The functional role of microRNAs in alcoholic liver injury

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Abstract

The function of microRNAs (miRNAs) during alcoholic liver disease (ALD) has recently become of great interest in biological research. Studies have shown that ALD associated miRNAs play a crucial role in the regulation of liver-inflammatory agents such as tumour necrosis factor-alpha (TNF-α), one of the key inflammatory agents responsible for liver fibrosis (liver scarring) and the critical contributor of alcoholic liver disease. Lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, is responsible for TNF-α release by Kupffer cells. miRNAs are the critical mediators of LPS signalling in Kupffer cells, hepatocytes and hepatic stellate cells. Certain miRNAs, in particular miR-155 and miR-21, show a positive correlation in up-regulation of LPS signalling when they are exposed to ethanol. ALD is related to enhanced gut permeability that allows the levels of LPS to increase, leads to increased secretion of TNF-α by the Kupffer cells and subsequently promotes alcoholic liver injury through specific miRNAs. Meanwhile, two of the most frequently dysregulated miRNAs in steatohepatitis, miR-122 and miR-34a are the critical mediators in ethanol/LPS activated survival signalling during ALD. In this review, we summarize recent findings regarding the experimental and clinical aspects of functions of specific microRNAs, focusing mainly on inflammation and cell survival after ethanol/LPS treatment, and advances on the role of circulating miRNAs in human alcoholic disorders.

Keywords: alcoholic liver diseases • microRNAs • Kupffer cells • TLR4 • TNF-α • LPS • apoptosis

Introduction

Alcoholic liver disease presents a global health concern [1]. This disease ranges from alcoholic fatty liver and steatohepatitis to alcoholic cirrhosis, and includes hepatocellular carcinoma [2, 3]. It is the 12th leading cause of death in the United States, according to the National Institute on Alcohol Abuse and Alcoholism and accounted for a total of 14,364 deaths in 2007. Nearly 14 million Americans-abuse

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alcoholic or are alcoholics. Several million more adults could be on their way to alcohol problems. More notably, alcohol is implicated in more than 200 diseases and is a direct causal factor in 60 types of diseases and injury [4]. The liver is the main site of alcohol metabolism and a major target organ of alcohol-induced injury [1]. Alcoholic liver disease has been the leading cause of liver-related disease worldwide [5–7]. Alcoholic liver disease is defined by scarring of the liver by the inflammatory agent tumour necrosis factor-alpha (TNF-α) which is secreted by Kupffer cells in the liver [8]. The release of TNF-α is triggered by the binding of lipopolysaccharide (LPS) by toll-like receptor 4 (TLR4) on Kupffer cells [9]. It is the translocation of these bacterial products in the lumen of the intestine that causes homeostatic imbalances in the liver. Because of the liver's importance to homeostasis and the worldwide prevalence of alcoholic liver disease, liver function has been given extra emphasis in biomedical research.

One control point regulating inflammatory response and cellular apoptosis in ALD involves microRNA targeting of critical inflammation/apoptosis signalling proteins. MicroRNAs are short functional RNAs that cause reduced expression of their target genes through post-transcriptional mechanisms. In general, this targeting involves imperfect base-pairing between the microRNA and the cognate mRNA target, resulting in altered protein production [10, 11]. In human ALDs, microRNAs are intimately involved in development and progression of liver injury, and act to alter expression of disease-related targets [12, 13]. Much attention has been devoted to microRNA function in recent years, in part because microRNA biology is still being elucidated, and in part because of their promise in diagnosis and treatment of disease. Modulation of microRNA function is an attractive emerging approach to ALD treatment, and studies to understand the underlying mechanisms of altered microRNA expression and functions are necessary to pursue this treatment strategy.

LPS

Lipopolysaccharide exposure is initiated by the liver's detoxification process upon exposure to gram-negative bacterial cell wall [14–16]. Lipopolysaccharide is released into the blood stream when gram-negative bacterial cells die or lyse and are then transported to the liver for detoxification [17]. The LPS alone has been found to increase with alcoholic liver consumption when gut permeability is severely compromised. The experiments with alcohol-fed mice revealed that endotoxin levels increased in alcohol-fed mice when compared with normal mice [14–16]. It was concluded from this experiment that such results were a direct result of the weakening of the intestinal tight junctions by ethanol [18]. The excess of endotoxins is absorbed by the intestinal lumen and later passes through the liver, where monocytes and macrophages, such as Kupffer cells are then exposed to the toxin [19, 20]. In addition to the increased permeability of the intestinal lumen due to ethanol exposure, ethanol exposure also seems to be correlated with an increased amount of gram-negative bacterial growth in the lumen of alcoholics' intestines. Although this increase in bacterial growth is not certain, suggestive evidence has led to further research on the subject [21]. Since alcohol can significantly increase the translocation of LPS from the gut. The study of

these interactions may provide potential new targets for therapeutic intervention.

TLR4

Toll-like receptors were first discovered in Drosophila and later as corresponding human homologs [9]. One particular toll-like receptor, TLR4, identifies and binds LPS through its co-receptors CD14 or MD-2 [22, 23]. MD-2 is a soluble protein that non-covalently associates with TLR4 and binds LPS directly to form a complex with LPS in the absence of TLRs [24]. MD-2 has been shown to have increased concentrations in alcohol-fed mice [25]. It is known to exist in a soluble form which, when in high concentrations, has shown to be an inhibitor of endotoxin-activated TNF-α secretion [9]. CD14, however, is found in monocytes, macrophages, parenchymal cells and fibroblasts [26, 27]. Its use in the LPS induced TNF-α pathway was confirmed during CD14 inhibition in mice. CD14 inhibited mice became resistant to endotoxin shock [28]. Upon contact with TLR4 receptors and co-receptors, LPS induces Kupffer cells to release TNF-α [6]. Because of this, the TLR4 and LPS relation plays a key role in the mediation of inflammatory agents in the liver.

There is ample evidence for increased inflammatory cascade activation in ALD. Toll-like receptor 4 is expressed in all cell types of the liver; thus, gut-derived endotoxin can modulate the function of all liver cells in ALD [9, 29, 30]. Over the years, compelling evidence has revealed that the TLR4-LPS signalling pathway plays a critical role in alcohol-induced liver injury [31]. Both chronic and acute (or binge) alcohol use affects the various components of TLR4 signalling. There is increased expression of TLR4 and its co-receptors, as well as other TLRs, in ALD in mice [9]. The studies in TLR4 mutant mice demonstrated protection from early ALD, and recent reports using TLR4 deficient mice validated the important role of TLR4 in the pathogenesis of ALD [25].

Some feedback mechanisms exist to limit LPS mediated toxic effects. Several soluble decoy receptors, such as soluble TLR4, and splice variants of signal-transduction proteins, including MyD88-s, IRAK-M and TAG, are the key regulators [32]. Toll-like receptor 4 mediates LPS signalling with the assistance of its co-receptors, CD14 or MD-2 [22, 23]. Lipopolysaccharide/TLR4 enlists the adaptor molecules MyD88 and TRIF, and subsequently activates downstream signalling pathways, respectively. Activation of NF-kB by TLR4-MyD88 complex enhances the production of pro-inflammatory cytokines, such as TNF-α, interleukin (IL)-6 and IL-1b [23]. Tank Binding Kinase-1/IkB kinase-e/IKKi (TBK/IKKe) phosphorylation and activation of the interferon regulatory factor-3 by TRIF signalling lead to the production of IFN-γ-interferons [33]. Within 4 days of ethanol exposure, there was a striking spike in expression of IFN-γ, along with TNF-α and IL-6 – prior to hepatic triglyceride accumulation or increased plasma alanine aminotransferase (ALT) activities, as well as before the induction of cytochrome P450 2E1 or oxidative stress [34]. Therefore, activation of both of the pro-inflammatory and IFN-γ pathways could be either LPS/TLR4 dependent or independent, and evaluation of these specific pathways may have translational impact on ALD [9, 34]. microRNAs have also been introduced, such as miR-146a

and miR-21, which is induced by LPS and negatively targets signalling proteins such as IRAK1, TRAF6 and PDCD4 at the post-transcriptional level [32, 35, 36].

TNF-α

Tumour necrosis factor-alpha is an inflammatory agent in the liver which has been found to be a key contributor to alcoholic liver disease [5]. Tumour necrosis factor-alpha is released by macrophages in the liver upon introduction of LPS, which binds to TLR4. The release of TNF-α causes inflammation, which leads to liver fibrosis [37]. A recent study determined that the production of TNF-α in the liver by Kupffer cells was increased both by the introduction of ethanol and LPS individually. In this study, the levels of TNF-α increased when a dosage of LPS was introduced to the liver macrophages while keeping the ethanol levels unchanged. The reverse experiment was conducted where ethanol was increased and no additional LPS was introduced into the system. In both experiments the TNF-α production rose. Since LPS is the known component to activate the TLR4 pathway, this experiment confirmed that it is not ethanol's direct involvement in the pathway's activation, but rather ethanol's mediation of LPS and other factors that leads to the liver scarring [31]. The TLR4 pathway plays a key role in the activation of TNF-α, but it is the TNF-α protein, which regulates the inflammation.

Kupffer cells and increase in TNF-α secretion

Kupffer cells are resident macrophages in the liver that become activated upon recognition of an LPS signal both *in vivo* and *in vitro* [9,31]. Kupffer cells were identified as a key component of alcoholic liver disease through studies of their activation and deactivation. *Inactivation of Kupffer cells* with gadolinium *chloride or clodronate* injection can ameliorate *alcohol-* induced liver disease [38, 39]. It was concluded, thereafter, that Kupffer cells are, in part, responsible for alcoholic liver disease [38, 39]. LPS signalling to TLR4 and its co-receptors stimulates Kupffer cells to release TNF-α, which causes scarring of liver tissue and ultimately alcoholic liver disease [31]. The damage caused by TNF-α from Kupffer cells in the liver ranges from steatosis and inflammation to hepatocyte damage in alcoholic liver disease [40, 41]. There exist three particular miRNAs that regulate the production of TNF-α in Kupffer cells: miR-155 and miR-146a [36, 42], although only one shows positive up-regulation of TNF-α: miR-155 [43]. As the major macrophage population of the body that has direct contact with the blood, Kupffer cells have been of great interest in recent research.

microRNAs

microRNAs are segments of RNA, which serve as epigenetic regulators. The first discovery and observation of miRNA's function occurred in 1993, and over the subsequent 10 years, 300+ miRNA sequences have been discovered. Today, ~17,000 miRNAs have been described in 142 species. Of these, around 1000 are present in the human genome [44, 45]. MicroRNAs (miRNAs) belong to a group of non-protein-coding RNAs (ncRNAs) about 20–22 nucleotides long [46–48] that have been found to be key regulators of gene expression [5, 6]. They are transcribed from RNA polymerase II or Poly II. They begin their existence in the nucleus, and then travel to the cytoplasm where they mature (Fig. 1). They are responsible for the alternation of hundreds of genes by binding to the untranslated regions of mRNA [49]. miRNAs have also been found to have influence on the modulation of methylation [50, 51]. Certain miRNAs have been linked to specific diseases such as hepatocellular carcinoma and various types of hepatitis. These miRNAs are the first responders when cells signal damage or pathogenic infection. They are important in the body's immune response. [6] Due to their vast amount of influence on the body, the study of miRNAs has gained a significant amount of interest in recent research.

miRNA and epigenetics

Epigenetics, in its essence, defines the alternation of gene expression without disruption of DNA sequences. Epigenetic regulation is altered and manipulated by several factors, including DNA methylation, modifications of histones and RNA silencing by non-protein-coding RNAs (ncRNAs) [46, 47]. DNA methylation is an epigenetic modification that can regulate gene expression and is tightly regulated by at least three DNA methyltransferases (DNMT-1, DNMT-3A and DNMT-3B). Aberrant DNA methylation has been implicated in many human diseases including alcoholic liver disease [52–54]. Alcohol consumption causes cellular injury. Recent developments indicate that ethanol induces epigenetic alterations, particularly acetylation, methylation of histones, and hypo- and hypermethylation of DNA [55–57]. This has opened up a new area of interest in ethanol research and is providing novel insight into actions of ethanol at the nucleosome level in relation to gene expression and pathophysiological consequences. Although DNA methylation has been tightly linked to liver injury and poor disease outcome in many hepatic disorders, including human ALD, its application to ethanol-dependent ncRNA expression is novel. A better understanding of how ethanol interacts with specific DNA methyl transferases and contributes to aberrant ncRNA expression will clearly advance the field and increase our understanding of the mechanisms involved in the development of ALD.

Altered DNA methylation occurs after alcohol consumption during initial periods of alcohol abuse. Global hypomethylation of DNA in liver after long-term ethanol exposure has been reported [58]. Regional hypomethylation of the c-myc gene occurs in the liver after long-term consumption of alcohol. Decreased DNA methylation with a concomitant decrease in DNMT activity after ethanol exposure of pregnant rats has been reported in foetal tissues [59]. Decreased activity of DNMT has also been discovered in peripheral blood cells from ALDs. Moreover, ethanol consumption has also been shown to be associated with reduced DNMT transcript levels and altered methylation of imprinted DNA regions in sperm [60–62]. In addition, chronic ethanol consumption can impair 1-carbon metabolism,
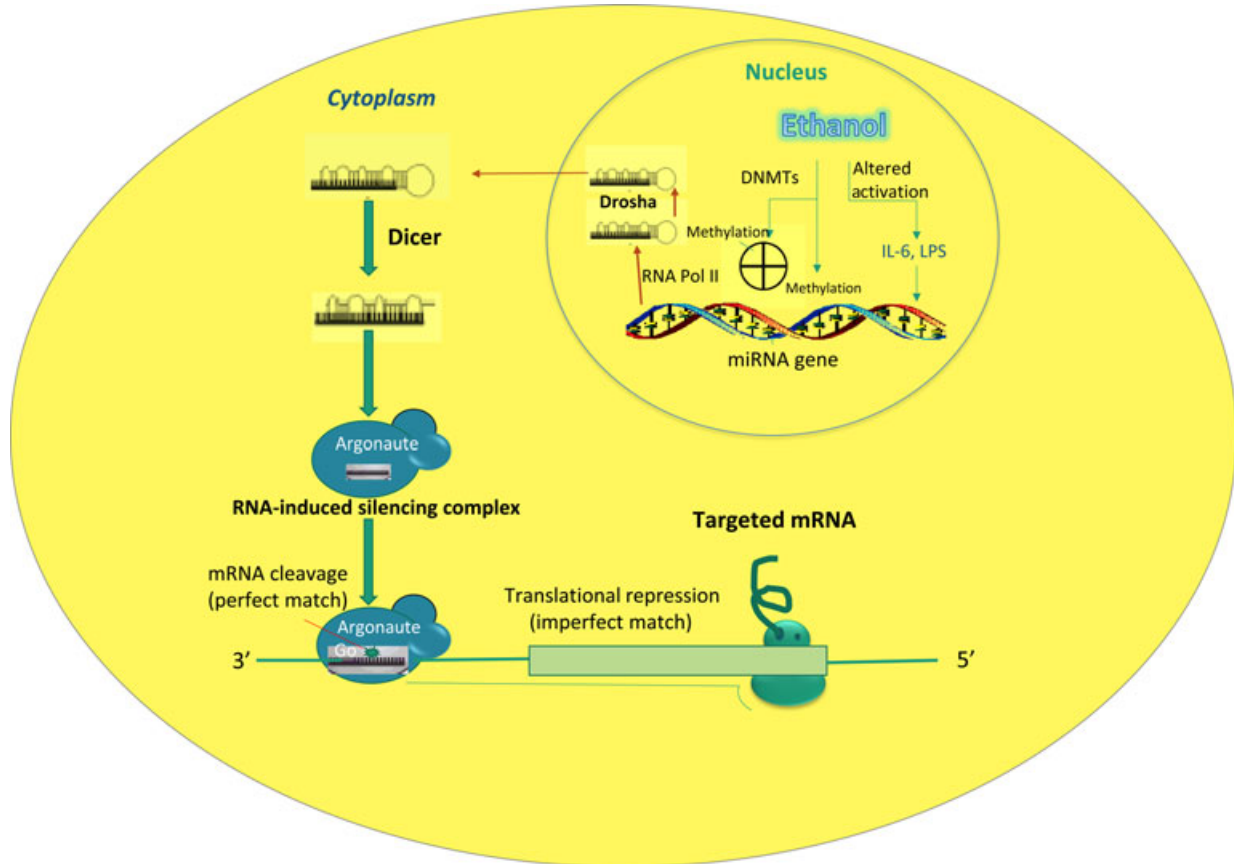
Fig. 1 Aberrant expression and functional changes of specific miRNAs during alcoholic liver injury. During alcoholic liver disease, Ethanol affects miRNA expression by altering activation of transcription factors including lipopolysaccharide, interleukin-6 and tumour necrosis factor-alpha, and/or epigenetic modification enzymes such as methyltransferases, such as DNA methyltransferases 1 (DNMT1), DNMT3A and DNMT3B. miRNA precursors are cleaved by RNases Drosha and Dicer while undergoing transport from the nucleus to the cytoplasm. Association of the mature miRNA with an Argonaut protein (Ago) directs the complex to complementary target sequences in specific messenger RNAs. If the target is perfectly complementary to the miRNA, Argonaute 2, a ribonucleoprotein associated with the miRNA, can mediate its cleavage. However, the imperfect match between miRNA and target may only result in translational repression without mRNA alterations [104–106].

consequently diminishing the availability of S-adenosyl-methionine. This methyl donor is required for both DNA and histone methylation [63, 64]. Ethanol-mediated reductions in DNA methylation could be expected to increase the expression of affected genes, including ncRNAs. Therefore, the intriguing possibility worthy of investigation is that epigenetic changes as a result of ethanol may account for altered expression of some miRNAs.

miRNA is also a modulator of epigenetics in the liver at a post-transcriptional level. It is responsible for gene expression regulation. They are responsible for the hindering of several translational elements to include: initiation, elongation, degradation, and degradation of target mRNA [65–67]. The role of miRNA epigenesis does not only involve the regulation of LPS signalling or TNF-α production, miRNA is also a major component in regulating intestinal permeability [67]. Ethanol has a prolonged effect on the regulation of miRNA (Table 1). Several studies showed that ethanol alters miRNA concentration in alcohol-fed mice. Several different miRNAs were found to be aberrantly expressed with ethanol exposure. While the predisposing risk factors and aetiologies of ALDs were varied, the deregulation of some specific miRNAs was commonly identified in the published studies, suggesting their importance in alcoholic liver injury. Among these, the over-expression of miR-21, miR-34a, miR-155, miR-320 and the under-expression of miR-122, miR-181a, miR-199a, miR-200a were reported by more than one publication. These miRNAs are described in Table 2. Three of the more notable ones are miR-122, miR-34a and miR-21. It accounts for over 70% of the liver's total miRNA content. The other notable one is, of course, miR-155, which regulates inflammatory agent secretion in the liver [68]. One of the miRNA's responsible for maintaining intestinal permeability is the miR-122, which as was mentioned before is negatively affected by ethanol exposure. Ethanol also up-regulates miR-155, which is responsible for the mediation of LPS induced TNF-α secretion [69].

Table 1 miRNA gene expression studies in ALD.

Year	Profiling method	Main conclusions of the studies	References
2008	Microarray/Northern blot	HCC cases associated with alcohol consumption displayed a decrease in miR-126 expression	[98]
2009	qPCR	Ethanol-induced miR-199 down-regulation may contribute to augmented HIF-1alpha and ET-1 expression	[99]
2009	Microarray	Hepatic specimens from mice fed with an ethanol-containing diet (Lieber–DeCarli) indicated features of alcoholic steatohepatitis and had an increased expression of miR-320, miR-486, miR-705, and miR-1224 and a decreased expression for miR-27b, miR-214, miR-199a-3p, miR-182, miR-183, miR-200a, and miR-322	[100]
2009	qPCR	Expression of miR-375 was shown to be highly expressed and was shown to increase with alcohol consumption, suggesting that this miRNA could represent a molecular fingerprint of alcohol consumption	[101]
2011	qPCR	Chronic alcohol consumption increases miR-155 in macrophages *via* NF-κB and the increased miR-155 contribute to alcohol-induced elevation in TNF-α production *via* increased mRNA stability	[31]
2012	Microarray/Northern blot/qPCR	Methylation-associated miRNA, miR-34a, was increased in ethanol feeding mice liver	[52]
2012	qPCR	miR-217 is increased after ethanol treatment and is a specific target of ethanol action in the liver	[91]
2012	qPCR	The increase of miR-21 expression during liver regeneration is more robust in ethanol-fed rats	[102]
2013	Microarray/qPCR	Several miRNAs that were significantly altered by chronic EtOH feeding, including miR-34a, miR-103, miR-107 and miR-122 have been reported to play a role in regulating hepatic metabolism and the onset of these miRNA changes occurred gradually during the time course of EtOH feeding	[103]

miR-155 and ethanol exposure

miRNA is a known regulator of Kupffer cell response to LPS. Three miRNAs in particular, miR-155, miR-125b and miR-146a, have been of interest in studies and shown to be key contributors to LPS regulation [69]. Only miR-155 and miR-146a, however, seemed to show up-regulation in macrophages such as Kupffer cells. Although both miR-146a and miR-155 was shown unregulated, only miR-155 showed to have a positive regulatory effect on the secretion of the inflammatory agent TNF-α by enhancing its translation (Fig. 2). Because of these findings, miR-155 received particular attention in the study of miRNAs' effect on alcoholic liver disease [42, 70]. Recent studies have shown that alcohol targets miR-155 and induces miR-155 in RAW264.7 macrophages and Kupffer cells. It was concluded from this study that miR-155 is directly correlated with TNF-α levels. Alcohol-fed mice showed increased levels of TNF-α and miR-155 [31]. This same study tested other miRNA's such as miR-125b and miR146a, but only miR-155 showed a significant affect in the inflammatory response of RAW 264.7 macrophages [31]. Similar studies have also shown that alcohol treatment increases miR-155 in the same RAW 264.7 macrophages *in vitro*. Since miR-155 plays such a crucial role in the stimulation of LPS-induced TNF-α production, this study stated that the inhibition of miR-155 prevents alcohol-induced sensitization to LPS. Inversely, it was found that the up-regulation of miR-155 increased Kupffer cell sensitivity to LPS signalling. Experimentation on alcohol-fed mice showed that alcohol exposure up-regulates miR-155 [43], which leads to Kupffer macrophage activation through miRNA mediation, causing the cells to release more TNF-α [31]. Earlier reports using pharmacological inhibitors of NF-κB [31] have demonstrated that alcohol-induced up-regulation of miR-155 is *via* NF-κB, thus linking NF-κB with miR-155.

miR-155 and TNF-α

The up-regulation of TNF-α production in Kupffer cells by LPS and alcohol was determined in two individual experiments: one in which LPS alone was increased, the other in which ethanol exposure alone was increased. In both experiments the production of TNF-α by resident macrophages was increased. A tertiary experiment also proved that miR-155 is the bridge step between ethanol and LPS up-regulation. In this experiment, miR-155 induced cells produced more TNF-α than the controls with no miR-155 overexposure. Lipopolysaccharide levels were also higher though no LPS was introduced. This led to the

Table 2 Most commonly dysregulated miRNAs in ALD.

| miRNA	Chromosome location	Dysregulation	References
miR-122	18q21.3	Decreased/Increased	[52, 103]
miR-125b	11q24.1	Decreased	[103]
miR-126	9q34.3	Decreased	[98]
miR-155	21q21.3	Increased	[31, 99]
miR-181a	1q32.1	Decreased	[52, 100]
miR-199a	1q24.3	Decreased	[99, 100]
miR-200a	1p36.33	Decreased	[100, 103]
miR-21	17q23.2	Increased	[52, 102]
miR-217	2p16.1	Increased	[91]
miR-320	8p21.3	Increased	[100, 103]
miR-34a	1p36.22	Increased	[52, 103]
miR-375	2q35	Increased	[101]
miR-486	8p11.21	Increased	[100]
let-7b	22q13	Decreased	[52]
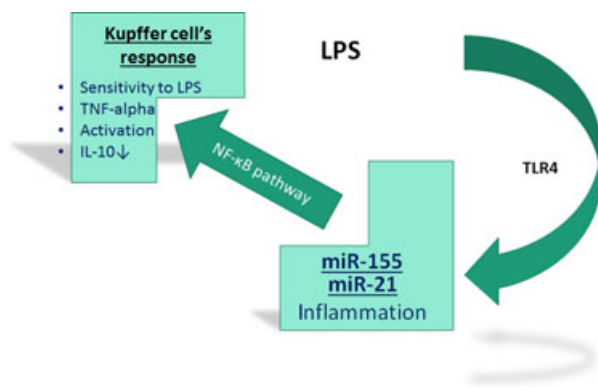
Fig. 2 microRNAs mediated lipopolysaccharide (LPS)/toll-like receptor 4 signalling in Kupffer's cells during alcoholic liver injury. Alcohol consumption may increase gut permeability and subsequent bacterial or microbial translocation into intestinal lumen and result in the increase of LPS in the portal circulation. The excess of LPS in the liver affects Kupffer's cells through miR-155/miR-21, and in response there is the activation of NF-κB signaling as well as the alterations of its downstream effects.

conclusion that miR-155 is directly involved in LPS-induced TNF-α production, not ethanol [31]. The effects of LPS on Kupffer cells, however, become saturated past a point where alcohol-induced miR-

155 no longer has any significant effect on the production on TNF-α after LPS stimulation [31].

miR-155 and NF-κB

Studies have linked the inhibition of miR-155 to the NF-κB inhibitor. NF-κB inhibitors have proven to mediate the up-regulation of miR-155 in Kupffer cells [31]. Furthermore, NF-κB has shown to be activated with chronic ethanol exposure and LPS stimulation [42, 71]. This leads to the studies to prove that miR-155 is regulated by NF-κB [72]. NF-κB is a heterodimeric transcription factor usually composed of p50 and p65 subunits and is a pleiotropic regulator of various inflammatory and immune responses during alcoholic liver injury. Under unactivated condition, p50/p65 dimers are sequestered in the cytoplasm bound to its inhibitors, the IκBs, which prevent the translocation into the nucleus. Following various stimulations, the IκBs are rapidly degraded, activating NF-κB. The active form of NF-κB rapidly translocates into the nucleus, binding to consensus sequences in the promoter/enhancer region of various genes, promoting their transcription [73]. The increase in NF-κB nuclear binding activity of p65/p50 and p50/p50 in prolonged alcohol treatment has been demonstrated, the same as the increase in LPS-induced NF-κB activation. The NF-κB and LPS activation relation was proven during an experiment where NF-κB was inhibited by MG-132 or Bay11-1782. The inhibition of NF-κB decreased miR-155 in ethanol, LPS and alcohol + LPS treated macrophages. This gave the conclusion that NF-κB is indeed the mediator of miR-155 expression in alcohol-induction [31].

miRNA and endotoxin altered permeability

Another regulator of gut-endotoxin permeability, other than ethanol, is miRNA. It was discovered that miRNA increases the permeability of the intestinal lumen in a similar way to ethanol. miRNA, however, does so by affecting the Zonula occludens 1 (ZO-1) protein negatively to induce intestinal lumen permeability. The ZO-1 protein is a critical component that insures the permeable response of the intestinal lumen to endotoxins. Just like with alcohol, the permeability of the intestinal lumen leads to higher absorption of endotoxins that are later transported to the liver to be detoxified. In this process, the detoxification of these endotoxins leads to an LPS-TNF-α chain reaction that causes alcoholic liver disease. In addition to its effects on gut permeability, ethanol also up-regulates miRNA responsible for the reduction of lumen permeability [74]. One particular miRNA, miR-212, has been identified as a contributor to the loss of tight junctions in the intestinal lumen through the ZO-1 protein. Another miRNA strand identifies as a key contributor to endotoxin permeability is the miR-122a, which also interacts with ZO-1 protein to regulate lumen permeability [6]. Such findings suggest that miRNA has more than one role in the development of alcoholic liver disease, first as a mediator of the endotoxins that activate the inflammatory scarring agents and then as a mediator to such specific signalling in liver macrophages.

microRNA and hepatic cell survival

Several investigations have demonstrated the regeneration and remodelling potentials of the adult cells in the liver after injury including hepatocytes and cholangiocytes and with newer studies showing the plasticity of hepatic stellate cell (HSC) and perhaps other cell types as well [75–78]. The persistence of endotoxin during ALDs not only activates the liver immune cells of the liver, but also affects the function of other liver cells (hepatocytes, cholangiocytes and HSCs) [24, 78]. Habitual alcohol consumption promotes hepatocyte death and inhibits the proliferation of mature hepatocytes that survive, leading to chronic liver damage. Alcoholic liver damage is generally accompanied by a ductular reaction that is characterized by periportal accumulation of atypical cholangiocytes. As in many other types of chronic liver disease [79], in alcoholic liver disease the intensity of this ductular reaction closely parallels the severity of liver injury [80]. Cholangiocytes and HSCs have been defined as unique subpopulations in ALDs that possess the ability to initiate regeneration processes as well as liver fibrosis [78]. Although the evidence has been provided to support the role of liver parenchymal and HSC in ALDs, the identity and functions of bile duct cells remains a mystery. Changes in the survival and remodelling activities may be used to characterize certain liver regeneration and fibrotic processes. The new and innovative technique to functionally characterize the remodelling properties of specific hepatic cells may ultimately allow the development of new diagnostic and therapeutic strategies for ALD patients.

The key pathophysiological features of ALD are altered lipid metabolism and hepatocyte apoptosis. The results of recent studies show a significant down-regulation of miR-122, a liver-specific miRNA [81, 82], which is important for normal lipid metabolism, and the marked up-regulation of miR-34a, a critical regulator of apoptosis [83], in the livers of mice fed with ethanol (Fig. 3). Several recent reports have shown that miRNAs miR-122 and miR-34a are two of the most frequently dysregulated miRNAs in steatohepatitis [84, 85]. Serum/plasma miR-122 has been correlated with ALT increases in the liver damage caused by alcohol, and was predominantly associated with the exosome-rich fraction [86]. Both miR-122 and miR-34a were aberrantly expressed in both alcoholic steatohepatitis and non-alcoholic fatty liver disease [52, 84, 87]. Sirtuin (silent mating type information regulation 2 homolog) 1 (SIRT1) is a verified target protein of miR-34a [88]. SIRT1 plays an important role in protecting cells from cellular oxidative stress and DNA damage [89, 90]. Specific miRNA promotes ethanol-induced fat accumulation in hepatocytes by down-regulating SIRT1 [91]. Once SIRT1 is activated, SIRT1 deacetylates histones and histone methyl-transferases. SIRT1 also deacetylates a variety of non-histone target proteins, such as p53, the retinoblastoma protein (Rb), FoxO transcription factors, Ku70, NF-kB and PGC-1alpha. SIRT1-mediated deacetylation of Lys382 decreases p53-mediated transcriptional activation and reduced the downstream protein such as p21 and PUMA levels [92]. Therefore, SIRT1 mediates the survival of cells during periods of severe stress through the inhibition of apoptosis. Overexpression of miR-34a decreased SIRT1 expression, leading to an increase in acetylated p53 levels and p53
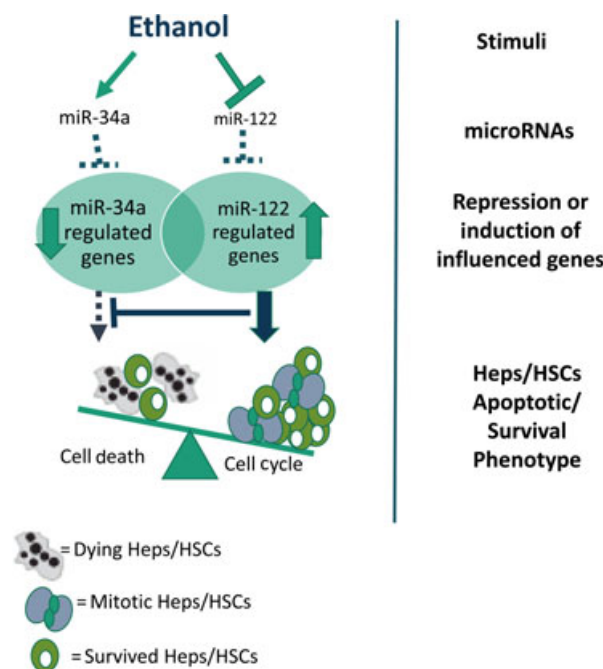

Fig. 3 microRNA mediated survival mechanisms in alcoholic liver injury. miR-34a is anti-apoptotic, while miR-122, the liver specific miRNA, is the critical regulator of cell cycle. In normal liver, miR-34a and miR-122 cooperatively repress gene expression to balance cell survival and proliferation. Ethanol increases miR-34a and decreases miR-122 in the liver, resulting in altered target gene expressions, and consequently, increased cell proliferation while maintaining overall apoptosis resistance.

targets, such as p21 and PUMA [93]. miR-34a overexpression also induced apoptosis in cancer cells expressing p53, but not cancer cells not expressing p53. These results suggest that miR-34a induces apoptosis in part through a pathway that involves: miR-34a—SIRT1—p53 acetylation [93]. However, the other relevant targets of miR-34a largely remain to be identified. Luciferase-based analysis has implicated E2F3, Foxp1, the Notch1 receptor, as well as its ligand Delta1, as potential miR-34a targets [94].

Circulating miRNAs as stable blood-based markers for ALDs

The development of minimally invasive tests for the detection and monitoring of ALD could greatly reduce the worldwide health burden of alcoholic liver injury. The demonstration that miRNA profiles could reveal smoke-related effects in the liver of mice exposed to ethanol established a proof-of-principle for the use of miRNAs to evaluate early steps of ALD process, which could be extended to other risk factors. However, the most useful approach would be to distinguish specific biomarkers circulating in the bloodstream before alcoholic liver

injury become clinically apparent. To this end, miRNAs can potentially become useful biomarkers for human ALD. Actually, deregulated miRNAs are found in liver as well as in the blood of ALD patients. Because of their innate stability, miRNAs may be detected blood based assays for ALD. miRNAs are present in plasma in a stable form, making them feasible biomarkers for the detection of ALD and other liver disorders [95, 96]. Recent proof-of-principle studies established that the analysis of miRNA expression in serum or plasma may be a promising approach for blood-based diagnosis of a number of human ALDs and other liver diseases [86, 97]. These studies suggest that the effect of ethanol consumption may be revealed by quantifying miRNAs circulating in serum/plasma. Further investigations will explore possibilities for circulating miRNAs as stable blood-based diagnostic markers and predicting progression for human ALDs.

Conclusion

The role of miRNA in alcoholic liver disease is certainly a crucial one. In the past decade and in recent years its role in alcoholic liver disease has found keen interest. miRNA's interaction with alcohol plays a pivotal role in alcohol-induced liver scarring and fibrosis. The process that leads to alcoholic liver disease begins, of course, with ethanol consumption and exposure. Ethanol exposed mice have shown loss of permeability of the intestinal lumen. This permeability alternation occurs in two ways: First, with the direct influence of ethanol on the permeability of the intestinal lumen and allowing endotoxins to enter the liver. Second, ethanol induces miRNA, a known mediator of intestinal permeability, in the intestinal lumen to do the same. Once in the liver these endotoxins, produced by the decomposition of gram-negative bacteria and pathogens, follow a pathway that leads to liver inflammation. The endotoxins that are taken in by the intestinal lumen find their way to the liver, where they are detoxified. The detoxification of the endotoxins from gram-negative bacteria releases a particular kind of endotoxin called LPS, which once in the liver, attaches to TLR4, and sets in action the pathway that leads to alcoholic liver disease. Toll-like receptor 4 is not composed of two coreceptors, MD-2 and CD14, but these two proteins may be required for response of cell to LPS stimulation *via* TLR4. Both of these receptors have been identified as key components of alcoholic liver disease since both their deactivations help reduce progression of the disease. Upon activation of TLR4, macrophages in the liver release inflammatory agents that cause liver fibrosis. One particular macrophage of interest is the Kupffer cell, which releases TNF-α. The role miRNA takes part in this process involves the regulation of the LPS signalling to the TLR4 receptor. It is miRNA that regulates the LPS reception in Kupffer cells. It is also known that ethanol regulates miRNA (miR-122 and miR-34a) in the liver that contributes to hepatocytes/HSC survival. Therefore, as alcohol consumption increases, so does miRNA in the liver and thus the reception of LPS and TNF-α release. Although still under study, microRNAs have been shown to play a pivotal role in the development, causation and regulation of alcoholic liver disease.

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Conflicts of interest

All authors declare that they have no conflicts of interests.

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