation were enriched at the elastin gene in alcohol-treated HSCs.[37]

Non-coding RNA

Non-coding RNA refers to a wide range of RNA molecules of varying lengths and functions (Fig. 2). We will focus on the role of microRNA (miRNA) and long non-coding RNA (lncRNA) in the pathogenesis of ARLD.

MicroRNA and ARLD

Alcohol promotes inflammation, steatosis, and subsequently fibrosis by regulating multiple miRNAs.[84–91] In alcohol-fed murine models, miR-132 and miR-155 levels were upregulated in Kupffer cells.[92] MiR-155 enhances the proinflammatory effects of alcohol on Kupffer cells.[93] Overexpression of miR-217 worsens ASH through SIRT1 inhibition. Targeting miR- 217 using miRIDIAN hairpin inhibitor ameliorated these effects.[94] Multiple miRNAs are downregulated in the pathogenesis of ARLD (*e.g.*, miR-122, miR-148a, miR-708).[95–97] miR-122 plays a protective role against alcohol-mediated liver injury by reducing the level of hypoxia inducible factor-1α (HIF1α). Little is known about the regulation of miR-122 in ARLD.[95] A recent study recognised the transcriptional regulator (GRHL2) to be responsible for the downregulation of miR-122 and subsequent increase in HIF1α in

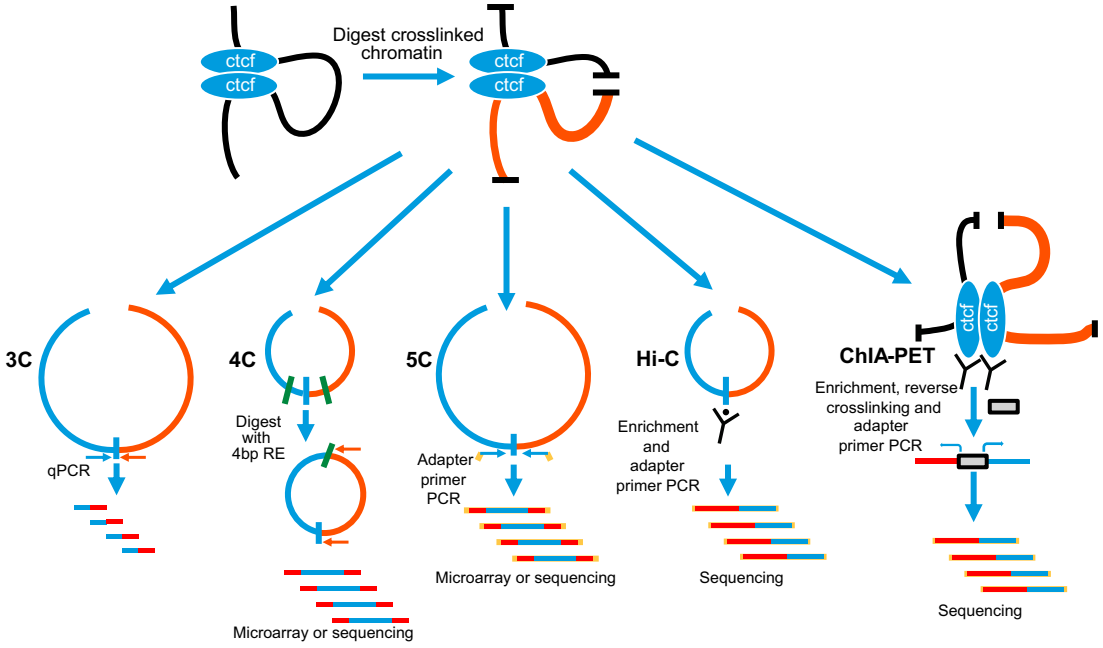


Fig. 3. Chromosome conformation capture (3C) methods study the interactions between genetic loci in 3D structure. 3C methods study the interactions between genetic loci in 3D structure. These 3D interactions can be hundreds or thousands of bases to megabases apart in sequence and can heavily regulate gene expression. Most 3C assays share the same basic principles, mainly the fixation of genomic DNA-DNA and DNA-protein interactions through chemical crosslinking. The DNA is then digested, proteins disassociated, and the crosslinked fragments are ligated. Fragments are sequenced and mapped onto the genome to localise these interactions,[104] 3C, chromosome conformation capture; 4C, chromosome conformation capture-on-chip; 5C, carbon copy chromosome conformation capture; ChIA-PET, chromatin interaction analysis by paired-end tag sequencing; CTCF, CCCTC-binding factor; Hi-C, chromosome capture followed by high-throughput sequencing.

murine alcohol disease models.[95] Similarly, miR-148a protects against inflammasome activation and pyroptosis via thioredoxin-interacting protein inhibition. Forkhead box O1 and hepatocyte nuclear factor 4α (HNF4α) have recently been described as novel transcriptional regulators of miR-148a in the context of ARLD.[96,98] miR-148a has also been shown to regulate the expression of multiple enzymes essential for the metabolism of various substances including alcohol (*e.g.*, cytochrome P450 and alcohol dehydrogenase 4.[98,99] Also, miR-708 is suggested to inhibit hepatic inflammation and steatosis through its effect on ZEB1.[97]

lncRNA and ARLD

Not much is known about the contribution of lncRNA in the context of ARLD. Multiple studies have linked lncRNA to the development of ARLD and progression to HCC. Dou *et al.*[100] analysed the effect of alcohol on lncRNA expression profiles in a murine ARLD model. In total, 29 lncRNAs were identified, 17 of which were downregulated. Pathway analysis of the top 5 downregulated lncRNAs (mou_lnc_0610005C13Rik, mou_lnc_1700023H06Rik, mou _ lnc _ Gm12265, mou _ lnc _ AW495222(39,807), and mou_lnc_Gm45724) showed an association with alcohol-induced hepatic oxidative damage and cellular inflammation. Furthermore, 5 regulatory networks were constructed to provide a deeper understanding of the mechanism of action of these lncRNAs in ARLD, but validation studies are awaited.[100] Also, through its interaction with SIRT1, the lncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) has been shown to propagate fibrosis and

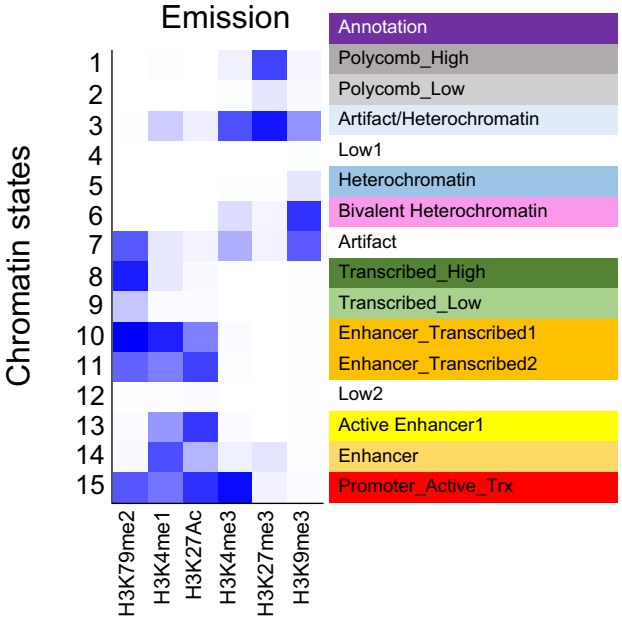


Fig. 4. ChromHMM model and chromatin transcriptional states. "ChromHMM model based on melanoma tumour samples. Emission profile from a 15-State LearnModel based on the 6 histone modifications studied. ChromHMM identifies functionally distinct chromatin states representing both repressive and active domains, such as polycomb repression (State 1), heterochromatic repression (State 5), active transcription (State 8 and 9) and active enhancers (State 13 and 14)." (From Terranova *et al.*[106]).

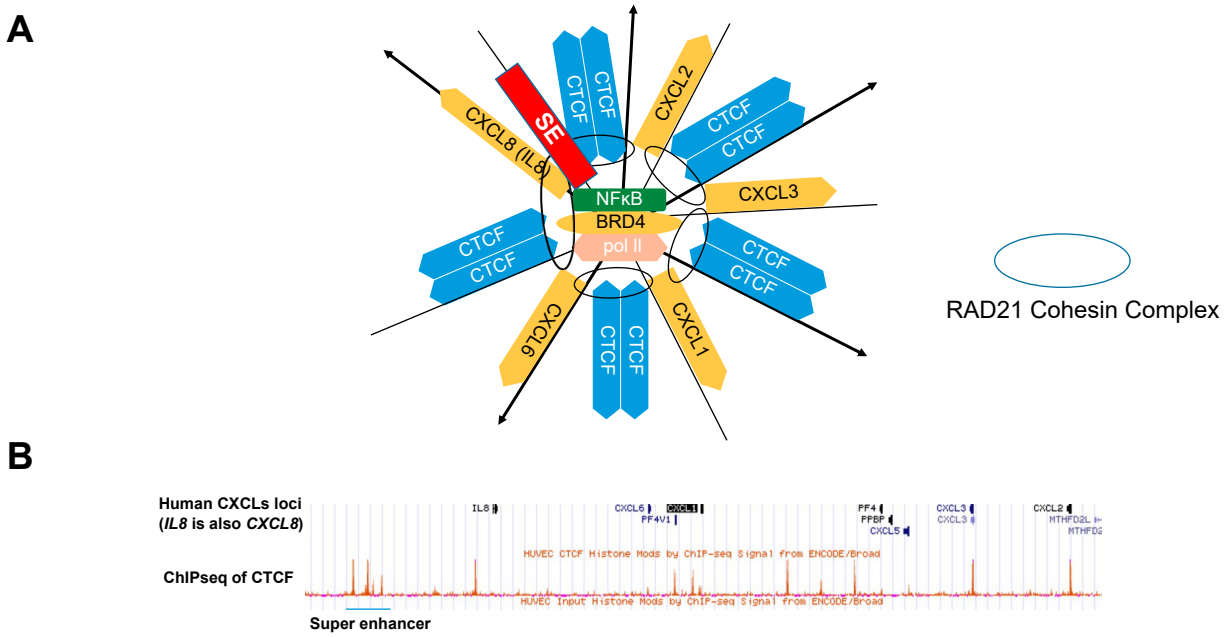


Fig. 5. Chromatin interactions around CXCL super-enhancer and co-induced gene promoter clusters. (A) TNF-α–induced SE and its interaction with the promoters of gene clusters, including IL-8, CXCL1, CXCL2, and CXCL3. CTCF, NF-κB p65, and BRD4 peaks are observed at the super-enhancer and the promoters of all these genes. (B) A snapshot of ENCODE data on the same gene loci. Chromatin immunoprecipitation-sequencing of CTCF in HUVEC shows the proximity of CTCF binding sites to the transcription start sites of all *CXCL* genes. BRD, bromodomain-containing; ChIP-Seq, chromatin immunoprecipitation-sequencing; CTCF, CCCTC-binding factor; CXCL, C-X-C motif chemokine ligand; HUVEC, human umbilical vein endothelial cell; IL8, interleukin-8; SE, super-enhancer.

inflammation. During liver injury, lnc-MALAT1 is overexpressed. Lnc-MALAT1 binds SIRT1, leading to its inactivation, which subsequently activates HSCs and results in extracellular matrix deposition and fibrosis.[101]

Advances in the study of epigenetics in ARLD

Recent advances in the technologies utilised in the study of epigenetics improved our appreciation of the epigenetic landscape during health and disease. In the next section we will describe current (chromatin confirmation capture assays) and more advanced (single-cell epigenome assays) technologies used in the study of ARLD.

3D genome structure, E-P interactions, and epigenetic gene regulation in ARLD

Technologies that study the interactions between genetic loci in 3D chromatin configuration reshaped our thinking of the role epigenetics plays in certain disease states (Fig. 3).[33,102–104] Chromatin state annotation was developed by analysing chromatin modification patterns and has been a powerful tool in the discovery of regulatory patterns.[105] ChromHMM – a java-based programme that uses multivariate Hidden Markov Mode – can recognise abnormal chromatin states and their correlations to biological functions from a large scale functional database, and can enable visualisation of the whole genome.[105] Fig. 4 is an example of the application of ChromHMM.[106]

Chromosome conformation capture assays and E-P interactions

Original chromosome conformation capture (3C) studies established the existence of E-P contacts.[104] Chromosome capture followed by high-throughput sequencing (Hi-C) data showed the enhancer and associated promoter interaction within the boundaries of a tissue topologically associating domain (TAD).[107] DNA sequences within a TAD can physically interact with each other more frequently than with sequences outside the TAD. TNF-α and LPS are the 2 main upstream regulators in the course of ARLD. There are a few studies on the effects of TNF-α and LPS on E-P interactions. In fact, our study showed that pre-existing loops within a TAD can affect TNF-α-dependent transcriptional regulation in ARLD for the first time.[120,154] Along the same lines, the human genes C-C motif chemokine ligand 2 (*CCL2*) and *CXCLs* responded to TNF-α signalling in ARLD and in Hi-C experiments designed to study dynamic chromatin interactions in primary human fibroblasts (IMR-90); the chemokine genes were arranged collinearly within the *CCL2* or *CXCL* gene clusters, which reflected their relative spatial-temporal expression patterns.[108–110] The genes appear to rely on long-range enhancer and promoter DNA contacts. Unexpectedly, 3C, chromosome conformation capture-on-chip (4C), and Hi-C studies showed that TNF-α-responsive enhancers are prelooped with their target promoters before signalling. Such pre-existing chromatin looping, which also exists in other cell types with different extracellular signalling, is a strong indicator of gene induction (Fig. 5). These observations suggest that the 3D chromatin landscape is stable and can influence the selection or activation of target genes by a ubiquitous transcriptional activator in a cell-specific manner, with the spatiotemporal deposition of active histone modifications.[108–111] The systematic mapping of chromatin loops by high-resolution Hi-C helps us to understand loop formation dynamics. Studies show that about a billion Hi-C ligation junctions are found per cell type, and up to 10,000 long-range

contacts or loops were called per cell line.[112] Approximately 30% of the loops involved genes. Applying modified chromatin interaction analysis with a paired-end tag sequencing protocol and Hi-C also demonstrated that regulatory chromatin loops involve CCCTC-binding factor (CTCF). Cohesin, a chromosome-associated multi-subunit protein complex is critical and highly associated with looped enhancers (Fig. 5).[113]

3D epigenomics and ARLD

Research into 3D gene regulation has greatly improved in the past few decades.[114] One of the earliest topological analyses was a 3C study demonstrating that a variant destabilised an E-P loop with the *OCA2* gene and caused its downregulation.[115] Recently, researchers have also established defined physiologic responses that lead to dynamic activation of pre-existing E-P interactions and the formation of new E-P loops in liver samples in response to physiologic stimuli (*e.g.*, diet).[116] For example, response to a fat rich diet was mediated largely by activation of preformed E-P loops interacting with nuclear receptors including HNF4α.[116] Peculiarly, studies on the 3D epigenome and transcriptome in AH are scarce. However, regulation of HNF4α E-P interactions through looping might be of relevance in ARLD. Analysis of RNA-sequencing of hepatic samples from patients with AH linked the development of AH to dysfunction of liver-enriched transcription factors with HNF4α being one of the most dysregulated.[117] Two promoter-driven, HNF4α-spliced isoforms in hepatocytes have been studied in detail using multiple epigenetic approaches such as whole-genome DNA methylome analysis, chromatin immunoprecipitation-sequencing (ChIP-seq) of histone markers, and single-nucleotide variation analysis. These studies show that AH livers underwent major alterations in DNA methylation patterns that resulted in chromatin remodelling.[117] For instance, HNF4α has 12 isoforms, which are expressed under the control of 2 promoters and result from alternative splicing. These isoforms can be categorised into 2 types; the adult isoforms, HNF4α-P1, and the foetal isoforms, HNF4α-P2, which are driven by a ∼45-kb upstream alternative promoter. The relevance of the P2 isoforms in adult human liver disease is not clear. The authors[117] found that *HNF4α-P1* mRNA was unchanged in AH, but expression of the *HNF4α-P2* isoforms was significantly increased in livers from patients with AH. They[117] showed that the expression of the lncRNA HNF4A-AS1, which uses the same P1 promoter region of *HNF4α*, was decreased in patients with AH. The function of this antisense lncRNA was not previously known and seemed to be related to HNF4α regulation and cell differentiation and possibly *HNF4α* E-P looping.[118] Thus, targeting epigenetic drivers that modulate HNF4α-dependent gene expression could be beneficial in patients with AH.[117]

Another facet of epigenomic regulation in ARLD is the role of super-enhancers. Super-enhancers is a term that denotes 'groups of putative enhancers in close genomic proximity with unusually high levels of Mediator binding, as measured by ChIP-seq'.[119] Our group and others demonstrated activation of cytokine pathways and chemokine production in AH.[120,121] Our initial transcriptomic study showed remarkable changes in the transcriptome and epigenome of AH cirrhotic livers, which were also accompanied by the upregulation of several CXCL chemokines. By using 3C, 4C, and analysis of histone markers such as H3K27ac, H3K4m1, H3K4m3, along with NF-κB ChIP-seq, our group also identified the existence of a super-enhancer governing CXCL chemokines that is located upstream of the *CXCL* locus in liver cells (Fig. 5). Similarly, we identified H3K27ac enrichment on the promoter and super-enhancer of CXCL chemokines in response to TNF-α stimulation in AH livers. Interestingly, pharmacologic inhibition of NF-κB and bromodomain-containing (BRD)4 binding can attenuate TNF-α-induced H3K27ac enrichment and downregulate CXCL expression. These findings and the favourable effects of suppressing the CXCL super-enhancer highlight the significance of epigenetic regulation in AH and a potential new treatment approach.[120]

Single-cell epigenome applications in ARLD

Traditionally, studies describing epigenetic changes and regulators in the pathogenesis of human disease have been limited to bulk assessment of tissues. Recently, one study combined single-cell RNA-sequencing data from healthy livers and peripheral immune cells to measure cell proportions in early AH, severe AH, HCV, HCV with cirrhosis, and NAFLD;[122] these analyses showed that patients with severe AH had the greatest change in cell composition. In addition, this study also identified a new group of inflammatory macrophages that is increased in patients with HCV. Network and signalling analysis also found that these changes are highly correlated with liver function tests. This evidence proved that only single-cell RNA-sequencing technology can provide this kind of statistical power in clinical disease studies.[122] Although other techniques, such as assay for transposase-accessible chromatin (ATAC)-sequencing, can provide more useful information about the overall chromatic accessibility state, they are limited by tissue and disease heterogeneity.[123] Liver tissue heterogeneity is being increasingly appreciated thanks to new state-of-the-art technologies that allow disease conditions to be discerned at the single-cell level. Similarly, the pathologic process of ARLD includes injury not only to hepatocytes but also to HSCs, Kupffer cells, liver sinusoidal endothelial cells, and others.[120,124,125] Until recently, few technologies were available that enabled the determination of epigenomic changes in individual cell types. Single-cell technologies are now available such as RNA-sequencing for the transcriptome, ATAC-sequencing for chromatin-accessibility and potential epigenomic regulatory elements, and single-nucleus multiome that combines RNA and ATAC-sequencing data from individual nuclei. These technologies may also help explain differences in presentation and outcome in patients at different points on the ARLD spectrum.[126–128]

Response to injury and fate of various cell lineages are determined in part by sequence-specific transcription factors interacting with cis-regulatory elements in a cell- and tissue-dependent manner. This is a guiding principle to understanding heterogeneity in normal and diseased tissue. Single-cell ATAC and DNase (DNase I–hypersensitive sites) sequencing leverage the hypersensitivity of cis-regulatory elements to transposases and nucleases in poised-to-act or active states and can be used to generate genome-wide regulome maps.[129] Some other nuanced and less-widely used technologies such as single-cell transposome hypersensitive site sequencing, or studying individual cells using cells isolated via microfluidic devices or nanowell arrays, are also now available for the study of chromatin landscapes.[130–132]

As noted earlier, dysregulation of master transcription factors such as HNF4a is well described.[117] Furthermore, an altered immune response in ARLD has been shown to have an epigenetic reprogramming function.[133,134] Transcription factors from the ETS, CCAAT/enhancer binding protein, and interferon-regulatory factor 1 families have been implicated in these changes.[134]

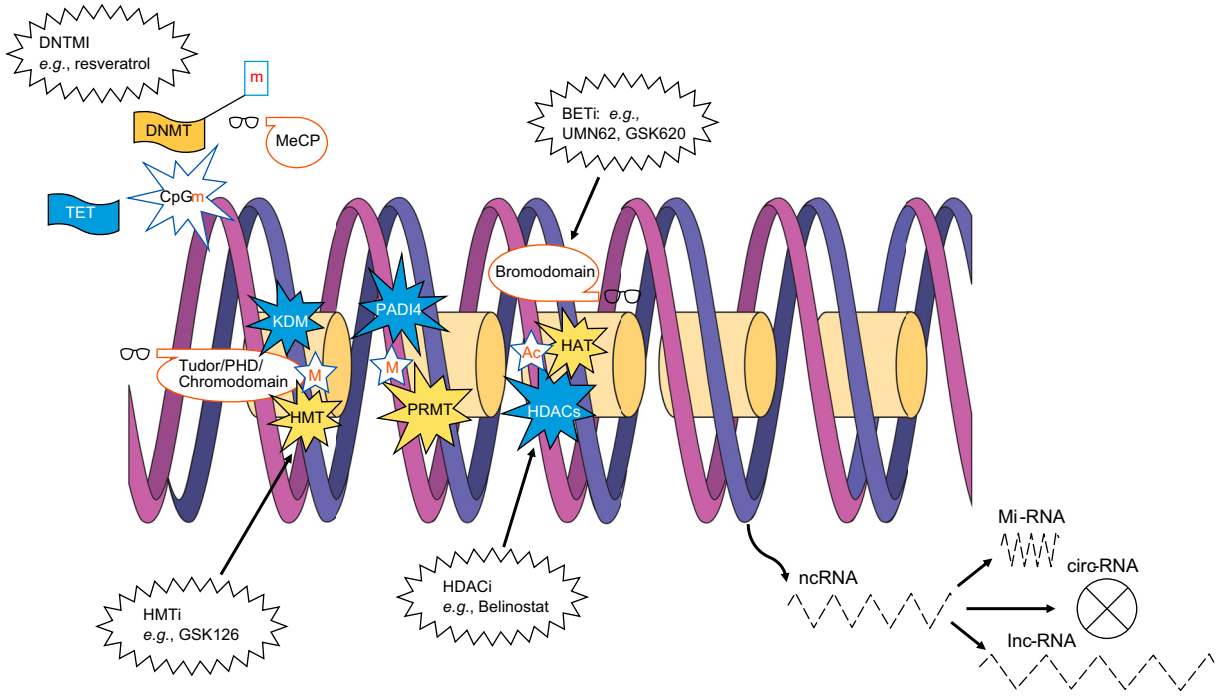


Fig. 6. Epidrugs in alcohol-related liver disease. Based on their mechanism of action, epidrugs can be divided into 8 broad categories: DNMTi, HATi, HDACi, HMTi, histone demethylase inhibitor, proteins binding to methylated histones inhibitor, proteins binding to acetylated histones inhibitor, and ncRNAs (such as antisense-RNAs, small interfering RNAs, and miRNAs). Ac, acetyl group; BETi, bromodomain and extraterminal motif inhibitor; circRNA, circular RNA; DNMT(i), DNA methyltransferase (inhibitor); HAT(i), histone acetyltransferase (inhibitor); HDAC(i), histone deacetylase; HMT(i), histone methyltransferase (inhibitor); KDM, lysine demethylase; lncRNA, long non-coding RNA; MECP2, methyl-CpG binding protein 2; miRNA, microRNA; ncRNA, non-coding RNA; PADI4, peptidyl arginine deiminase 4; PHD, plant homeodomain; PRMT, protein arginine methyltransferase; TET, ten eleven translocation.

Similarly, endothelial GATA4 has been shown to control liver fibrosis and regeneration by preventing a pathogenic switch in angiocrine signalling.[135] These cis-regulatory elements then lead to tissue-specific alterations in the transcriptome. Thus, identifying the role of one or a group of transcription factors in each cell type will help to identify new therapeutic targets, monitor responses, and provide insight into cell-cell interactions. Meanwhile, a lot of effort has also been put into the study of these pioneer transcription factors in cis-regulatory elements and E-P regulation, which are being recognised as druggable targets against disease onset and progression,[120,136,137] owing to their activity in a cell-identity and state-dependent manner. Advances in single-cell technologies will facilitate recognition of these interactions on a genome-wide level and provide new epigenomic target regions for known and novel genes of interest.

Clinical implications of the study of epigenetics in ARLD

The study of the epigenetics of ARLD has led to discoveries that are now entering everyday clinical practice in the form of either diagnostic tests or medications.

Epigenetics and the diagnosis of liver disease: Liquid biopsy

Liver biopsy remains the standard for the diagnosis and staging of acute and chronic liver diseases. The search for reliable non-invasive methods to diagnose and monitor disease progression has always been at the forefront of medical research.[25,88] Candidate serum biomarkers should fulfil certain criteria – they should be sensitive and specific and should correlate well with tissue-based tests. *Liquid biopsies* can be broadly defined as any body fluid-derived biomarker that can inform medical decision-making.[138] One example of liquid biopsy is miRNA and lncRNA profiling. In a recent study, Eguchi *et al.*[139] showed a specific miRNA signature that was released from hepatocytes during early ASH in a mouse model. Specifically, miRNAs Let7f, miR-29a, and miR-340 were increased in ASH mice but not in other chronic liver injury models. The same 3 miRNAs were increased in the serum of patients with mild ARLD.[139] Similarly, global profiling of sera from patients with and without ARLD showed a unique lncRNA signature. Further analysis identified 244 upregulated lncRNAs; lncRNAs AK128652 and AK054921 were significantly increased. To determine the prognostic value of AK128652 and AK054921, 48 patients with alcohol-related cirrhosis were followed up for 520 days, and these 2 lncRNAs were linked to shortened survival.[140]

Detecting and staging the degree of hepatic fibrosis is essential for practicing hepatologists. Given the invasive nature of liver biopsy, many alternatives have been sought.[141] As mentioned previously, *PPARG* is methylated during the trans-differentiation of HSCs into activated HSCs in the context of hepatic fibrosis. DNA methylation of the *PPARG* promoter was detected in cell-free DNA in patients with ARLD,[142] and the level correlated with progression to cirrhosis in ARLD and NAFLD. Also, the hypermethylation of *PPARG* was specific to liver fibrosis. These findings are promising and may herald the development of cost-effective blood-based liquid biomarkers for the assessment of liver fibrosis.[142]

Drugs targeting epigenetic regulation and ARLDs

Epigenetic drugs (epidrugs) are a group of compounds that target perturbed epigenetic changes in different disease states. The first class of epidrugs, DNMT inhibitors (*e.g.*, azacitidine, decitabine), were in use for many years before their epigenetic mechanism of action was elucidated.[27] Intriguingly, the list of medications with previously unknown epigenetic modulatory function is expanding, which has broadened their therapeutic indications. A noteworthy example is the antiepileptic drug valproic acid, which has been shown to have HDAC inhibitor capabilities, with possible implications for the treatment of HCC[143] (Fig. 6). ARLD is a leading cause of HCC.[144] The role of environmental factors – including alcohol consumption – and the epigenetic changes that promote the development of HCC have been studied extensively.[42,144,145] The role of epidrugs in the management of liver disease is most established in the field of HCC. This topic has been reviewed recently by Fernandez-Barrena *et al.*[28] The field of epidrugs for the treatment of ARLD and NAFLD is not as developed.[28] However, recent advances have been made, particularly with the use of novel selective BRD inhibitors to counteract inflammation in ARLD. Chemokines are small chemotactic molecules that promote inflammation. In ARLD, CXCL chemokines facilitate neutrophil tissue infiltration and are linked to poor clinical outcome.

Suppression of CXCLs mitigated alcohol-induced liver injury in mouse models.[146] The regulation and function of chemokines in liver disease was reviewed thoroughly by Cao *et al.*[146]

As described previously, our group described the role of super-enhancers in regulating inflammation in ARLD, particularly upregulation of several CXCL chemokines.[120] The bromodomain and extraterminal (BET) family comprises epigenetic reader proteins. BET proteins are known to bind with super-enhancers and modulate super-enhancers' function in inflammatory conditions.[111,147–150] BET proteins recognise the acetyl group on tagged histone lysine residues through their BRD. Four BET proteins have been described, one of which is germ cell-specific (BRDT), with the other 3 being expressed ubiquitously (BRD2, BRD3, and BRD4). Each BET protein contains 2 BRDs that are structurally homologous (BD1 and BD2). The role of BET proteins in modulating the expression of inflammatory mediators drew attention to their therapeutic potential, leading to the design of multiple BET inhibitors (BETis). The original BETi molecules targeted both BD1 and BD2 in a non-selective manner.[151,152] The lack of selectivity limited the development of medications owing to the pleiotropic effects exerted by these inhibitors.[153] Accordingly, the development of selective BD1 and BD2 BETis represents a turning point in our understanding of the roles of BET proteins in health and disease states. In fact, our group found suppression of CXCL expression in mice undergoing alcohol binges/LPS injection using the novel selective BD1 inhibitor, UMN627.[120] UMN627 is a novel BD1-selective inhibitor, with a 20-fold higher affinity for BRD4 BD1 over BRD4 BD2.[154,155] Treatment with UMN627 not only decreased the expression of CXCL but also attenuated neutrophil infiltration.[120] Another relevant example is the BRD2-selective BETi GSK620 developed by Gilan *et al.*[153] In NAFLD mouse models, GSK620 resulted in reduced levels of steatosis, lobular inflammation, and hepatocyte ballooning.[153] Taken together, the findings of our group[120] and Gilan *et al.*[153] may facilitate a new era of therapeutic approaches for the treatment of liver disease. In contrast, salvianolic acid A (SAA), a phenolic acid compound found in Danshen (used in Chinese herbal medicine), is a non-specific BRD4 inhibitor. In a recent study, SAA appeared to protect against AH and fibrosis in mouse models.[156] The effects of SAA were mediated by the inhibition of BRD4 and the translocation of its downstream inflammatory mediator, high-mobility group box protein 1 (HMGB1), which is secreted primarily by inflammatory cells. HMGB1 is believed to regulate NF-kβ via the modulation of Toll-like receptor (TLR)2, TLR4, and TLR9, which subsequently culminates in the expression of inflammatory mediators such as IL-6 and TNF-α.[156]

Conclusion

In the past couple of decades, our understanding of epigenetics and its molecular mechanisms has increased significantly. This knowledge has added a new layer to our understanding of disease mechanisms, facilitated innovative diagnostic avenues and capabilities, and, most intriguingly, paved the way for a new class of medications (epidrugs). Despite all these advances, the field of epigenetics and its clinical applications are still in their early stages. For example, adverse reactions to epidrugs have emerged because they have pleiotropic effects and off-target issues. Furthermore, advances in single-cell epigenomics will allow for recognition of these interactions on a genome-wide level and provide new target regions for known and novel genes of interest. A great deal remains to be determined, but the advances made thus far are promising.

Abbreviations

3C, chromosome conformation capture; 4C, chromosome conformation capture-on-chip; AH, alcohol-related hepatitis; ARLD, alcohol-related liver disease; ASH, alcohol-related steatohepatitis; ATAC, assay for transposase-accessible chromatin; BET, bromodomain and extraterminal motif; BETi, BET inhibitor; BRD, bromodomain; CCL2, C-C motif chemokine ligand 2; CTCF, CCCTC-binding factor; CXCL, C-X-C motif chemokine ligand; DNMT, DNA methyltransferase; E-P, enhancer-promoter; FKBP5, FK506-binding protein 5; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; Hi-C, chromosome capture followed by high-throughput sequencing; HIF1α, hypoxia inducible factor-1α; HMGB1, high-mobility group box protein 1; HNF4α, hepatocyte nuclear factor 4α; HSC, hepatic stellate cell; IL, interleukin; lncRNA, long non-coding RNA; LPS, lipopolysaccharide; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; MECP2, methyl-CpG binding protein 2; miRNA, microRNA; NAFLD, non-alcohol-related fatty liver disease; PPARG, peroxisome proliferator activated receptor-γ; SAA, salvianolic acid A; SIRT, sirtuin; SREBPs, sterol regulatory element-binding proteins; TAD, topologically associating domain; TEAD, TEA domain transcription factor; TLR, Toll-like receptor; TNF, tumour necrosis factor; YAP, Yes-associated protein.

Financial support

This work is supported by funding provided by the National Institutes of Health (USA NIH): R01 AA21171 and R01 DK59615 (V.H.S.)

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

N.W.S. contributed to literature analysis, interpretation, intellectual input, drafting and editing of the manuscript. T.S.S. contributed to drafting part

of the manuscript. V.H.S. and S.C. contributed to writing conception and design, analysis and interpretation of literatures, intellectual input, and editing of the manuscript.

Acknowledgment

Alyssa B. Quiggle, PhD, Mayo Clinic, substantively edited the manuscript. The Scientific Publications staff at Mayo Clinic provided proofreading and administrative and clerical support.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2022.100466.

References

[1] Rehm J, Taylor B, Mohapatra S, Irving H, Baliunas D, Patra J, et al. Alcohol as a risk factor for liver cirrhosis: a systematic review and meta-analysis. Drug Alcohol Rev 2010;29:437–445. https://doi.org/10.1111/j.1465-3362.2009.00153.x.

[2] Seitz HK, Bataller R, Cortez-Pinto H, Gao B, Gual A, Lackner C, et al. Alcoholic liver disease. Nat Rev Dis Prim 2018;4. https://doi.org/10.1038/s41572-018-0014-7.

[3] Stickel F, Hampe J. Genetic determinants of alcoholic liver disease. Gut 2012;61:150–159. https://doi.org/10.1136/gutjnl-2011-301239.

[4] Tian C, Stokowski RP, Kershenobich D, Ballinger DG, Hinds DA. Variant in PNPLA3 is associated with alcoholic liver disease. Nat Genet 2010;42:21–23. https://doi.org/10.1038/ng.488.

[5] Salameh H, Raff E, Erwin A, Seth D, Nischalke HD, Falleti E, et al. PNPLA3 gene polymorphism is associated with predisposition to and severity of alcoholic liver disease. Am J Gastroenterol 2015;110:846–856. https://doi.org/10.1038/ajg.2015.137.

[6] Trépo E, Gustot T, Degré D, Lemmers A, Verset L, Demetter P, et al. Common polymorphism in the PNPLA3/adiponutrin gene confers higher risk of cirrhosis and liver damage in alcoholic liver disease. J Hepatol 2011;55:906–912. https://doi.org/10.1016/j.jhep.2011.01.028.

[7] Buch S, Stickel F, Trépo E, Way M, Herrmann A, Nischalke HD, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. Nat Genet 2015;47:1443–1448. https://doi.org/10.1038/ng.3417.

[8] Mancina RM, Ferri F, Farcomeni A, Molinaro A, Maffongelli A, Mischitelli M, et al. A two gene-based risk score predicts alcoholic cirrhosis development in males with at-risk alcohol consumption. Appl Clin Genet 2019;12:1–10. https://doi.org/10.2147/TACG.S187922.

[9] Ntandja Wandji LC, Gnemmi V, Mathurin P, Louvet A. Combined alcoholic and non-alcoholic steatohepatitis. JHEP Rep 2020;2:100101. https://doi.org/10.1016/j.jhepr.2020.100101.

[10] Stickel F, Buch S, Nischalke HD, Weiss KH, Gotthardt D, Fischer J, et al. Genetic variants in PNPLA3 and TM6SF2 predispose to the development of hepatocellular carcinoma in individuals with alcohol-related cirrhosis. Am J Gastroenterol 2018;113:1475–1483. https://doi.org/10.1038/s41395-018-0041-8.

[11] Yang J, Trépo E, Nahon P, Cao Q, Moreno C, Letouzé E, et al. A 17-beta-hydroxysteroid dehydrogenase 13 variant protects from hepatocellular carcinoma development in alcoholic liver disease. Hepatology 2019;70:231–240. https://doi.org/10.1002/hep.30623.

[12] Stickel F, Lutz P, Buch S, Nischalke HD, Silva I, Rausch V, et al. Genetic variation in HSD17B13 reduces the risk of developing cirrhosis and hepatocellular carcinoma in alcohol misusers. Hepatology 2020;72:88–102. https://doi.org/10.1002/hep.30996.

[13] Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. N Engl J Med 2018;378:1096–1106. https://doi.org/10.1056/nejmoa1712191.

[14] Choudhary NS, Duseja A. Genetic and epigenetic disease modifiers: non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD). Transl Gastroenterol Hepatol 2021;6. https://doi.org/10.21037/TGH.2019.09.06.

[15] Singal AK, Bataller R, Ahn J, Kamath PS, Shah VH. ACG clinical guideline: alcoholic liver disease. Am J Gastroenterol 2018;113:175–194. https://doi.org/10.1038/ajg.2017.469.

[16] Mathurin P, Bataller R. Trends in the management and burden of alcoholic liver disease. J Hepatol 2015;62:S38–46. https://doi.org/10.1016/j.jhep.2015.03.006.

[17] Crabb DW, Im GY, Szabo G, Mellinger JL, Lucey MR. Diagnosis and treatment of alcohol-associated liver diseases: 2019 practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2020;71:306–333. https://doi.org/10.1002/hep.30866.

[18] Majeed MB, Agrawal R, Attar BM, Abu Omar Y, Gandhi SR. Safety and efficacy of infliximab in severe alcoholic hepatitis: a systematic review. Cureus 2019;11. https://doi.org/10.7759/cureus.5082.

[19] Boetticher NC, Peine CJ, Kwo P, Abrams GA, Patel T, Aqel B, et al. A randomized, double-blinded, placebo-controlled multicenter trial of etanercept in the treatment of alcoholic hepatitis. Gastroenterology 2008;135:1953–1960. https://doi.org/10.1053/j.gastro.2008.08.057.

[20] Goldberg D, Ditah IC, Saeian K, Lalehzari M, Aronsohn A, Gorospe EC, et al. Changes in the prevalence of hepatitis C virus infection, nonalcoholic steatohepatitis, and alcoholic liver disease among patients with cirrhosis or liver failure on the waitlist for liver transplantation. Gastroenterology 2017;152:1090–1099.e1. https://doi.org/10.1053/j.gastro.2017.01.003.

[21] Mellinger JL, Volk ML. Transplantation for alcohol-related liver disease: is it fair? Alcohol Alcohol 2018;53:173–177. https://doi.org/10.1093/alcalc/agx105.

[22] Rogal S, Shenai N, Kruckenberg K, Rosenberger E, Dew MA, DiMartini A. Post-transplant outcomes of persons receiving a liver graft for alcoholic liver disease. Alcohol Alcohol 2018;53:157–165. https://doi.org/10.1093/alcalc/agx100.

[23] Mann J, Reeves HL, Feldstein AE. Liquid biopsy for liver diseases. Gut 2018;2204–12. https://doi.org/10.1136/gutjnl-2017-315846.

[24] Barrera-Saldaña HA, Fernández-Garza LE, Barrera-Barrera SA. Liquid biopsy in chronic liver disease. Ann Hepatol 2021;20. https://doi.org/10.1016/j.aohep.2020.03.008.

[25] Trevisan França de Lima L, Broszczak D, Zhang X, Bridle K, Crawford D, Punyadeera C. The use of minimally invasive biomarkers for the diagnosis and prognosis of hepatocellular carcinoma. Biochim Biophys Acta - Rev Cancer 2020;1874:188451. https://doi.org/10.1016/j.bbcan.2020.188451.

[26] Bissonnette J, Altamirano J, Devue C, Roux O, Payancé A, Lebrec D, et al. A prospective study of the utility of plasma biomarkers to diagnose alcoholic hepatitis. Hepatology 2017;66:555–563. https://doi.org/10.1002/hep.29080.

[27] Ganesan A, Arimondo PB, Rots MG, Jeronimo C, Berdasco M. The timeline of epigenetic drug discovery: from reality to dreams. Clin Epigenetics 2019;11:1–17. https://doi.org/10.1186/s13148-019-0776-0.

[28] Fernández-Barrena MG, Arechederra M, Colyn L, Berasain C, Avila MA. Epigenetics in hepatocellular carcinoma development and therapy: the tip of the iceberg. JHEP Rep 2020;2:100167. https://doi.org/10.1016/j.jhepr.2020.100167.

[29] Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. Nature 2019;571:489–499. https://doi.org/10.1038/s41586-019-1411-0.

[30] Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet 2016;17:487–500. https://doi.org/10.1038/nrg.2016.59.

[31] Berdasco M, Esteller M. Clinical epigenetics: seizing opportunities for translation. Nat Rev Genet 2019;20:109–127. https://doi.org/10.1038/s41576-018-0074-2.

[32] Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, et al. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. Signal Transduct Target Ther 2019;4. https://doi.org/10.1038/s41392-019-0095-0.

[33] Bohnsack JP, Pandey SC. Histone modifications, DNA methylation, and the epigenetic code of alcohol use disorder. Int Rev Neurobiol 2021;156:1–62. https://doi.org/10.1016/bs.irn.2020.08.005.

[34] Hardy T, Mann DA. Epigenetics in liver disease: from biology to therapeutics. Gut 2016;65:1895–1905. https://doi.org/10.1136/gutjnl-2015-311292.

[35] Lin XX, Lian GH, Peng SF, Zhao Q, Xu Y, Ou-Yang DS, et al. Reversing epigenetic alterations caused by alcohol: a promising therapeutic direction for alcoholic liver disease. Alcohol Clin Exp Res 2018;42:1863–1873. https://doi.org/10.1111/acer.13863.

[36] Zeybel M, Hardy T, Robinson SM, Fox C, Anstee QM, Ness T, et al. Differential DNA methylation of genes involved in fibrosis progression in non-alcoholic fatty liver disease and alcoholic liver disease. Clin Epigenetics 2015;7:1–11. https://doi.org/10.1186/s13148-015-0056-6.

[37] Page A, Paoli PP, Hill SJ, Howarth R, Wu R, Kweon SM, et al. Alcohol directly stimulates epigenetic modifications in hepatic stellate cells. J Hepatol 2015;62:388–397. https://doi.org/10.1016/j.jhep.2014.09.033.

[38] Bönsch D, Lenz B, Reulbach U, Kornhuber J, Bleich S. Homocysteine associated genomic DNA hypermethylation in patients with chronic alcoholism. J Neural Transm 2004;111:1611–1616. https://doi.org/10.1007/s00702-004-0232-x.
[39] Liu H, Li J, Tillman B, French BA, French SW. Ufmylation and FATylation pathways are downregulated in human alcoholic and nonalcoholic steatohepatitis, and mice fed DDC, where Mallory-Denk bodies (MDBs) form. Exp Mol Pathol 2014;97:81–88. https://doi.org/10.1016/j.yexmp.2014.05.010.
[40] Liu H, Gong M, French BA, Li J, Tillman B, French SW. Mallory-Denk Body (MDB) formation modulates ufmylation expression epigenetically in alcoholic hepatitis (AH) and non-alcoholic steatohepatitis (NASH). Exp Mol Pathol 2014;97:477–483. https://doi.org/10.1016/j.yexmp.2014.10.001.
[41] Shen H, French BA, Tillman BC, Li J, French SW. Increased DNA methylation in the livers of patients with alcoholic hepatitis. Exp Mol Pathol 2015;99:326–329. https://doi.org/10.1016/j.yexmp.2015.08.001.
[42] French SW. Epigenetic events in liver cancer resulting from alcoholic liver disease. Alcohol Res Curr Rev 2012;35:57–67.
[43] Kim HG, Cho JH, Kim J, Kim SJ. The role of epigenetic changes in the progression of alcoholic steatohepatitis. Front Physiol 2021;12:1–10. https://doi.org/10.3389/fphys.2021.691738.
[44] Kusumanchi P, Liang T, Zhang T, Ross RA, Han S, Chandler K, et al. Stress-responsive gene FK506-binding protein 51 mediates alcohol-induced liver injury through the hippo pathway and chemokine (C-X-C Motif) Ligand 1 signaling. Hepatology 2021;74:1234–1250. https://doi.org/10.1002/hep.31800.
[45] Li H, Su P, Lai TKY, Jiang A, Liu J, Zhai D, et al. The glucocorticoid receptor-FKBP51 complex contributes to fear conditioning and posttraumatic stress disorder. J Clin Invest 2020;130:877–889. https://doi.org/10.1172/JCI130363.
[46] Liu K, Wang FS, Xu R. Neutrophils in liver diseases: pathogenesis and therapeutic targets. Cell Mol Immunol 2021;18:38–44. https://doi.org/10.1038/s41423-020-00560-0.
[47] Takeuchi M, Vidigal PT, Guerra MT, Hundt MA, Robert ME, Olave-Martinez M, et al. Neutrophils interact with cholangiocytes to cause cholestatic changes in alcoholic hepatitis. Gut 2021;70:342–356. https://doi.org/10.1136/gutjnl-2020-322540.
[48] Lowe PP, Gyongyosi B, Satishchandran A, Iracheta-Vellve A, Ambade A, Kodys K, et al. Alcohol-related changes in the intestinal microbiome influence neutrophil infiltration, inflammation and steatosis in early alcoholic hepatitis in mice. PLos One 2017;12:1–16. https://doi.org/10.1371/journal.pone.0174544.
[49] Friedman SL. Evolving challenges in hepatic fibrosis. Nat Rev Gastroenterol Hepatol 2010;7:425–436. https://doi.org/10.1038/nrgastro.2010.97.
[50] Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol 2021;18:151–166. https://doi.org/10.1038/s41575-020-00372-7.
[51] Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol 2017;14:397–411. https://doi.org/10.1038/nrgastro.2017.38.
[52] Chen X, Li WX, Chen Y, Li XF, Li H Di, Huang HM, et al. Suppression of SUN2 by DNA methylation is associated with HSCs activation and hepatic fibrosis. Cell Death Dis 2018;9. https://doi.org/10.1038/s41419-018-1032-9.
[53] Mann J, Oakley F, Akiboye F, Elsharkawy A, Thorne AW, Mann DA. Regulation of myofibroblast transdifferentiation by DNA methylation and MeCP2: implications for wound healing and fibrogenesis. Cell Death Differ 2007;14:275–285. https://doi.org/10.1038/sj.cdd.4401979.
[54] Mann J, Chu DCK, Maxwell A, Oakley F, Zhu NL, Tsukamoto H, et al. MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. Gastroenterology 2010;138:705–714.e4. https://doi.org/10.1053/j.gastro.2009.10.002.
[55] Moran-Salvador E, Garcia-Macia M, Sivaharan A, Sabater L, Zaki MYW, Oakley F, et al. Fibrogenic activity of MECP2 is regulated by phosphorylation in hepatic stellate cells. Gastroenterology 2019;157:1398–1412.e9. https://doi.org/10.1053/j.gastro.2019.07.029.
[56] Barcena-Varela M, Paish H, Alvarez L, Uriarte I, Latasa MU, Santamaria E, et al. Epigenetic mechanisms and metabolic reprogramming in fibrogenesis: dual targeting of G9a and DNMT1 for the inhibition of liver fibrosis. Gut 2021;70:388–400. https://doi.org/10.1136/gutjnl-2019-320205.
[57] Hardy T, Zeybel M, Day CP, Dipper C, Masson S, McPherson S, et al. Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in non-alcoholic fatty liver disease. Gut 2017;66:1321–1328. https://doi.org/10.1136/gutjnl-2016-311526.
[58] Legoff L, D'Cruz SC, Tevosian S, Primig M, Smagulova F. Transgenerational inheritance of environmentally induced epigenetic alterations during mammalian development. Cells 2019;8:1–26. https://doi.org/10.3390/cells8121559.
[59] Horsthemke B. A critical view on transgenerational epigenetic inheritance in humans. Nat Commun 2018;9:1–4. https://doi.org/10.1038/s41467-018-05445-5.
[60] Zeybel M, Hardy T, Wong YK, Mathers JC, Fox CR, Gackowska A, et al. Multigenerational epigenetic adaptation of the hepatic wound-healing response. Nat Med 2012;18:1369–1377. https://doi.org/10.1038/nm.2893.
[61] Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693–705. https://doi.org/10.1016/j.cell.2007.02.005.
[62] Won KJ, Chepelev I, Ren B, Wang W. Prediction of regulatory elements in mammalian genomes using chromatin signatures. BMC Bioinformatics 2008;9:1–18. https://doi.org/10.1186/1471-2105-9-547.
[63] Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet 2007;39:311–318. https://doi.org/10.1038/ng1966.
[64] Heerboth S, Lapinska K, Snyder N, Leary M, Rollinson S, Sarkar S. Use of epigenetic drugs in disease: an overview. Genet Epigenetics 2014;1:9–19. https://doi.org/10.4137/GeG.s12270.
[65] Park PH, Miller R, Shukla SD. Acetylation of histone H3 at lysine 9 by ethanol in rat hepatocytes. Biochem Biophys Res Commun 2003;306:501–504. https://doi.org/10.1016/S0006-291X(03)01040-4.
[66] Park PH, Lim RW, Shukla SD. Involvement of histone acetyltransferase (HAT) in ethanol-induced acetylation of histone H3 in hepatocytes: potential mechanism for gene expression. Am J Physiol - Gastrointest Liver Physiol 2005;289:1124–1136. https://doi.org/10.1152/ajpgi.00091.2005.
[67] Bardag-Gorce F, French BA, Joyce M, Baires M, Montgomery RO, Li J, et al. Histone acetyltransferase p300 modulates gene expression in an epigenetic manner at high blood alcohol levels. Exp Mol Pathol 2007;82:197–202. https://doi.org/10.1016/j.yexmp.2006.10.006.
[68] Park PH, Lim RW, Shukla SD. Gene-selective histone H3 acetylation in the absence of increase in global histone acetylation in liver of rats chronically fed alcohol. Alcohol Alcohol 2012;47:233–239. https://doi.org/10.1093/alcalc/ags004.
[69] Kirpich I, Ghare S, Zhang J, Gobejishvili L, Kharebava G, Barve SJ, et al. Binge alcohol-induced microvesicular liver steatosis and injury are associated with down-regulation of hepatic Hdac 1, 7, 9, 10, 11 and up-regulation of Hdac 3. Alcohol Clin Exp Res 2012;36:1578–1586. https://doi.org/10.1111/j.1530-0277.2012.01751.x.
[70] Restrepo RJ, Lim RW, Korthuis RJ, Shukla SD. Binge alcohol alters PNPLA3 levels in liver through epigenetic mechanism involving histone H3 acetylation. Alcohol 2017;60:77–82. https://doi.org/10.1016/j.alcohol.2017.01.009.
[71] Ferré P, Phan F, Foufelle F. SREBP-1c and lipogenesis in the liver: an update1. Biochem J 2021;478:3723–3739. https://doi.org/10.1042/BCJ20210071.
[72] You M, Liang X, Ajmo JM, Ness GC. Involvement of mammalian sirtuin 1 in the action of ethanol in the liver. Am J Physiol - Gastrointest Liver Physiol 2008;294:892–898. https://doi.org/10.1152/ajpgi.00575.2007.
[73] Zhang Y, Long X, Ruan X, Wei Q, Zhang L, Wo L, et al. SIRT2-mediated deacetylation and deubiquitination of C/EBPβ prevents ethanol-induced liver injury. Cell Discov 2021;7. https://doi.org/10.1038/s41421-021-00326-6.
[74] Donde H, Ghare S, Joshi-Barve S, Zhang JW, Vadhanam MV, Gobejishvili L, et al. Tributyrin inhibits ethanol-induced epigenetic repression of CPT-1A and attenuates hepatic steatosis and injury. Cmgh 2020;9:569–585. https://doi.org/10.1016/j.jcmgh.2019.10.005.
[75] Mandrekar P, Szabo G. Signalling pathways in alcohol-induced liver inflammation. J Hepatol 2009;50:1258–1266. https://doi.org/10.1016/j.jhep.2009.03.007.
[76] Gao B, Ahmad MF, Nagy LE, Tsukamoto H. Inflammatory pathways in alcoholic steatohepatitis. J Hepatol 2019;70:249–259. https://doi.org/10.1016/j.jhep.2018.10.023.
[77] Kendrick SFW, O'Boyle G, Mann J, Zeybel M, Palmer J, Jones DEJ, et al. Acetate, the key modulator of inflammatory responses in acute alcoholic hepatitis. Hepatology 2010;51:1988–1997. https://doi.org/10.1002/hep.23572.

[78] Shen Z, Ajmo JM, Rogers CQ, Liang X, Le L, Murr MM, et al. Role of SIRT1 in regulation of LPS- or two ethanol metabolites-induced TNF-α production in cultured macrophage cell lines. Am J Physiol - Gastrointest Liver Physiol 2009;296:1047–1053. https://doi.org/10.1152/ajpgi.00016.2009.
[79] Völkel P, Angrand PO. The control of histone lysine methylation in epigenetic regulation. Biochimie 2007;89:1–20. https://doi.org/10.1016/j.biochi.2006.07.009.
[80] Bassett SA, Barnett MPG. The role of dietary histone deacetylases (HDACs) inhibitors in health and disease. Nutrients 2014;6:4273–4301. https://doi.org/10.3390/nu6104273.
[81] Pal-Bhadra M, Bhadra U, Jackson DE, Mamatha L, Park PH, Shukla SD. Distinct methylation patterns in histone H3 at Lys-4 and Lys-9 correlate with up- & down-regulation of genes by ethanol in hepatocytes. Life Sci 2007;81:979–987. https://doi.org/10.1016/j.lfs.2007.07.030.
[82] Bardag-Gorce F, Oliva J, Dedes J, Li J, French BA, French SW. Chronic ethanol feeding alters hepatocyte memory which is not altered by acute feeding. Alcohol Clin Exp Res 2009;33:684–692.
[83] Ara AI, Xia M, Ramani K, Mato JM, Lu SC. S-adenosylmethionine inhibits lipopolysaccharide-induced gene expression via modulation of histone methylation. Hepatology 2008;47:1655–1666. https://doi.org/10.1002/hep.22231.
[84] Szabo G, Bala S. MicroRNAs in liver disease. Nat Rev Gastroenterol Hepatol 2013;10:542–552. https://doi.org/10.1038/nrgastro.2013.87.
[85] Szabo G. Gut-liver axis in alcoholic liver disease. Gastroenterology 2015;148:30–36. https://doi.org/10.1053/j.gastro.2014.10.042.
[86] Szabo G, Petrasek J. Gut-liver axis and sterile signals in the development of alcoholic liver disease. Alcohol Alcohol 2017;52:414–424. https://doi.org/10.1093/alcalc/agx025.
[87] Gholizadeh M, Szelag-Pieniek S, Post M, Kurzawski M, Prieto J, Argemi J, et al. Identifying differentially expressed micrornas, target genes, and key pathways deregulated in patients with liver diseases. Int J Mol Sci 2020;21:1–16. https://doi.org/10.3390/ijms21197368.
[88] Arechederra M, Recalde M, María Gárate-Rascón MGF-B, Matías A, Ávila CB. Epigenetic biomarkers for the diagnosis and treatment of liver disease. Cancers (Basel) 2021:285–307.
[89] Babuta M, Szabo G. Extracellular vesicles in inflammation: focus on the microRNA cargo of EVs in modulation of liver diseases. J Leukoc Biol 2022;111:75–92. https://doi.org/10.1002/JLB.3MIR0321-156R.
[90] Liu SY, Tsai IT, Hsu YC. Alcohol-related liver disease: basic mechanisms and clinical perspectives. Int J Mol Sci 2021;22. https://doi.org/10.3390/ijms22105170.
[91] Wang X, He Y, MacKowiak B, Gao B. MicroRNAs as regulators, biomarkers and therapeutic targets in liver diseases. Gut 2021;70:784–795. https://doi.org/10.1136/gutjnl-2020-322526.
[92] Bala S, Szabo G. MicroRNA signature in alcoholic liver disease. Int J Hepatol 2012;2012:1–6. https://doi.org/10.1155/2012/498232.
[93] Bala S, Marcos M, Kodys K, Csak T, Catalano D, Mandrekar P, et al. Up-regulation of microRNA-155 in macrophages contributes to increased Tumor Necrosis Factor α (TNFα) production via increased mRNA half-life in alcoholic liver disease. J Biol Chem 2011;286:1436–1444. https://doi.org/10.1074/jbc.M110.145870.
[94] Yin H, Liang X, Jogasuria A, Davidson NO, You M. miR-217 regulates ethanol-induced hepatic inflammation by disrupting sirtuin 1 - Lipin-1 signaling. Am J Pathol 2015;185:1286–1296. https://doi.org/10.1016/j.ajpath.2015.01.030.
[95] Satishchandran A, Ambade A, Rao S, Hsueh YC, Iracheta-Vellve A, Tornai D, et al. MicroRNA 122, regulated by GRLH2, protects livers of mice and patients from ethanol-induced liver disease. Gastroenterology 2018;154:238–252.e7. https://doi.org/10.1053/j.gastro.2017.09.022.
[96] Heo MJ, Kim TH, You JS, Blaya D, Sancho-Bru P, Kim SG. Alcohol dysregulates miR-148a in hepatocytes through FoxO1, facilitating pyroptosis via TXNIP overexpression. Gut 2019;68:708–720. https://doi.org/10.1136/gutjnl-2017-315123.
[97] Hu S, Liu YM, Chen-chen, Li LY, Zhang BY, Yang JF, et al. MicroRNA-708 prevents ethanol-induced hepatic lipid accumulation and inflammatory reaction via direct targeting ZEB1. Life Sci 2020;258:118147. https://doi.org/10.1016/j.lfs.2020.118147.
[98] Luo J, Xie M, Hou Y, Ma W, Jin Y, Chen J, et al. A novel epigenetic mechanism unravels hsa-miR-148a-3p-mediated CYP2B6 downregulation in alcoholic hepatitis disease. Biochem Pharmacol 2021;188:114582. https://doi.org/10.1016/j.bcp.2021.114582.
[99] Luo J, Hou Y, Ma W, Xie M, Jin Y, Xu L, et al. A novel mechanism underlying alcohol dehydrogenase expression: hsa-miR-148a-3p promotes ADH4 expression via an AGO1-dependent manner in control and ethanol-exposed hepatic cells. Biochem Pharmacol 2021;189:114458. https://doi.org/10.1016/j.bcp.2021.114458.
[100] Dou X, Yang W, Ding Q, Han Q, Qian Q, Du Z, et al. Comprehensive analysis of the expression profiles of hepatic lncRNAs in the mouse model of alcoholic liver disease. Front Pharmacol 2021;12:1–11. https://doi.org/10.3389/fphar.2021.709287.
[101] Wu Y, Liu X, Zhou Q, Huang C, Meng X, Xu F, et al. Silent information regulator 1 (SIRT1) ameliorates liver fibrosis via promoting activated stellate cell apoptosis and reversion. Toxicol Appl Pharmacol 2015;289:163–176. https://doi.org/10.1016/j.taap.2015.09.028.
[102] Roadmap Epigenomics Consortium, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, et al. Integrative analysis of 111 reference human epigenomes. Nature 2015;518:317–329. https://doi.org/10.1038/nature14248.
[103] Pucéat M. Capturing chromosome conformation. Methods Mol Biol 2021;2157:1–7. https://doi.org/10.1007/978-1-0716-0664-3_1.
[104] de Wit E, de Laat W. A decade of 3C technologies: insights into nuclear organization. Genes Dev 2012;26:11–24. https://doi.org/10.1101/gad.179804.111.
[105] Ernst J, Kellis M. ChromHMM: automating chromatin-state discovery and characterization. Nat Methods 2012;9:215–216. https://doi.org/10.1038/nmeth.1906.
[106] Terranova C, Tang M, Orouji E, Maitituoheti M, Raman A, Amin S, et al. An integrated platform for genome-wide mapping of chromatin states using high-throughput chip-sequencing in tumor tissues. J Vis Exp 2018;2018:1–11. https://doi.org/10.3791/56972.
[107] Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 2012;485:376–380. https://doi.org/10.1038/nature11082.
[108] Jin F, Li Y, Dixon JR, Selvaraj S, Ye Z, Lee AY, et al. A high-resolution map of the three-dimensional chromatin interactome in human cells. Nature 2013;503:290–294. https://doi.org/10.1038/nature12644.
[109] Fanucchi S, Fok ET, Dalla E, Shibayama Y, Börner K, Chang EY, et al. Immune genes are primed for robust transcription by proximal long noncoding RNAs located in nuclear compartments. Nat Genet 2019;51:138–150. https://doi.org/10.1038/s41588-018-0298-2.
[110] Higashijima Y, Matsui Y, Shimamura T, Nakaki R, Nagai N, Tsutsumi S, et al. Coordinated demethylation of H3K9 and H3K27 is required for rapid inflammatory responses of endothelial cells. EMBO J 2020;39:1–24. https://doi.org/10.15252/embj.2019103949.
[111] Brown JD, Lin CY, Duan Q, Griffin G, Federation AJ, Paranal RM, et al. Nf-kb directs dynamic super enhancer formation in inflammation and atherogenesis. Mol Cel 2014;56:219–231. https://doi.org/10.1016/j.molcel.2014.08.024.
[112] Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell 2014;159:1665–1680. https://doi.org/10.1016/j.cell.2014.11.021.
[113] Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, Van Berkum NL, et al. Mediator and cohesin connect gene expression and chromatin architecture. Nature 2010;467:430–435. https://doi.org/10.1038/nature09380.
[114] Lupiáñez DG, Spielmann M, Mundlos S. Breaking TADs: how alterations of chromatin domains result in disease. Trends Genet 2016;32:225–237. https://doi.org/10.1016/j.tig.2016.01.003.
[115] Visser M, Kayser M, Palstra RJ. HERC2 rs12913832 modulates human pigmentation by attenuating chromatin-loop formation between a long-range enhancer and the OCA2 promoter. Genome Res 2012;22:446–455. https://doi.org/10.1101/gr.128652.111.
[116] Qin Y, Grimm SA, Roberts JD, Chrysovergis K, Wade PA. Alterations in promoter interaction landscape and transcriptional network underlying metabolic adaptation to diet. Nat Commun 2020;11. https://doi.org/10.1038/s41467-020-14796-x.
[117] Argemi J, Latasa MU, Atkinson SR, Blokhin IO, Massey V, Gue JP, et al. Defective HNF4alpha-dependent gene expression as a driver of

hepatocellular failure in alcoholic hepatitis. Nat Commun 2019;10. https://doi.org/10.1038/s41467-019-11004-3.
[118] Sandovici I, Smith NH, Nitert MD, Ackers-Johnson M, Uribe-Lewis S, Ito Y, et al. Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the Hnf4a gene in rat pancreatic islets. Proc Natl Acad Sci U S A 2011;108:5449–5454. https://doi.org/10.1073/PNAS.1019007108/-/DCSUPPLEMENTAL/PNAS.201019007SI.PDF.
[119] Pott S, Lieb JD. What are super-enhancers? Nat Genet 2015;47:8–12. https://doi.org/10.1038/ng.3167.
[120] Liu M, Cao S, He L, Gao J, Arab JP, Cui H, et al. Super enhancer regulation of cytokine-induced chemokine production in alcoholic hepatitis. Nat Commun 2021;12:1–14. https://doi.org/10.1038/s41467-021-24843-w.
[121] Dominguez M, Miquel R, Colmenero J, Moreno M, García-Pagán JC, Bosch J, et al. Hepatic expression of CXC chemokines predicts portal hypertension and survival in patients with alcoholic hepatitis. Gastroenterology 2009;136:1639–1650. https://doi.org/10.1053/j.gastro.2009.01.056.
[122] Kim A, Wu X, Allende DS, Nagy LE. Gene deconvolution reveals aberrant liver regeneration and immune cell infiltration in alcohol-associated hepatitis. Hepatology 2021;74:987–1002. https://doi.org/10.1002/hep.31759.
[123] Chen T, Oh S, Gregory S, Shen X, Diehl AM. Single-cell omics analysis reveals functional diversification of hepatocytes during liver regeneration. JCI Insight 2020;5:1–16. https://doi.org/10.1172/jci.insight.141024.
[124] He L, Sehrawat TS, Verma VK, Navarro-Corcuera A, Sidhu G, Mauer A, et al. XIAP knockdown in alcohol-associated liver disease models exhibits divergent in vitro and in vivo phenotypes owing to a potential zonal inhibitory role of SMAC. Front Physiol 2021;12:1–16. https://doi.org/10.3389/fphys.2021.664222.
[125] Arab JP, Cabrera D, Sehrawat TS, Jalan-Sakrikar N, Verma VK, Simonetto D, et al. Hepatic stellate cell activation promotes alcohol-induced steatohepatitis through Igfbp3 and SerpinA12. J Hepatol 2020;73:149–160. https://doi.org/10.1016/j.jhep.2020.02.005.
[126] Yang L, Wang WH, Qiu WL, Guo Z, Bi E, Xu CR. A single-cell transcriptomic analysis reveals precise pathways and regulatory mechanisms underlying hepatoblast differentiation. Hepatology 2017;66:1387–1401. https://doi.org/10.1002/hep.29353.
[127] Terkelsen MK, Bendixen SM, Hansen D, Scott EAH, Moeller AF, Nielsen R, et al. Transcriptional dynamics of hepatic sinusoid-associated cells after liver injury. Hepatology 2020;72:2119–2133. https://doi.org/10.1002/hep.31215.
[128] Sarropoulos I, Sepp M, Frömel R, Leiss K, Trost N, Leushkin E, et al. Developmental and evolutionary dynamics of cis-regulatory elements in mouse cerebellar cells. Science (80-) 2021:373. https://doi.org/10.1126/science.abg4696.
[129] Liu C, Wang M, Wei X, Wu L, Xu J, Dai X, et al. An ATAC-seq atlas of chromatin accessibility in mouse tissues. Sci Data 2019;6:1–10. https://doi.org/10.1038/s41597-019-0071-0.
[130] Lake BB, Chen S, Sos BC, Fan J, Kaeser GE, Yung YC, et al. Integrative single-cell analysis of transcriptional and epigenetic states in the human adult brain. Nat Biotechnol 2018;36:70–80. https://doi.org/10.1038/nbt.4038.
[131] Buenrostro JD, Wu B, Litzenburger UM, Ruff D, Gonzales ML, Snyder MP, et al. Single-cell chromatin accessibility reveals principles of regulatory variation. Nature 2015;523:486–490. https://doi.org/10.1038/nature14590.
[132] Mezger A, Klemm S, Mann I, Brower K, Mir A, Bostick M, et al. High-throughput chromatin accessibility profiling at single-cell resolution. Nat Commun 2018;9:6–11. https://doi.org/10.1038/s41467-018-05887-x.
[133] Sureshchandra S, Stull C, Ligh BJK, Nguyen SB, Grant KA, Messaoudi I. Chronic heavy drinking drives distinct transcriptional and epigenetic changes in splenic macrophages. EBioMedicine 2019;43:594–606. https://doi.org/10.1016/j.ebiom.2019.04.027.
[134] Weichselbaum L, Azouz A, Smolen KK, Das J, Splittgerber M, Lepida A, et al. Epigenetic basis for monocyte dysfunction in patients with severe alcoholic hepatitis. J Hepatol 2020;73:303–314. https://doi.org/10.1016/j.jhep.2020.02.017.
[135] Winkler M, Staniczek T, Kürschner SW, Schmid CD, Schönhaber H, Cordero J, et al. Endothelial GATA4 controls liver fibrosis and regeneration by preventing a pathogenic switch in angiocrine signaling. J Hepatol 2021;74:380–393. https://doi.org/10.1016/j.jhep.2020.08.033.
[136] Tang F, Yang Z, Tan Y, Li Y. Super-enhancer function and its application in cancer targeted therapy. Npj Precis Oncol 2020;4:1–7. https://doi.org/10.1038/s41698-020-0108-z.
[137] Claringbould A, Zaugg JB. Enhancers in disease: molecular basis and emerging treatment strategies. Trends Mol Med 2021;27:1060–1073. https://doi.org/10.1016/j.molmed.2021.07.012.
[138] Olson DR. The 'liquid' biopsy. College of American Pathologists. Accessed December 20, 2021. https://www.cap.org/member-resources/articles/the-liquid-biopsy.
[139] Eguchi A, Lazaro RG, Wang J, Kim J, Povero D, Willliams B, et al. Extracellular vesicles released by hepatocytes from gastric infusion model of alcoholic liver disease contain a MicroRNA barcode that can be detected in blood. Hepatology 2017;65:475–490. https://doi.org/10.1002/hep.28838.
[140] Yang Z, Ross RA, Zhao S, Tu W, Liangpunsakul S, Wang L. LncRNA AK054921 and AK128652 are potential serum biomarkers and predictors of patient survival with alcoholic cirrhosis. Hepatol Commun 2017;1:513–523. https://doi.org/10.1002/hep4.1061.
[141] Loomba R, Adams LA. Advances in non-invasive assessment of hepatic fibrosis. Gut 2020;69:1343–1352. https://doi.org/10.1136/gutjnl-2018-317593.
[142] Yiğit B, Boyle M, Özler O, Erden N, Tutucu F, Hardy T, et al. Plasma cell-free DNA methylation: a liquid biomarker of hepatic fibrosis. Gut 2018;67:1907–1908. https://doi.org/10.1136/gutjnl-2017-315668.
[143] Lötsch J, Schneider G, Reker D, Parnham MJ, Schneider P, Geisslinger G, et al. Common non-epigenetic drugs as epigenetic modulators. Trends Mol Med 2013;19:742–753. https://doi.org/10.1016/j.molmed.2013.08.006.
[144] Nagaraju GP, Dariya B, Kasa P, Peela S, El-Rayes BF. Epigenetics in hepatocellular carcinoma. Semin Cancer Biol 2021. https://doi.org/10.1016/j.semcancer.2021.07.017.
[145] Wolinska E, Skrzypczak M. Epigenetic changes affecting the development of hepatocellular carcinoma. Cancers (Basel) 2021;13:1–15. https://doi.org/10.3390/cancers13164237.
[146] Cao S, Liu M, Sehrawat TS, Shah VH. Regulation and functional roles of chemokines in liver diseases. Nat Rev Gastroenterol Hepatol 2021;18:630–647. https://doi.org/10.1038/s41575-021-00444-2.
[147] Gibbons HR, Mi DJ, Farley VM, Esmond T, Kaood MB, Aune TM. Bromodomain inhibitor JQ1 reversibly blocks IFN-γ production. Sci Rep 2019;9:1–10. https://doi.org/10.1038/s41598-019-46516-x.
[148] Peeters JGC, Vervoort SJ, Tan SC, Mijnheer G, de Roock S, Vastert SJ, et al. Inhibition of super-enhancer activity in autoinflammatory site-derived T cells reduces disease-associated gene expression. Cell Rep 2015;12:1986–1996. https://doi.org/10.1016/j.celrep.2015.08.046.
[149] Krishna V, Yin X, Song Q, Walsh A, Pocalyko D, Bachman K, et al. Integration of the transcriptome and genome-wide landscape of BRD2 and BRD4 binding motifs identifies key superenhancer genes and reveals the mechanism of Bet inhibitor action in rheumatoid arthritis synovial fibroblasts. J Immunol 2021;206:422–431. https://doi.org/10.4049/jimmunol.2000286.
[150] Higashijima Y, Kanki Y. Potential roles of super enhancers in inflammatory gene transcription. FEBS J 2021:1–14. https://doi.org/10.1111/febs.16089.
[151] Biswas S, Rao CM. Epigenetic tools (The Writers, The Readers and The Erasers) and their implications in cancer therapy. Eur J Pharmacol 2018;837:8–24. https://doi.org/10.1016/j.ejphar.2018.08.021.
[152] Shorstova T, Foulkes WD, Witcher M. Achieving clinical success with BET inhibitors as anti-cancer agents. Br J Cancer 2021;124:1478–1490. https://doi.org/10.1038/s41416-021-01321-0.
[153] Gilan O, Rioja I, Knezevic K, Bell MJ, Yeung MM, Harker NR, et al. Selective targeting of BD1 and BD2 of the BET proteins in cancer and immunoinflammation. Science (80-) 2020;368:387–394. https://doi.org/10.1126/science.aaz8455.
[154] Gao J, Wei B, Liu M, Hirsova P, Sehrawat TS, Cao S, et al. Endothelial p300 promotes portal hypertension and hepatic fibrosis through C-C Motif chemokine ligand 2–mediated angiocrine signaling. Hepatology 2021;73:2468–2483. https://doi.org/10.1002/hep.31617.
[155] Divakaran A, Talluri SK, Ayoub AM, Mishra NK, Cui H, Widen JC, et al. Molecular basis for the N-terminal bromodomain-and-extra-terminal-family selectivity of a dual kinase-bromodomain inhibitor. J Med Chem 2018;61:9316–9334. https://doi.org/10.1021/acs.jmedchem.8b01248.
[156] Lan Y, Yan R, Shan W, Chu J, Sun R, Wang R, et al. Salvianic acid A alleviates chronic alcoholic liver disease by inhibiting HMGB1 translocation via down-regulating BRD4. J Cel Mol Med 2020;24:8518–8531. https://doi.org/10.1111/jcmm.15473.
[157] Torres JL, Novo-Veleiro I, Manzanedo L, Suárez LA, MacÍas R, Laso FJ, et al. Role of microRNAs in alcohol-induced liver disorders and non-alcoholic

fatty liver disease. World J Gastroenterol 2018;24:4104–4118. https://doi.org/10.3748/wjg.v24.i36.4104.
[158] Szabo G, Satishchandran A. MicroRNAs in alcoholic liver disease. Semin Liver Dis 2015;35:36–42. https://doi.org/10.1055/s-0034-1397347.
[159] Natarajan SK, Pachunka JM, Mott JL. Role of microRNAs in alcohol-induced multi-organ injury. Biomolecules 2015;5:3309–3338. https://doi.org/10.3390/biom5043309.
[160] Greuter T, Malhi H, Gores GJ, Shah VH. Therapeutic opportunities for alcoholic steatohepatitis and nonalcoholic steatohepatitis: exploiting similarities and differences in pathogenesis. JCI Insight 2017;2. https://doi.org/10.1172/JCI.INSIGHT.95354.
[161] Tadokoro T, Morishita A, Masaki T. Diagnosis and therapeutic management of liver fibrosis by microrna. Int J Mol Sci 2021;22. https://doi.org/10.3390/ijms22158139.
[162] Dippold RP, Vadigepalli R, Gonye GE, Hoek JB. Chronic ethanol feeding enhances miR-21 induction during liver regeneration while inhibiting proliferation in rats. Am J Physiol Gastrointest Liver Physiol 2012;303:733–743. https://doi.org/10.1152/ajpgi.00019.2012.-Liver.
[163] Francis H, McDaniel K, Han Y, Liu X, Kennedy L, Yang F, et al. Regulation of the extrinsic apoptotic pathway by microRNA-21 in alcoholic liver injury. J Biol Chem 2014;289:27526–27539. https://doi.org/10.1074/jbc.M114.602383.
[164] Juskeviciute E, Dippold RP, Antony AN, Swarup A, Vadigepalli R, Hoek JB. Inhibition of miR-21 rescues liver regeneration after partial hepatectomy in ethanol-fed rats. Am J Physiol - Gastrointest Liver Physiol 2016;311:G794. https://doi.org/10.1152/AJPGI.00292.2016.
[165] Han W, Fu X, Xie J, Meng Z, Gu Y, Wang X, et al. MiR-26a enhances autophagy to protect against ethanol-induced acute liver injury. J Mol Med (Berl) 2015;93:1045–1055. https://doi.org/10.1007/S00109-015-1282-2.
[166] Saha B, Momen-Heravi F, Kodys K, Szabo G. MicroRNA cargo of extracellular vesicles from alcohol-exposed monocytes signals naive monocytes to differentiate into M2 macrophages. J Biol Chem 2016;291:149–159. https://doi.org/10.1074/JBC.M115.694133.
[167] Saha B, Bruneau JC, Kodys K, Szabo G. Alcohol-induced miR-27a regulates differentiation and M2 macrophage polarization of normal human monocytes. J Immunol 2015;194:3079–3087. https://doi.org/10.4049/jimmunol.1402190.
[168] Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, et al. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. Hepatology 2011;53:209–218. https://doi.org/10.1002/HEP.23922.
[169] Wan Y, McDaniel K, Wu N, Ramos-Lorenzo S, Glaser T, Venter J, et al. Regulation of cellular senescence by miR-34a in alcoholic liver injury. Am J Pathol 2017;187:2788–2798. https://doi.org/10.1016/J.AJPATH.2017.08.027.
[170] Dippold RP, Vadigepalli R, Gonye GE, Patra B, Hoek JB. Chronic ethanol feeding alters miRNA expression dynamics during liver regeneration. 2012. https://doi.org/10.1111/j.1530-0277.2012.01852.x.
[171] Meng F, Glaser SS, Francis H, Yang F, Han Y, Stokes A, et al. Epigenetic regulation of miR-34a expression in alcoholic liver injury. Am J Pathol 2012;181:804–817. https://doi.org/10.1016/j.ajpath.2012.06.010.
[172] Ambade A, Satishchandran A, Szabo G. Alcoholic hepatitis accelerates early hepatobiliary cancer by increasing stemness and miR-122-mediated HIF-1α activation OPEN. Nat Publ Gr 2016. https://doi.org/10.1038/srep21340.
[173] Momen-Heravi F, Bala S, Kodys K, Szabo G. Exosomes derived from alcohol-treated hepatocytes horizontally transfer liver specific miRNA-122 and sensitize monocytes to LPS. Nat Publ Gr 2015. https://doi.org/10.1038/srep09991.
[174] Bala S, Csak T, Kodys K, Catalano D, Ambade A, Furi I, et al. Alcohol-induced miR-155 and HDAC11 inhibit negative regulators of the TLR4 pathway and lead to increased LPS responsiveness of Kupffer cells in alcoholic liver disease. J Leukoc Biol 2016;102:487–498. https://doi.org/10.1189/jlb.3A0716-310R.
[175] Bala S, Csak T, Saha B, Zatsiorsky J, Kodys K, Catalano D, et al. The pro-inflammatory effects of miR-155 promote liver fibrosis and alcohol-induced steatohepatitis. J Hepatol 2016;64:1378–1387. https://doi.org/10.1016/j.jhep.2016.01.035.
[176] Csak T, Bala S, Lippai D, Kodys K, Catalano D, Iracheta-Vellve A, et al. MicroRNA-155 deficiency attenuates liver steatosis and fibrosis without reducing inflammation in a mouse model of steatohepatitis. PLos One 2015;10. https://doi.org/10.1371/journal.pone.0129251.
[177] Lippai D, Bala S, Catalano D, Kodys K, Szabo G. Micro-RNA-155 deficiency prevents alcohol-induced serum endotoxin increase and small bowel inflammation in mice. 2014. https://doi.org/10.1111/acer.12483.
[178] Alpini G, Glaser SS, Zhang J-P, Francis H, Han Y, Gong J, *et al.* Regulation of placenta growth factor by microRNA-125b in hepatocellular cancer. n.d.
[179] Dolganiuc A, Petrasek J, Kodys K, Catalano D, Mandrekar P, Velayudham A, et al. MicroRNA expression profile in lieber-decarli diet-induced alcoholic and methionine choline deficient diet-induced nonalcoholic steatohepatitis models in mice. Alcohol Clin Exp Res 2009;33:1704–1710. https://doi.org/10.1111/j.1530-0277.2009.01007.x.
[180] Blaya D, Coll M, Rodrigo-Torres D, Vila-Casadesús M, Altamirano J, Llopis M, *et al.* Integrative microRNA profiling in alcoholic hepatitis reveals a role for microRNA-182 in liver injury and inflammation n.d. https://doi.org/10.1136/gutjnl-2015-311314.
[181] Ladeiro Y, Couchy G, Balabaud C, Bioulac-Sage P, Pelletier L, Rebouissou S, et al. MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. 2008. https://doi.org/10.1002/hep.22256.
[182] Huang GH, Shan H, Li D, Zhou B, Pang PF. MiR-199a-5p suppresses tumorigenesis by targeting clathrin heavy chain in hepatocellular carcinoma. Cell Biochem Funct 2017;35:98–104. https://doi.org/10.1002/CBF.3252.
[183] Saikia P, Bellos D, Mcmullen MR, Pollard KA, De La Motte C, Nagy LE. MicroRNA 181b-3p and its target importin a5 regulate toll-like receptor 4 signaling in Kupffer cells and liver injury in mice in response to ethanol. 2017. https://doi.org/10.1002/hep.29144/suppinfo.
[184] Dong X, Liu H, Chen F, Li D, Zhao Y. MiR-214 promotes the alcohol-induced oxidative stress via down-regulation of glutathione reductase and cytochrome P450 oxidoreductase in liver cells. 2013. https://doi.org/10.1111/acer.12209.
[185] Yeligar S, Tsukamoto H, Kalra VK. MicroRNA-199 and α hypoxia-inducible factor-1 and human endothelial cells involves ET-BR in liver sinusoidal endothelial cells ethanol-induced expression of ET-1 and. J Immunol Ref 2009;183:5232–5243. https://doi.org/10.4049/jimmunol.0901084.
[186] Li M, He Y, Zhou Z, Ramirez T, Gao Y, Gao Y, et al. MicroRNA-223 ameliorates alcoholic liver injury by inhibiting the IL-6-p47phox-oxidative stress pathway in neutrophils. Gut 2017;66:705–715. https://doi.org/10.1136/gutjnl-2016-311861.
[187] Saikia P, Roychowdhury S, Bellos D, Pollard KA, Mcmullen MR, Mccullough RL, *et al.* Hyaluronic acid 35 normalizes TLR4 signaling in Kupffer cells from ethanol-fed rats via regulation of microRNA291b and its target Tollip OPEN n.d. https://doi.org/10.1038/s41598-017-15760-4.
[188] Hyun J, Wang S, Kim J, Rao KM, Park SY, Chung I, et al. ARTICLE MicroRNA-378 limits activation of hepatic stellate cells and liver fibrosis by suppressing Gli3 expression. 2016. https://doi.org/10.1038/ncomms10993.