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MicroRNAs in Alcoholic Liver Disease

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Abstract

Alcoholic liver disease (ALD) is characterized by hepatocyte damage, inflammatory cell activation and increased intestinal permeability leading to the clinical manifestations of alcoholic hepatitis. Selected members of the family of microRNAs are affected by alcohol, resulting in an abnormal miRNA profile in the liver and circulation in ALD. Increasing evidence suggests that mRNAs that regulate inflammation, lipid metabolism and promote cancer are affected by excessive alcohol administration in mouse models of ALD. This communication highlights recent findings in miRNA expression and functions as they relate to the pathogenesis of ALD. The cell-specific distribution of miRNAs, as well as the significance of circulating extracellular miRNAs, is discussed as potential biomarkers. Finally, the prospects of miRNA-based therapies are evaluated in ALD.

Keywords

- ▶ microRNA
- ▶ miR-122
- ▶ miR-155
- ▶ Kupffer cell
- ▶ gut permeability

Biogenesis and Function of MicroRNAs

MicroRNAs (miRNAs) are small noncoding RNAs that act at the posttranscriptional level to regulate expression of their respective target mRNAs and encoded proteins.^{1–3} MicroRNAs are transcribed from DNA as pri-miRNAs, transported from the nucleus to the cytosol as pre-miRNAs where they are processed to mature miRNAs, usually into 22 nucleic acid sequences. MicroRNAs act in the RISC (RNA-induced silencing complex) and the significance of their intracellular localization is under active investigation.⁴ Owing to the usually large number of target genes of individual miRNAs, miRNAs fine-tune all important biological processes in the liver including regeneration, metabolism, immunity, bile secretion, fibrosis, and hepatocellular cancer.^{5–8}

In addition to intracellular localization, miRNAs are also found in body fluids including serum, plasma, urine, and saliva.^{9–11} Increasing evidence suggests that miRNAs are present in the protein fraction as well as in exosomes in the circulation.¹² The high stability of miRNAs in the circulation makes them attractive for biomarker discovery in liver diseases.¹³ In this review, we summarize the central components of current knowledge on the role of microRNAs in alcoholic liver disease (ALD) (▶ **Table 1**, ▶ **Fig. 1**).

Pathogenic Features of Alcoholic Liver Disease and Alcoholic Hepatitis

Alcoholic liver disease is caused by prolonged and excessive alcohol use in susceptible individuals. It is characterized by hepatic steatosis, steatohepatitis, and fibrosis/cirrhosis in advanced cases that are all biological processes potentially regulated by miRNAs.¹⁴ The clinical progression and manifestations of ALD parallel its pathogenesis. Changes from normal liver to steatosis, steatohepatitis, steatohepatitis with fibrosis, and cirrhosis represent progression of ALD. Through its direct effects, as well as its metabolic byproducts, acetaldehyde and reactive oxygen species (ROS), alcohol triggers mitochondrial and intracellular damage mechanisms.¹⁵ Even in the early phase of ALD, hepatocyte damage and apoptosis results in the release of cellular danger molecules that are sensed by immune cells in the liver, triggering inflammation. In addition to these endogenous danger signals, alcohol use results in increased levels of bacterial lipopolysaccharide (LPS) in the portal and systemic circulation due to increased intestinal permeability, leading to the activation of Kupffer cells.^{16–19} Excessive alcohol use adversely affects multiple organ systems including the gastrointestinal tracts and the liver.^{20,21} The gut–liver axis plays a critical

Table 1 Distribution and function of microRNAs

Distribution and function of microRNAs
Distribution
Nucleus
Cytoplasm
Extracellular
Body fluids (saliva, urine, bile)
Serum, plasma
Exosomes
Functions
Intracellular: Posttranscriptional regulation
Extracellular
Cell-to-cell communication
Interorgan communications

role in the pathogenesis of ALD due to the alcohol-induced increase in gut permeability.^{22,23} Intake of excessive amounts of alcohol decreases the barrier function of the gut mucosa and results in increased levels of bacterial endotoxin in the portal circulation.^{24–27} MicroRNA-212 has been implicated in the alcohol-induced abnormal gut barrier function.²⁸ Studies found that gut-derived endotoxin contributes to activation of Kupffer cells in the liver, leading to increased production of proinflammatory cytokines including TNF α , IL-1, IL-6, and other inflammatory mediators.^{17,29–31} It has also been demonstrated that TLR4 and its downstream signaling molecule,

IRF3, are critical in alcohol-induced liver disease.^{15,32} Importantly, TLR4-mediated signaling is a major regulator of inflammation-associated microRNAs, and conversely, inflammation-associated microRNAs exert tight regulation on the mRNA levels of TLR4 and the majority of the signal transduction machinery downstream of TLR4.^{33,34} Thus, the components of the LPS/TLR4/inflammatory cascade are all closely regulated by miRNAs in ALD.^{13,35}

Inflammation-Associated MicroRNAs Are Induced in Alcoholic Liver Disease

Inflammation-associated microRNAs (inflammiRs) are a group of miRNAs that regulate inflammation. These include miR-223, miRNA-155, miRNA-146a, miRNA-146b, miRNA-125, and miRNA-132, which play important roles in the regulation of inflammation by modulating expression of multiple components of TLR signaling and cytokine production, as well as being negative regulators of inflammation (►Table 2).^{33,34} In the immediate early stage of the inflammatory processes, there is a rapid increase in the levels of miR-132, followed by increases in miR-155 and miR-146b. In the late stage of inflammation, miR-21 is induced and contributes to IL-10 production and is also involved in fibrosis.³⁶ Although the kinetics of miRNA induction in the various stages of ALD awaits investigation, prolonged alcohol feeding in mice was shown to increase levels of miR-155 in the total liver, isolated hepatocytes, and in Kupffer cells.^{35,37}

Kupffer Cell Activation and Sensitization to LPS Is Regulated by miRNA-155 in Alcoholic Liver Disease

Kupffer cells, resident liver macrophages, are important contributors to the inflammatory components of ALD. Previous studies showed that prolonged (5–7 days) exposure of human

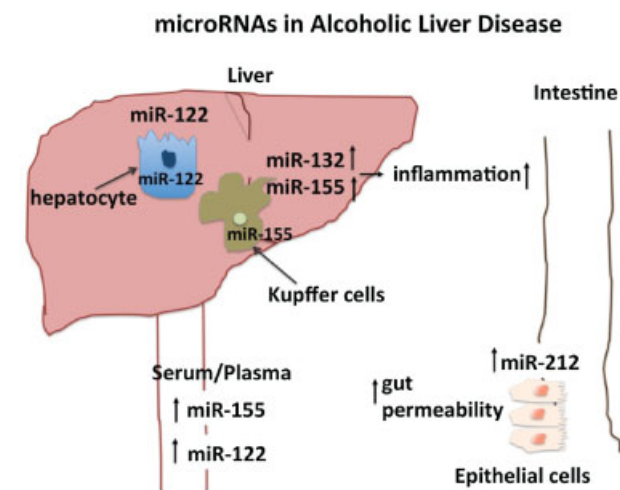


Fig. 1 The diverse roles microRNAs (miRNAs) in the pathogenesis of alcoholic liver disease (ALD) in regulating steatosis, inflammation, and gut permeability. Ethanol and its metabolites induce miRNA dysregulation in a variety of organ systems. In the liver, miRNAs in hepatocytes regulate steatosis, while those in Kupffer cells yield a proinflammatory state, perpetuating liver injury. Additionally, alcohol's effect on the intestines leads to increasing the permissiveness of the gut to inflammatory mediators such as lipopolysaccharide. The resulting endotoxemia contributes to hepatic damage and to the development of ALD.

Table 2 microRNAs in liver diseases

MicroRNA	Target genes
miR-155	TNF α (positive regulation), SOCS-1, SHIP-1
miR-132	Neuroimmune microRNA
miR-122	Lipid metabolism: HMGCoA, ApoE, MTTP, PGC1 α Fibrosis: P4HA1 Hepatocellular cancer: Igf1R, ADAM10, cyclin G1, KLF6
miR-21	Fibrosis
miR-26a	Hepatocellular cancer

Abbreviations: ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; ApoE, apolipoprotein E; HMGCoA, 3-hydroxy-3-methylglutaryl-coenzymeA; Igf1R, insulin-like growth factor 1 receptor; KLF6, Kruppel-like factor 6; PGC1 α , PPAR-gamma coactivator 1-alpha; SHIP-1, Src Homology-2 domain containing inositol 5-phosphate-1; SOCS, suppressor of cytokine signaling; TNF α , tumor necrosis factor alpha.

monocytes or RAW murine macrophages to alcohol results in a proinflammatory phenotype and sensitization to LPS.^{30,38} Interestingly, prolonged alcohol treatment of RAW macrophages resulted in increased miR-155 levels.³⁷ Kupffer cells isolated from mice after a 4-week chronic alcohol feeding have increased sensitivity to LPS by producing significantly higher levels of TNF α compared with KCs from nonalcohol-exposed mice.^{30,37} We found that the increased TNF α production by KCs from chronic alcohol-fed mice had significantly higher levels of miR-155 compared with KCs from paired control mice. Chronic alcohol increased miR-155 in KCs via NF- κ B induction.³⁷

MicroRNA-132 and miR-212

MicroRNAs-132 and -212 are products of the same gene and share substantial homology.³⁹ Independent studies discovered increases in miR-132 levels in the liver and brain and miR-212 increases in the intestine after chronic alcohol administration in mice.^{28,35,40,41} The complex roles of these microRNAs, both in the liver and in other organs, have yet to be evaluated in ALD. Increasing evidence suggests that alcohol-induced tissue damage, including ALD, is influenced not only by the direct effects of alcohol on the liver, but also by signals derived from other organs as a result of alcohol exposure. The best-studied evidence for this is the increased LPS in the portal circulation after excessive alcohol consumption. It remains to be seen whether alcohol-induced microRNAs generated in one organ could modify the function of another remote organ in alcohol-induced tissue damage.

The importance of miR-212 has been highlighted in alcohol-induced gut permeability. Alcohol has increased the levels of miR-212 in caco-2 cells and this has been correlated with a decrease in the expression of tight junction proteins including occludens and zona occludens-1 (ZO-1).²⁸

Liver Steatosis and miRNA-122 in Hepatocytes in Alcoholic Liver Disease

In hepatocytes, miR-122 is unique among hepatic microRNAs, representing approximately 70% of its miRnome.⁴²⁻⁴⁵ In hepatocytes, miRNA-122 directly and indirectly regulates a complex web of genes involved in lipid synthesis and export, as well as cholesterol homeostasis.^{44,46-49} Germline knockout (KO) and antisense oligonucleotides (ASO), or adeno-associated virus- (AAV) mediated knockdown (KD) models in mice have been shown to reduce serum cholesterol (high-density lipoprotein and low-density lipoprotein) and decrease serum triglycerides (TGs) through inhibition of critical regulatory steps involved in lipid metabolism such as HMG-CoA reductase, cytochrome P450 7a1, PGC1a, and ApoE.^{44,46,50} However, though both germline and liver-specific KO models demonstrated beneficial trends in serum lipid profiles, corroborating previous KD studies, both KO models were shown to have advanced steatohepatitis, contrary to previous ASO-mediated KD studies.^{44,46-48} In these KO mice, microsomal triglyceride transfer protein (MTTP) was found to be indirectly reduced, preventing the assembly and export of lipopro-

teins from the liver, thus leading to steatosis.^{47,48} It is key to note that while these pathways are significantly affected by modulation of miR-122 in vivo, a direct target or central mechanism has remained elusive. Studies have suggested that AMP-activated protein kinase (AMPK) and the PPAR family of proteins, both widely identified as master regulators of cellular metabolism, may act as primary effectors of this microRNA.^{46,51}

Alcohol and Hepatitis C Infection

The clinical course and outcomes of the combination of prolonged alcohol use and chronic hepatitis C virus (HCV) infection result in accelerated progression to fibrosis and cirrhosis.⁵²⁻⁵⁴ Multiple pathogenic mechanisms have been identified as contributors to the combined liver damage by alcohol plus HCV, including effects on hepatocytes, HCV replication, and host immune defense.^{55,56} Importantly, miR-122 is a key host factor in HCV replication by providing a binding site for the promoter-region of the HCV virus, stabilizing the vector genome, and promoting viral replication.⁵⁷ Because of the key role of miR-122 as host factor in HCV replication, it is not unexpected that miR-122 inhibition results in attenuation of HCV replication.⁵⁸ Inhibition of miR-122 using Miravirsin, has resulted in attractive inhibition of HCV viral load in a recently published study, and currently Miravirsin is in phase 2 clinical trials.^{59,60} Using in vitro approaches, it has recently been shown that alcohol administration to hepatoma cells increases miR-122 levels via NF- κ B activation and that miR-122-mediated upregulation of cyclinG1 also contributes to the alcohol-induced increase in HCV replication.⁶¹ Furthermore, alcohol increases GW182 and heat shock protein-90 in hepatoma cells and these proteins provide a stabilization platform for miR-122, thereby increasing HCV replication.⁶² Together, these data suggest that microRNA modulation by alcohol has an important role in the modulation of HCV infection and potentially other liver diseases.

Fibrosis in Alcoholic Liver Disease and the Role of MicroRNAs

miR-122 expression was significantly reduced in transactivated HSCs and in the livers of mice treated with CCl₄. In vitro, an overexpression of miR-122 in LX2 cells inhibited the proliferation. It was found that P4HA1 is a primary target of miR-122. Additionally, overexpression led to decreased collagen maturation and ECM production.⁶³ Ethanol has been shown to repress miRNA 21 in neural stem cells and neural progenitor cells that alter cellular development and maturation. Additionally, miR-21 influences PTEN/PI3K signaling, which ultimately contributes to the fibrotic response.⁶⁴

Hepatocellular Cancer, MicroRNAs, and Alcoholic Liver Disease

Cancer cells have changes in their miRNA expression profile and also secrete miRNAs that may provide specific signatures.

In hepatocellular cancer (HCC), decreases in miRNA-26a and miR-122 have been found in hepatocytes.⁶⁵ In contrast, miR-221 is increased in HCC.

The greatest body of literature has characterized the significance of miR-122 in the development of HCC. Genome-wide expression profiling has shown decreased miR-122 expression and increased miR-122 predicted target genes in tumor tissues.⁵⁰ This decrease of miR-122 expression in HCC has been found to be associated with worsening prognosis and metastasis by many studies.^{66–70} Additional studies have shown the involvement of miR-122 in cellular proliferation, hepatobiliary differentiation, apoptosis, and tumor invasion. Currently, several target genes of miR-122 have been identified to be involved in hepatocarcinogenesis, such as A disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), Kruppel-like-factor 6 (KLF6), insulin-like growth factor 1 receptor (Igf1R), cyclin G1, and Wnt1.^{68,71,72}

Circulating MicroRNAs in Alcoholic Liver Disease

MicroRNAs are present in various body fluids including blood, saliva, urine, bile, etc. This makes them attractive for biomarker discovery particularly because of their stability and easy detection. MicroRNAs are present in different compartments of the serum and plasma including the plasma protein fraction in association with Ago-2 or in extracellular vesicles, including exosomes that are small (20–150 nm) membrane-coated vesicles or in macrovesicles (size 150–1000 nm). The exact biological function of circulating microRNAs is under active investigation. Current knowledge suggests that they are involved in cell-to-cell communication and likely serve as markers of cellular damage, stress and disease. The latter provides potential for evaluation of miRNA “signatures” of tissue damage and injury in disease conditions including liver disease.

Much investigation focused on miRNA-122 as a maker of liver damage, and studies consistently demonstrate that circulating miRNA-122 levels are increased in various types of liver disease including alcoholic, non-alcoholic fatty liver disease, viral hepatitis (HCV and HBV), and drug (acetaminophen) induced liver injury.^{73–75} In most of these studies, correlation between serum ALT and miRNA-122 was linear suggesting that serum/plasma miR-122 is a good marker of liver injury.

MicroRNAs that regulate inflammation are also found in the circulation in ALD. In a recent study, we showed that in mouse models of liver diseases, inflammation-associated miRNAs are also present in the circulation. For example, miR-155 levels were increased both in ALD as well as in TLR-induced, but not in drug-induced, hepatocyte-damage models.¹²

A major limitation in current diagnostic tools is the lack of disease-specific biomarkers in alcoholic liver injury, and the lack of specific and/or non-invasive markers that could differentiate between liver injury and inflammation. It remains to be seen whether microRNAs (single or a panel of microRNA) will have the potential to provide such diagnostic tools as biomarkers.

MicroRNAs as Therapeutic Targets in Alcoholic Liver Disease

Therapeutic strategies targeting miRNAs can involve miRNA replacement or inhibition. To date, there have been no reports on miRNA-driven therapy in ALD.⁷⁶ However, we showed that inhibition of miRNA-155 in alcohol-treated Kupffer cells and macrophages attenuates LPS-induced TNF α production.³⁷ In HCC models, restoration of the reduced levels of miR-26a yielded an attenuation in the development of HCC.⁶⁵ Further studies may reveal miRNA targets for therapy of ALD.

Future Perspectives

Understanding the function and role of microRNAs in ALD has many potential aspects that can be translated to improved care and potential treatment of patients with ALD.

First, miRNAs may serve as biomarkers to distinguish patients who have severe or moderate disease and/or aid physicians in predicting clinical outcomes. Although it is unlikely that a particular circulating miRNA would become a marker of disease, it is conceivable that a specific microRNA “signature” would indicate association with ALD and/or its various clinical stages.

Second, beyond the potential biomarker, circulating microRNAs (ex-miRNA) may have a yet to be defined biological function in cell-to-cell communication. The biodistribution and function of extracellular miRNAs is a novel research area. In a recent study, we showed that injection of a MicroRNA mimic into miR-155 deficient mice resulted in its rapid distribution and clearance of miRNA in the circulation.⁷⁷ We also found that ex-miRNA-155 enters hepatocytes in miR-155-deficient mice, suggesting that miRs may have a potential to modulate functions of cells and/or organs outside of their origin.

Finally, miRNAs are potential therapeutic targets in ALD. For example, increased miR-155 expression in Kupffer cells that contributes to LPS sensitivity and increased TNF α production could be a feasible target to attenuate TNF α production in ALD. Given that the extent of TNF α increase is a predictor of mortality in acute alcoholic hepatitis, it is tempting to speculate that attenuation of TNF by targeting miRNAs would result in reduced, but not eliminated, TNF levels. Previous clinical trials using anti-TNF α antibodies that block TNF α resulted in increased infections; thus, moderate reduction of TNF levels though miRNA inhibition might be an attractive approach in this patient population. Because miRNAs affect only posttranslational processes, anti-miRNA therapies result only in partial changes in their target gene products. A technical barrier to the theoretical approach of inhibition of miR-155 in KCs is cell-specific delivery of the miRNA inhibitor.

Abbreviations

AAV	adeno-associated virus
ADAM10	a disintegrin and metalloproteinase domain-containing protein 10
ALD	alcoholic liver disease
AMPK	AMP-activated protein kinase

ApoE	apolipoprotein E
ASO	antisense oligonucleotides
HBV	hepatitis B virus
HCV	hepatitis C virus
HCC	hepatocellular cancer
HSC	hepatic stellate cell
Igf1R	insulin-like growth factor 1 receptor
IL-1	interleukin-1
IL-6	interleukin-6
IRF3	interferon regulatory factor
KC	Kupffer cells
KD	knockdown
KLF6	Kruppel-like factor 6
KO	knockout
LPS	lipopolysaccharide
miRNA and miR	microRNA
MTTP	microsomal triglyceride transfer protein
NF- κ B	nuclear factor kappa beta
RISC	RNA-induced silencing complex
ROS	reactive oxygen species
TG	triglyceride
TLR	toll-like receptor
TNF α	tumor necrosis factor alpha
ZO-1	zona occludens-1

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