Review

JOURNAL OF HEPATOLOGY

miR-122 – A key factor and therapeutic target in liver disease

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Summary

Being the largest internal organ of the human body with the unique ability of self-regeneration, the liver is involved in a wide variety of vital functions that require highly orchestrated and controlled biochemical processes. Increasing evidence suggests that microRNAs (miRNAs) are essential for the regulation of liver development, regeneration and metabolic functions. Hence, alterations in intrahepatic miRNA networks have been associated with liver disease including hepatitis, steatosis, cirrhosis and hepatocellular carcinoma (HCC). miR-122 is the most frequent miRNA in the adult liver, and a central player in liver biology and disease. Furthermore, miR-122 has been shown to be an essential host factor for hepatitis C virus (HCV) infection and an antiviral target, complementary to the standard of care using direct-acting antivirals or interferon-based treatment. This review summarizes our current understanding of the key role of miR-122 in liver physiology and disease, highlighting its role in HCC and viral hepatitis. We also discuss the perspectives of miRNA-based therapeutic approaches for viral hepatitis and liver disease.

Keywords: Hepatitis; HBV; HCV; miR-122; Liver disease pathogenesis; HCC.
Received 8 August 2014; received in revised form 26 September 2014; accepted 2 October 2014

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Abbreviations: miRNA, microRNA; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; RNAi, RNA interference; Ago, Argonaute; RISC, RNA-induced silencing complex; mRNA, messenger RNA; 3′ UTR, 3′ untranslated region; 5′ UTR, 5′ untranslated region; DAA, direct-acting antiviral; IFN, interferon; NAFLD, non-alcoholic fatty-liver disease; LEFT, liver-enriched transcription factor; HNF, hepatocyte nuclear factor; CUTL1, cut-like homeobox 1; APK, AMP-activated protein kinase; PPAR, peroxisome proliferator-activated receptor; KO, knock-out; KLF6, Krüppel-like factor 6; Ccl2, (C-C) motif ligand 2; AKT3, v-akt murine thymoma viral oncogene homolog 3; ADAM10, disintegrin and metalloproteinase domain-containing protein 10; IGF1R, insulin-like growth factor-1 receptor; SRF, serum response factor; Wnt1, wingless-type MMTV integration site family, member 1; PFV-1, primate foamy virus type 1; BACH1, BTB and CNC homology 1; HMOX1, heme oxygenase 1; KSHV, Kaposi's sarcoma-associated herpesvirus; HSV-1, *herpes simplex* virus-1; HCMV, human cytomegalovirus; HBsAg, hepatitis B surface antigen; IFITM1, interferon induced transmembrane protein 1; HBV, hepatitis B virus; HBx, hepatitis B virus X protein; Akt, v-akt murine thymoma viral oncogene homolog 1; IRES, internal ribosome entry site; SVR, sustained virological response; rcDNA, relaxed circular partially double-stranded genome; cccDNA, covalently closed circular DNA; Gld2, germline development 2; NDRG3, N-myc downstream regulated gene 3; PTTG1, pituitary tumor-transforming gene 1-binding factor.

Introduction

Among the wealth of recently discovered non-protein-coding RNAs, miRNAs constitute a class of endogenous post-transcriptional regulators of gene expression through RNA interference (RNAi), which relies on the sequence-specific pairing between a small non-protein-coding RNA and a target nucleic acid [1,2]. miRNAs have been identified in 206 organisms, ranging from microbes to animal species, including humans, where ~2000 miRNAs are currently reported by the official miRNA repository miRBase (release 20, [3]). In the canonical miRNA biogenesis pathway, a miRNA gene is first transcribed as a hairpin-shaped double-stranded primary RNA (the pri-miRNA), which is cleaved in the nucleus to generate a ~60–70 nt long precursor called pre-miRNA, that is then exported to the cytoplasm to be further processed by Dicer into a ~22 nt RNA duplex, of which one of the two strands represents the functional mature miRNA. Mature miRNAs are then sorted into one of the Argonaute (Ago) proteins to form the core of the effector RNA-induced silencing complex (RISC) (reviewed in [4]). The RISC-loaded miRNA ('guide' RNA) recognizes its target RNA, most likely a messenger RNA (mRNA), by base-pairing typically within its 3′ untranslated region (3′ UTR). This interaction can result in downregulation of the encoded protein via mRNA degradation and/or translational repression. Furthermore, miRNAs have also been shown to regulate their targets by binding to the 5′ UTR. Although miRNA-target interactions usually lead to target repression/decay, miRNAs can also stimulate the expression of target genes (reviewed in [5]). Since the minimal requirement of pairing consists of seven nucleotides within the 5′ proximal part of the miRNA (miRNA seed), a single miRNA may target a cohort of different mRNAs. Consistently, up to 60% of all human protein-coding genes were predicted to be subject to miRNA-mediated regulation [6]. Moreover, different miRNAs tend to act cooperatively to repress one specific gene [7,8] or several genes within the same pathway [9]. As such, miRNAs are part of complex regulatory networks, controlling gene expression in virtually every biological process including development, immune response, aging and cell death.

Key Points

- miR-122 is a key factor, involved in liver development, differentiation and homeostasis as well as in metabolic functions; loss of miR-122 has been associated with liver disease and HCC
- Restoration of miR-122 expression prevents development of liver disease and HCC in mouse models
- miR-122 also plays a role in the life cycle of liver-specific pathogens: it is an essential host factor for HCV replication but appears to restrict HBV replication
- Clinical proof-of-concept studies have demonstrated that miR-122 inhibitors efficiently reduced viral load in chronically infected HCV patients without detectable resistance but in light of the very high cure rates of orally administrated DAAs and a potential liver disease-promoting effect of miRNA depletion, the role of miR-122 in future treatment approaches for HCV infection remains to be determined
- Given the limited or absent strategies to impair the progression of liver disease and to prevent and treat HCC, miR-122 mimics may provide a novel strategy for the prevention and treatment of HCC with need for randomized clinical trials

miRNAs and disease biology

Given their involvement in regulating cell homeostasis and functions, miRNA expression is tightly controlled in a temporally restrained and tissue-specific manner [10,11]. This suggests that miRNAs may be involved in determining and maintaining tissue identity. These specific expression patterns are controlled by both transcriptional and post-transcriptional regulatory systems that may target different steps of miRNA biogenesis and turnover (for a detailed discussion, see [12]). It is thus not surprising that dysregulations of miRNA networks have been associated with various diseases. Indeed, several pieces of evidence have demonstrated that altered regulation of miRNA expression might contribute to disease processes, including genetic and infectious diseases as well as cancer. While some diseases have been linked to the altered functions of enzymes regulating miRNA biogenesis, others appear to involve altered modulation of miRNA expression or genetic alterations of genes, encoding miRNAs or their targets, including deletions and single-nucleotide polymorphisms that may ultimately lead to a gain or loss of miRNA-target interaction (reviewed in [13–15]). Therefore, miRNAs represent potentially interesting druggable targets. Indeed, a miR-122 inhibitor (miravirsen) and a miR-34 mimic (MRX34) were the first miRNA-based molecules to enter the clinic [16,17]. First, clinical trials have provided the proof-of-concept of the potential of miravirsen as a novel therapeutic strategy against chronic hepatitis C virus (HCV) infection, complementary to the standard of care using direct-acting antivirals (DAAs) or interferon (IFN)-based treatment [16]. MRX34 is currently in a phase 1 clinical trial in patients with unresectable primary liver cancer, and advanced or metastatic cancer with liver involvement (ClinicalTrials.gov identifier: NCT01829971A) [17]. Furthermore, given the association of differential miRNA expression patterns with diseases, both tissue and circulating miRNA expression profiles can also be used as biomarkers for diagnostic, prognostic and therapeutic purposes.

The liver is the largest internal organ of the human body with the unique ability of self-regeneration. It is involved in a wide variety of vital functions that require highly orchestrated and controlled biochemical processes. Increasing evidence suggests that miRNAs are essential for the regulation of liver development, regeneration and metabolic functions [18]. Hence, alterations in intrahepatic miRNA networks have been associated with all aspects of liver disease, including hepatitis, steatosis, cirrhosis and HCC (reviewed in [19]). miR-122 is the most frequent miRNA in the adult liver [20–22]. Interestingly, miR-122 can be detected in the circulation and serum miR-122 has been shown to serve as a biomarker of liver injury in chronic hepatitis B or C, non-alcoholic fatty-liver disease (NAFLD) and drug-induced liver disease [23–29]. Here, we review the key involvement of miR-122 in liver physiology and disease, highlighting its roles in HCC and viral hepatitis. We also discuss the perspectives of miRNA-based therapeutic approaches for viral hepatitis and liver disease.

miR-122 and liver physiology

miR-122 has a liver-enriched expression and is one of the most abundant miRNAs in the liver, accounting for about 70% and 52% of the whole hepatic miRNome in adult mouse and human, respectively [20–22]. Consequently, miR-122 plays a central role in liver development, differentiation, homeostasis and functions (Fig. 1). miR-122 expression is driven by liver-enriched transcription factors (LETFs), including hepatocyte nuclear factor (HNF) 6 and 4a [30–32] that also fine-tune miR-122 dosage during liver development *in vivo* [30–32]. Particularly in liver development, the concertized expression of miR-122 and LETFs was suggested to regulate the proper balance between cell proliferation and differentiation in both the hepatocyte and cholangiocyte lineages [30,31]. This temporal-regulation of miR-122 expression is particularly important as miR-122 promotes hepatobiliary segregation along with the acquisition and maintenance of a hepato-specific phenotype [30,31,33] (Fig. 1). Indeed, during mouse liver development, miR-122 was shown to gradually repress the transcription factor cut-like homeobox 1 (CUTL1), thus allowing terminal liver differentiation [30] (Fig. 1). This important role of miR-122 in liver development and differentiation was further demonstrated by studies reporting that antisense-mediated inhibition of miR-122 delayed liver development in zebrafish [31] and switched on the expression of genes that were normally repressed in the adult mouse liver [34]. This is also corroborated by the fact that the repression of miR-122 in primary HCC with poor prognosis was associated with suppression of the hepatic phenotype [33].

miR-122 also plays a crucial role in the regulation of cholesterol and fatty acid metabolism in the adult liver (Fig. 1). *In vivo* antisense studies, coupled with microarray analysis, have been instrumental to uncover the role of miR-122 in lipid metabolism [34–36]. Indeed, antisense-mediated inhibition of hepatic miR-122 markedly lowered plasma cholesterol levels in