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.

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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

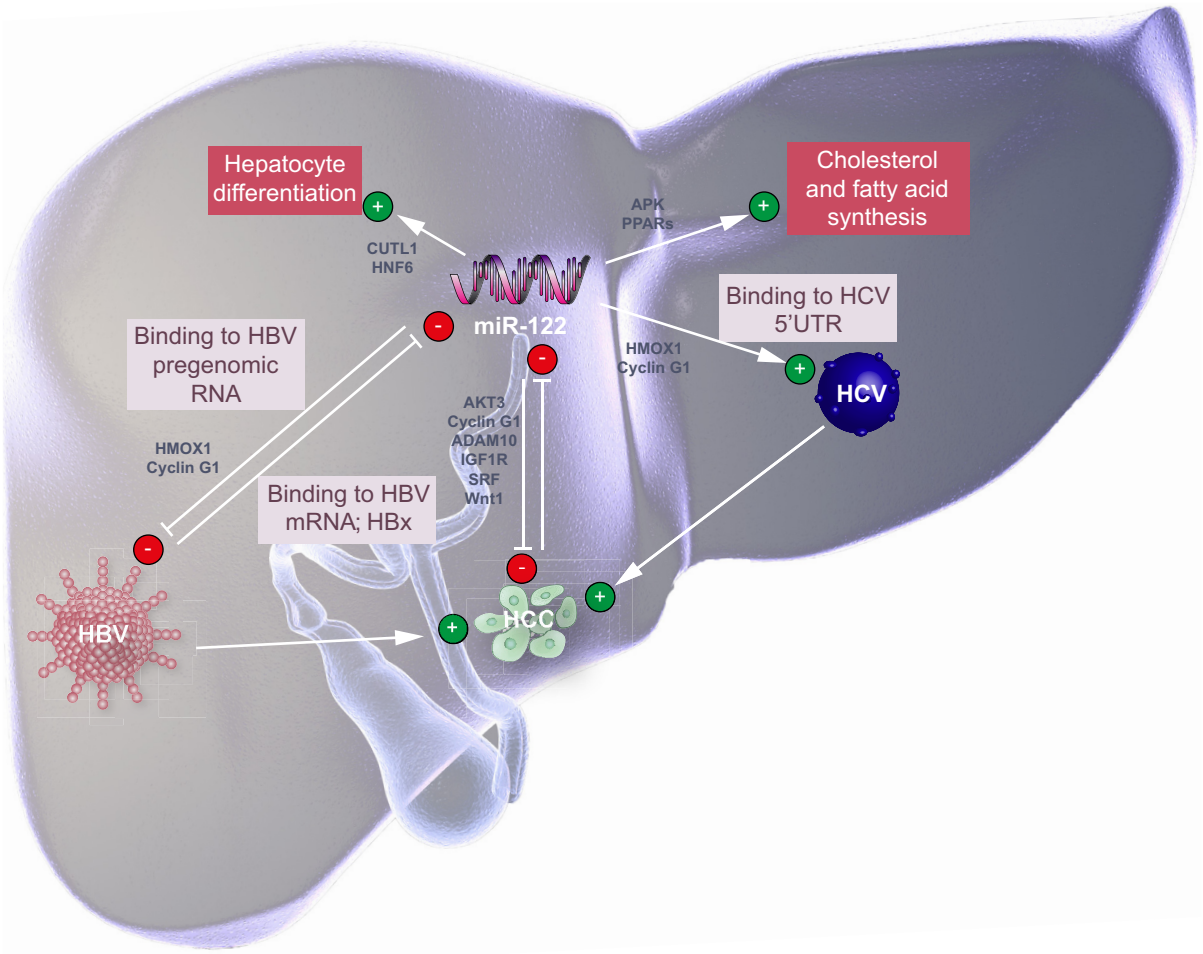

Fig. 1. miR-122 is a key regulator of liver physiology and disease biology. The scheme illustrates the different roles of miR-122 in liver development and metabolism (red boxes) as well as in viral hepatitis and liver disease. Activation (+) or inhibition (−) is indicated dependent on the effect of miR-122 on a specific process. While host miR-122 targets are depicted outside of boxes, miR-122 targets of viral origin are indicated within grey boxes.

both mice and non-human primates [34–36]. Transcriptomic analyses in mice further revealed that transient miR-122 sequestration downregulated the expression of genes involved in fatty acid metabolism as well as cholesterol biosynthesis, including the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-CoA-reductase [34,35]. Although the molecular mechanisms underlying regulation of lipid homeostasis by miR-122 are still unclear, both AMP-activated protein kinase (APK) and circadian metabolic regulators of the peroxisome proliferator-activated receptor (PPAR) family were suggested to be putative effectors of miR-122-mediated metabolic control [35,37] (Fig. 1). Interestingly, transcription of the miR-122 locus itself occurs in a circadian manner, suggesting the existence of a link between miR-122, circadian gene expression and hepatic lipid metabolism [37].

miR-122 and pathogenesis of liver disease and hepatocellular carcinoma

In line with its essential role in maintaining liver homeostasis and differentiation, reduced expression of miR-122 has been associated with liver disease. The generation of both germline knock-out (KO) mice and liver-specific KO mice has been pivotal to revealing a key involvement of miR-122 in liver disease [38–40]. Indeed, in contrast to transient miR-122 sequestration, genetic deletion of miR-122 was shown not only to severely impact on lipid metabolism, but also to drive microsteatosis and inflammation, which progressed to steatohepatitis and fibrosis as mice aged [38,39]. Consistently, miR-122 expression was also lowered in a carbon tetrachloride-induced mouse model of liver fibrosis [41]. Of note, the restoration of miR-122 levels in miR-122 KO mice reversed liver inflammation, at least in part, by repressing two miR-122 targets, namely the chemokine Ccl2, which was shown to recruit $CD11b^{hi}Gr1^{+}$ inflammatory cells intrahepatically [38] and the pro-fibrogenic Krüppel-like factor 6 (KLF6), whose expression was enhanced in the miR-122 KO mouse liver [39]. This piece of data clearly highlights the anti-inflammatory and anti-fibrotic properties of miR-122 in the liver (Fig. 2). Although this knowledge has been acquired using mouse models, it is important to note that reduced miR-122 expression has been associated with human non-alcoholic steatohepatitis [42] extending the relevance of these findings to

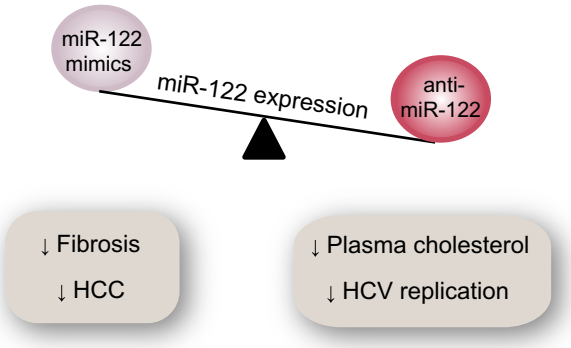

Fig. 2. Therapeutic effects of miR-122-modulating agents in liver disease. Current state-of-the-art approaches in modulating miRNAs *in vivo* comprise restoration of miRNA expression, using synthetic miRNA mimics or viral vectors driving miRNA expression, as well as inhibition of miRNA expression via chemically modified anti-miR oligonucleotides [123]. While antisense-mediated inhibition of miR-122 (anti-miR-122) has been demonstrated of clinical interest to treat chronic HCV infection and to represent a potential therapeutic strategy against hypercholesterolemia, restoration of miR-122 (miR-122 mimics), was suggested as a therapeutic approach against liver fibrosis and HCC development.

human liver disease. Furthermore, decreased miR-122 levels have been associated with poor prognosis and metastasis of liver cancer, and several targets of miR-122 have been implicated in tumourigenesis [38,43–49] (Fig. 1). Indeed, a number of validated miR-122 targets including cyclin G1, ADAM10, IGF1R, SRF, and Wnt1, were shown to be involved in hepatocarcinogenesis, epithelial-mesenchymal transition, and angiogenesis [49] (Fig. 1). Altogether, these data suggested that miR-122 acts as a tumour suppressor in the liver.

The proof-of-concept that miR-122 has an anti-tumour function in the liver was again provided using *miR-122* KO mice [38,39]. These mice spontaneously develop liver tumours and demonstrate abnormal expression of genes involved in cell growth and cell death, epithelial-mesenchymal transition and cancer [38,39]. Importantly, tumour development in these mice could be prevented by restoration of miR-122 expression *in vivo* [38,39]. Moreover, by using a mouse model where tumours developed in the absence of inflammation, it has been demonstrated that miR-122 has an anti-tumour function that is independent of its role in preventing liver disease and inflammation [38]. miR-122 may thus be used as a potential therapeutic tool against HCC. Indeed, given that the decrease of miR-122 can promote hepatocarcinogenesis, and that restoration of miR-122 in HCC cells can reverse the tumourigenic properties of these cells, preventing HCC development *in vivo* [38,39,43–45,50,51], miR-122 mimics represent an interesting strategy to prevent and treat HCC (Fig. 2). Furthermore, it has also been shown that restoration of miR-122 also sensitizes HCC cells to chemotherapy, suggesting that combination of miR-122 and chemotherapeutic agents may have an additive or synergistic effect against liver cancer [44,45,50]. It is worth noting that the first miRNA mimic reached phase 1 clinical studies, indicating the feasibility of modulating miRNA expression in human liver (ClinicalTrials.gov identifier: NCT01829971A) [17]. Taken together, results from these studies broadened our understanding of HCC-development, enabled researchers to draw arresting conclusions regarding the association between loss of miR-122 and diverse aspects of liver disease as well as HCC, and highlighted important implications regarding the therapeutic potential of miR-122 [38–40].

Despite the fact that proof-of-concept studies have elegantly demonstrated the tumour suppressor function of miR-122 [38,39], it is important to point out that HCC is not consistently associated with loss of miR-122. Indeed, HCC is a multifactorial and heterogeneous disease and miR-122 expression appears to be dependent on the aetiology of the liver cancer. Interestingly, reduced miR-122 expression has been associated with hepatitis B virus (HBV)-related HCC, while miR-122 levels appear normal or increased in HCV-related HCC [52,53]. One can hypothesize that this is due to different roles of miR-122 in the life cycle of these two viruses (see below), and at least with respect to miR-122, each of the two viruses causes HCC in different ways (Fig. 1). These data underscore that HCC is not the result of the deregulated expression of a single gene, and rather several lines of evidence indicate that various signalling pathways are deregulated in HCC (reviewed in [19,54,55]). Further studies are required to better understand the molecular mechanisms underlying HCC and the role of miRNAs in this disease.

miRNAs and virus-host interactions

Chronic viral hepatitis due to HBV or HCV infection is a major cause of chronic liver disease and HCC. HBV and HCV are both characterized by a tight species and tissue tropism, almost exclusively infecting human hepatocytes. This cell specificity may be explained by the fact that both viruses depend at each step of their respective life cycle on several host factors, which happen to be expressed in hepatocytes. Within the past years, numerous proteins have been uncovered to be required for either the HBV or the HCV life cycle, and increasing evidence indicates that non-protein-coding RNAs, such as miRNAs also plays important roles in these processes (reviewed in [56–61]). Furthermore, in addition of using host miRNAs for their replicative cycle, HBV and HCV have also been reported to modulate the expression profile of the cellular miRNome to favour viral persistence, which may contribute to pathogenesis of liver disease (reviewed in [62,63]). Accumulating evidence points to a role of human miRNAs in modulating viral infectivity, cell tropism and host immune responses [64,65]. The outcome of this miRNA-virus interplay can have either a positive (proviral) or negative (antiviral) effect on the virus. In addition, there are different levels of interactions, which are not mutually exclusive, as described below.

Cellular miRNAs have been demonstrated to directly target defined viral genomes or transcripts (Table 1). The best described example so far is the binding of miR-122 within the HCV genomic RNA that has a positive effect on viral translation, replication and infectious particle production (see below) [66,67]. Actually, the positive outcome of miR-122 for HCV is more of an oddity than the rule, as most direct binding of miRNAs to viral RNAs is deleterious. Indeed, miR-199a also directly targets HCV RNA but this leads to an inhibition of HCV replication [68]. Likewise, HBV transcripts have also been reported to contain binding sites for cellular miRNAs, including miR-122, miR-199a, and miR-210 that all repress HBV mRNA expression [69,70]. Noteworthy, to counteract inhibition by cellular miRNAs, RNA viruses appear to have evolved strategies to escape direct miRNA-mediated repression. Indeed, a recent comprehensive survey on the roles of miRNAs

Table 1. miRNA-mediated regulation of viral infection.

Concept	Mechanism	Examples	References
miRNA-binding to viral genome or transcript	miRNA-induced stability or decay/ translational repression of the viral RNA	• miR-122 binds to the HCV genome and enhances viral translation and replication	[66,73,89,97]
		• miR-199a and miR-210 bind to HBsAg mRNA leading to reduced HBsAg expression	[69,70]
		• miR-32 binds PFV-1 mRNA and inhibits viral translation	[124]
miRNA-mediated regulation of host factors	miRNA-induced translational repression or decay of host mRNAs involved in restriction of the viral life cycle and/or antiviral reponses	• miR-196 translationally represses BACH1, thus enhancing HMOX1-mediated antioxidant and anti-inflammatory response against HCV	[22]
		• miR-141 inhibits HBV replication by targeting PPARα	[74]
Virus-mediated modulation of host miRNA	Viral transcripts or proteins modulate expression of host miRNAs that in turn modulate expression of viral or host proteins	• HCV increases miR-130a expression to reduce IFITM1 expression to promote viral replication	[77]
		• HBx decreases miR-15b to increase HNF1α expression in order to moderate HBV replication during acute infection	[80]
		• KSHV, HSV-1 and HCMV enhance transcription of miR-132 that represses innate immunity through p300	[125]
		• Herpes virus saimiri non-protein-coding RNA HSUR 1 binds miR-27a to induce its degradation	[81]
Virus-encoded miRNA-mediated modulation of host or viral factors	Virus-encoded miRNAs usually promote the viral infection by modulating viral or host factors to limit the lytic cycle of the virus, prolong the longevity of infected cells and/or inhibit immune responses	• HSV-1-encoded miRNAs regulates the viral gene ICP0, to switch between lytic and latent cycles	[126]
		• No experimental evidence for any HBV- or HCV-encoded miRNA	[87]

in different virus infections using Dicer KO HEK293 cells indicated that miRNAs had only a limited impact on the viruses tested, and hence that most of the viruses have evolved to be resistant to cellular miRNAs [71]. While the molecular mechanisms underlying viral evasion from miRNAs remain to be determined, first evidence indicated that HIV-1 was able to adopt extensive RNA secondary structures to avoid efficient inhibition by host miRNAs [72]. Taken together, these data suggest that the crosstalk between miRNAs and viral RNAs likely lead viruses to develop strategies to escape antiviral immunity and indicate that the dependence on a host miRNA as seen with miR-122 and HCV is rare [71].

Host miRNAs are also able to indirectly target a virus through the miRNA-mediated regulation of specific host factors (Table 1). This kind of interaction has for example been described in the context of the antiviral response to HCV infection. Indeed, recent studies indicated that miR-196 may play a role in counteracting HCV infection *in vitro* by both enhancing antioxidant and anti-inflammatory responses and direct targeting of the HCV genome [22,73], which merits further validation *in vivo*. Furthermore, HBV replication has been shown to be regulated by different miRNAs, which modulate the expression of transcription factors, having an impact on the virus life cycle [74,75]. Given the widely spread regulation of cellular proteins by cellular miRNAs, it is likely that the tuning of host cell gene expression by host miRNAs contributes to modulating viral life cycles.

If host miRNAs modulate viral RNA expression, likewise viruses can impact on host miRNA expression, which in turn could target either host or viral RNAs (Table 1). Viral infection has been reported to modulate the expression of miRNAs that can promote viral replication and/or contribute to viral evasion as well as pathogenesis. For instance, HCV infection promotes the expression of miRNAs that suppress the innate immune response pathways, thereby leading to an increase of viral replication [76–78]. Furthermore, the HBV X protein (HBx) has been reported to modulate the expression of cellular miRNAs that likely contribute to the pathogenesis of liver disease [79,80]. Beside viral proteins, virus-encoded transcripts can also play a role in regulating miRNA abundance in host cells by degrading miRNAs or interfering with their biogenesis [81–83] (Table 1). Taken together, these data demonstrate that viruses have evolved several strategies to modulate cellular miRNAs. While this may allow the virus to escape antiviral immunity and establish persistent infection, virus-induced changes in the host miRNome may ultimately also contribute to cellular transformation and oncogenesis.

Finally, viruses can also encode miRNAs, which can target either host or viral RNAs [65,84,85] (Table 1). Virus-encoded miRNAs can either be specific to a virus or be analogues of host miRNAs, and they usually promote viral infection by prolonging the longevity of infected cells, inhibiting immune responses, and/or regulating host or viral genes to limit the lytic cycle (reviewed in [86]). Interestingly, although a computational approach indicated that HBV putatively encodes a candidate pre-miRNA that might yield a mature miRNA with putative binding sites within the HBV mRNA [87], to date there is no experimental evidence for any HBV- or HCV-encoded miRNA.

miR-122 and HCV infection: Host-dependency factor and antiviral target

HCV is a single-stranded RNA virus of positive polarity [88]. The role for miR-122 in HCV infection was first demonstrated by sequestration of endogenous miR-122, which led to a substantial reduction in HCV RNA abundance [66]. Unlike most miRNAs that repress their targets through binding the 3′ UTR of mRNAs, miR-122 directly pairs with two adjacent sites in the 5′ UTR of the viral RNA, thus enhancing viral replication [73,89–92] (Fig. 1). These target sites are located upstream of the HCV internal ribosome entry site (IRES), and are conserved across HCV genotypes. Recent studies indicated that miR-122 positively acts on the HCV life cycle by enhancing viral translation and genome stabilization. Indeed, it has been shown that miR-122 binding to the 5′ UTR of the HCV genome enhances the association of ribosomes with the viral RNA [90,93,94]. Furthermore, the association of miR-122 and the HCV genome together with Ago2 within the RISC complex also stabilizes viral RNA by protecting it from degradation by exonucleases [92,95–97]. The importance of miR-122 in HCV infection is also underscored by a number of studies, which indicated the involvement of this miRNA in allowing HCV replication in non-HCV permissive cell lines. Indeed, hepatoma cell lines as well as non-liver derived HEK-293T or HeLa cells, which do not express significant amounts of miR-122 and are unable to sustain HCV replication, were rendered permissive to HCV replication upon ectopic miR-122 expression [98–103]. Interestingly, in addition to the direct effect, mediated by miR-122 targeting of the HCV RNA, an indirect effect has been reported that involves the downregulation of HMOX1, the latter having been shown to inhibit HCV replication [104] (Fig. 1). miR-122 was also discovered to prompt alcohol-induced HCV RNA replication [105,106]. In particular, acute alcohol exposure in HCC cell lines was shown to enhance HCV replication by upregulating miR-122 expression while downregulating the miR-122 target cyclin G1 [105]. Taken together, these data indicate that miR-122 represents an essential hepatocyte-specific host factor for HCV infection.

Counter-intuitively, the beneficial role of miR-122 for the virus *in vitro* does not translate into a positive correlation between its expression and HCV load in patients. Particularly non-responders to IFN-based therapy have lower miR-122 pre-treatment levels [107–110], suggesting that pre-treatment miR-122 levels could be used as a biomarker to predict the therapeutic outcome. While it has been shown that IFN-based therapy does not appear to decrease intrahepatic miR-122 in patients [107], another study reported that reduced serum miR-122 correlates with therapeutic success, probably by reflecting reduced liver damage [27].

Given its essential role in the HCV life cycle and its liver-enriched expression, miR-122 represents a target for antiviral therapy (Fig. 2). The first animal studies using antisense miR-122 oligonucleotides of different chemistry were encouraging as they indicated that targeting miR-122 did not result in liver toxicity in mice and African green monkeys [35,111]. In addition, the treatment decreased their plasma cholesterol levels and this effect was sustained for several weeks but reversible following withdrawal of the inhibitor [35,111], suggesting that targeting miR-122 might also be a potential therapeutic strategy for hypercholesterolemia (Fig. 2). A study using chronically HCV-infected chimpanzees then provided the first proof-of-concept for the potential of the miR-122 inhibitor SPC3649, now known as miravirsen, as an efficient antiviral. Indeed, the inhibitor reduced HCV RNA levels in the majority of treated animals and its effect was gradually lost once the inhibitor was withdrawn [112], confirming a sustained but reversible inhibition of miR-122 *in vivo*. The potential of this inhibitor has recently been confirmed in a phase 2a clinical trial [16]. Administration of this inhibitor for 5 weeks resulted in a dose-dependent and sustained reduction of HCV RNA levels up to 3 logs for the highest dose of 7 mg/kg with several patients transiently achieving undetectable HCV RNA levels. However, viral RNA levels rebounded in patients that did not start an IFN-based therapy at the end of the trial. No dose-limiting adverse events were observed but patients exhibited a sustained and reversible decrease in serum cholesterol levels. Nevertheless, the miR-122 inhibitor half-life and long-term implications of miR-122 inhibition *in vivo* may merit further studies. Very importantly, no adaptive mutations were detected with in the HCV miR-122 binding regions, indicating that miR-122 inhibitors have a high barrier to resistance [16]. Despite these interesting results, given the recent tremendous advances in the treatment of chronic HCV infection with the approval of orally administered DAAs with pan-genotypic activity and high barrier to resistance (reviewed in [113]) that enable very high rates of sustained virological response (SVR), it is likely that miR-122 inhibitors that require parenteral administration will not play a major role in the future antiviral therapy against HCV. However, since patients who cleared HCV remain at risk for HCC (reviewed in [113]), a better understanding of the miRNA networks, modulated in the course of HCV infection and involved in development of HCC, will allow to ultimately uncover pathways that may represent potential therapeutic targets to prevent/treat HCC.

miR-122 and HBV infection: A viral restriction factor?

HBV is a DNA virus with a relaxed circular partially double-stranded genome (rcDNA) that is converted into a covalently closed circular DNA (cccDNA) in the host cell nucleus, following infection of human hepatocytes. The cccDNA serves as a template for the transcription of four viral RNAs that represent templates for the translation of the HBV proteins and for viral replication, involving reverse transcription [114]. In contrast to its role as a host-dependency factor for HCV, miR-122 appears to restrict HBV replication. Indeed, it has been shown that miR-122 directly targets a conserved region of the HBV pregenomic RNA that functions as a bicistronic mRNA, encoding the HBV polymerase and core protein [69] (Fig. 1). However, the exact mechanisms by which miR-122 binding to HBV RNA results in the inhibition of HBV protein expression, transcription and replication remain to be determined. Furthermore, miR-122 has been shown to indirectly interfere with HBV replication by decreasing expression of cyclin G1, which results in p53-mediated inhibition of HBV transcription [115]. However, in human hepatoma cell lines, miR-122 was also observed to indirectly enhance HBV replication by repressing HMOX1, which in turn interfered with HBV replication by reducing the stability of the HBV core protein [116] (Fig. 1). In contrast to HCV, HBV infection downregulates miR-122 expression and viral load was shown to inversely correlate with miR-122 expression in HBV-infected patients [69,115,117]. The exact underlying mechanisms are not fully understood, but one possibility could be that all HBV mRNAs

contain a miR-122 binding site and could act as sponges to sequester miR-122 [117]. Moreover, a recent study demonstrated that the HBx protein could bind PPARγ, thereby leading to inhibition of miR-122 transcription [118]. HBx can also decrease the stability of miR-122 by downregulating germline development 2 (*Gld2*) that is involved in miR-122 adenylation [119].

Given the important role of miR-122 in liver physiology, this virus-induced suppression of miR-122 may alter liver function and contribute to the development of liver disease including HCC. Indeed, it has been reported that the HBV-mediated downregulation of miR-122 increases the expression of the tumour promoter N-myc downstream regulated gene 3 (*NDRG3*) [120]. Furthermore, this increases expression of the miR-122 target cyclin G1 (*CCNG1*) that results in enhanced Akt activation leading to epithelial-mesenchymal transition [121]. Moreover, HBV-induced inhibition of miR-122 also results in an increase in pituitary tumour-transforming gene 1-binding factor (*PTTG1*) that promotes tumour growth and cell invasion [117]. Taken together, these HBV-induced changes in regulatory networks may contribute to the development of HCC (Fig. 1). Given that restoration of miR-122 has been shown to reverse the tumourigenic properties of hepatoma cells and to prevent HCC development *in vivo* [38,39,43–45,50,51], potential future therapeutic strategies, aiming at restoring miR-122 to prevent/treat HCC in patients with reduced/absent miR-122 levels might be an interesting strategy for patients with HBV-induced HCC.

Conclusions and perspectives

Given its central role in liver biology and disease, miR-122 represents an interesting therapeutic target for the treatment of liver disease including viral hepatitis, fibrosis, steatosis and HCC. Proof-of-concept studies have elegantly demonstrated that a miR-122 inhibitor efficiently reduces viral load in chronically infected HCV patients without detectable resistance [16] (Fig. 2). However, given the very high cure rates of orally administrated DAAs with a high genetic barrier for resistance (reviewed in [113]), the need for parenteral administration of miRNA-122 inhibitors [16], and a potential HCC/liver disease-promoting effect of miRNA depletion, the role of miR-122 inhibitors in the future treatment approaches for HCV infection remains to be determined. The exploration of miR-122 as a therapeutic target for HBV infection is ongoing. While experimental studies suggest that miR-122 plays a role in the HBV life cycle as a potential restriction factor, further studies are needed to assess whether targeting miR-122 would result in cccDNA eradication and viral cure – the ultimate goal for novel HBV therapeutic approaches. Given the limited or absent strategies to impair progression of liver disease and to prevent and treat HCC (reviewed in [122]) and the association between loss of miR-122 and liver inflammation, fibrosis, steatosis and HCC, miR-122 mimics may provide a novel strategy to slow down liver disease progression and to prevent and treat HCC. Current and future randomized clinical trials with miRNA-based molecules will shed light on the perspective of this approach for advanced liver disease and HCC. Finally, given the major involvement of miR-122 in liver homeostasis, cholesterol biosynthesis and fatty acid metabolism, additional preclinical studies will be required to determine the optimal level of miRNA mimics in therapy and to assess the potential risks associated with miR-122 overexpression or depletion.

Financial support

The authors' work was supported by Inserm, University of Strasbourg, the European Union (ERC-2008-AdG-233130-HEPCENT, ERC-StG-260767-ncRNAVIR, INTERREG-IV-Rhin Supérieur-FEDER-Hepato-Regio-Net 2012, EU FP7 HepaMab), ANRS (2012/239, 2013/108), the Direction Générale de l'Offre de Soins (A12027MS) the Institut Hospitalo-Universitaire (IHU) Mix-Surg and ARC (TheraHCC, IHU201301187). This work has been published under the framework of the LABEX ANR-10-LABX-0028_HEPSYS and ANR-10-LABX-0036_NETRNA and benefits from a funding from the state, managed by the French National Research Agency as part of the investments for the future program.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 2000;404:293–296.
[2] Martinez J, Patkaniowska A, Urlaub H, Lührmann R, Tuschl T. Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. Cell 2002;110:563–574.
[3] Kozomara A, Griffiths-Jones S. MiRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 2011;39:D152–D157.
[4] Yang J-S, Lai EC. Dicer-independent, Ago2-mediated microRNA biogenesis in vertebrates. Cell Cycle 2010;9:4455–4460.
[5] Ameres SL, Zamore PD. Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol 2013;14:475–488.
[6] Friedman RC, Farh KK-H, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009;19:92–105.
[7] Bartel DP, Chen C-Z. Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs. Nat Rev Genet 2004;5:396–400.
[8] Doench JG, Sharp PA. Specificity of microRNA target selection in translational repression. Genes Dev 2004;18:504–511.
[9] Tsang JS, Ebert MS, van Oudenaarden A. Genome-wide dissection of microRNA functions and co-targeting networks using gene set signatures. Mol Cell 2010;38:140–153.
[10] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–297.
[11] Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007;129:1401–1414.
[12] Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010;11:597–610.
[13] Kloosterman WP, Plasterk RHA. The diverse functions of microRNAs in animal development and disease. Dev Cell 2006;11:441–450.
[14] Bandiera S, Hatem E, Lyonnet S, Henrion-Caude A. MicroRNAs in diseases: from candidate to modifier genes. Clin Genet 2010;77:306–313.
[15] Hrdlickova B, de Almeida RC, Borek Z, Withoff S. Genetic variation in the non-coding genome: involvement of micro-RNAs and long non-coding RNAs in disease. Biochim Biophys Acta 2014;1842:1910–1922.
[16] Janssen HLA, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al. Treatment of HCV infection by targeting microRNA. N Engl J Med 2013;368:1685–1694.
[17] Agostini M, Knight RA. MiR-34: from bench to bedside. Oncotarget 2014;5:872–881.
[18] Chen Y, Verfaillie CM. MicroRNAs: the fine modulators of liver development and function. Liver Int 2014;34:976–990.
[19] Szabo G, Bala S. MicroRNAs in liver disease. Nat Rev Gastroenterol Hepatol 2013;10:542–552.

[20] Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002;12:735–739.
[21] Girard M, Jacquemin E, Munnich A, Lyonnet S, Henrion-Caude A. MiR-122, a paradigm for the role of microRNAs in the liver. J Hepatol 2008;48:648–656.
[22] Hou W, Tian Q, Zheng J, Bonkovsky HL. MicroRNA-196 represses Bach1 protein and hepatitis C virus gene expression in human hepatoma cells expressing hepatitis C viral proteins. Hepatology 2010;51:1494–1504.
[23] Cermelli S, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. PLoS One 2011;6:e23937.
[24] Starkey Lewis PJ, Dear J, Platt V, Simpson KJ, Craig DGN, Antoine DJ, et al. Circulating microRNAs as potential markers of human drug-induced liver injury. Hepatology 2011;54:1767–1776.
[25] Waidmann O, Bihrer V, Pleli T, Farnik H, Berger A, Zeuzem S, et al. Serum microRNA-122 levels in different groups of patients with chronic hepatitis B virus infection. J Viral Hepat 2012;19:e58–e65.
[26] Van der Meer AJ, Farid WRR, Sonneveld MJ, de Ruiter PE, Boonstra A, van Vuuren AJ, et al. Sensitive detection of hepatocellular injury in chronic hepatitis C patients with circulating hepatocyte-derived microRNA-122. J Viral Hepat 2013;20:158–166.
[27] Köberle V, Waidmann O, Kronenberger B, Andrei A, Susser S, Füller C, et al. Serum microRNA-122 kinetics in patients with chronic hepatitis C virus infection during antiviral therapy. J Viral Hepat 2013;20:530–535.
[28] Miyaaki H, Ichikawa T, Kamo Y, Taura N, Honda T, Shibata H, et al. Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease. Liver Int 2014;34:e302–e307.
[29] Yamada H, Suzuki K, Ichino N, Ando Y, Sawada A, Osakabe K, et al. Associations between circulating microRNAs (miR-21, miR-34a, miR-122, and miR-451) and non-alcoholic fatty liver. Clin Chim Acta 2013;424:99–103.
[30] Xu H, He J-H, Xiao Z-D, Zhang Q-Q, Chen Y-Q, Zhou H, et al. Liver-enriched transcription factors regulate microRNA-122 that targets CUTL1 during liver development. Hepatology 2010;52:1431–1442.
[31] Laudadio I, Manfroid I, Achouri Y, Schmidt D, Wilson MD, Cordi S, et al. A feedback loop between the liver-enriched transcription factor network and miR-122 controls hepatocyte differentiation. Gastroenterology 2012;142:119–129.
[32] Deng X-G, Qiu R-L, Wu Y-H, Li Z-X, Xie P, Zhang J, et al. Overexpression of miR-122 promotes the hepatic differentiation and maturation of mouse ESCs through a miR-122/FoxA1/HNF4a-positive feedback loop. Liver Int 2014;34:281–295.
[33] Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene 2009;28:3526–3536.
[34] Krützfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, et al. Silencing of microRNAs in vivo with "antagomirs". Nature 2005;438:685–689.
[35] Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, et al. MiR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 2006;3:87–98.
[36] Elmén J, Lindow M, Schütz S, Lawrence M, Petri A, Obad S, et al. LNA-mediated microRNA silencing in non-human primates. Nature 2008;452:896–899.
[37] Gatfield D, Le Martelot G, Vejnar CE, Gerlach D, Schaad O, Fleury-Olela F, et al. Integration of microRNA miR-122 in hepatic circadian gene expression. Genes Dev 2009;23:1313–1326.
[38] Hsu S-H, Wang B, Kota J, Yu J, Costinean S, Kutay H, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest 2012;122:2871–2883.
[39] Tsai W-C, Hsu S-D, Hsu C-S, Lai T-C, Chen S-J, Shen R, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J Clin Invest 2012;122:2884–2897.
[40] Zeisel MB, Pfeffer S, Baumert TF. MiR-122 acts as a tumor suppressor in hepatocarcinogenesis in vivo. J Hepatol 2013;58:821–823.
[41] Li J, Ghazwani M, Zhang Y, Lu J, Li J, Fan J, et al. MiR-122 regulates collagen production via targeting hepatic stellate cells and suppressing P4HA1 expression. J Hepatol 2013;58:522–528.
[42] Cheung O, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, et al. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. Hepatology 2008;48:1810–1820.
[43] Coulouarn C, Corlu A, Glaise D, Guénon I, Thorgeirsson SS, Clément B. Hepatocyte-stellate cell cross-talk in the liver engenders a permissive inflammatory microenvironment that drives progression in hepatocellular carcinoma. Cancer Res 2012;72:2533–2542.
[44] Bai S, Nasser MW, Wang B, Hsu S-H, Datta J, Kutay H, et al. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. J Biol Chem 2009;284:32015–32027.
[45] Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, et al. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 2009;69:5761–5767.
[46] Zeng C, Wang R, Li D, Lin X-J, Wei Q-K, Yuan Y, et al. A novel GSK-3 beta-C/EBP alpha-miR-122-insulin-like growth factor 1 receptor regulatory circuitry in human hepatocellular carcinoma. Hepatology 2010;52:1702–1712.
[47] Nassirpour R, Mehta PP, Yin M-J. MiR-122 regulates tumorigenesis in hepatocellular carcinoma by targeting AKT3. PLoS One 2013;8:e79655.
[48] Xu J, Zhu X, Wu L, Yang R, Yang Z, Wang Q, et al. MicroRNA-122 suppresses cell proliferation and induces cell apoptosis in hepatocellular carcinoma by directly targeting Wnt/β-catenin pathway. Liver Int 2012;32:752–760.
[49] Nakao K, Miyaaki H, Ichikawa T. Antitumor function of microRNA-122 against hepatocellular carcinoma. J Gastroenterol 2014;49:589–593.
[50] Xu Y, Xia F, Ma L, Shan J, Shen J, Yang Z, et al. MicroRNA-122 sensitizes HCC cancer cells to adriamycin and vincristine through modulating expression of MDR and inducing cell cycle arrest. Cancer Lett 2011;310:160–169.
[51] Hsu S-H, Yu B, Wang X, Lu Y, Schmidt CR, Lee RJ, et al. Cationic lipid nanoparticles for therapeutic delivery of siRNA and miRNA to murine liver tumor. Nanomedicine Nanotechnol Biol Med 2013;9:1169–1180.
[52] Varnholt H, Drebber U, Schulze F, Wedemeyer I, Schirmacher P, Dienes H-P, et al. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. Hepatology 2008;47:1223–1232.
[53] Spaniel C, Honda M, Selitsky SR, Yamane D, Shimakami T, Kaneko S, et al. MicroRNA-122 abundance in hepatocellular carcinoma and non-tumor liver tissue from Japanese patients with persistent HCV versus HBV infection. PLoS One 2013;8:e76867.
[54] Negrini M, Gramantieri L, Sabbioni S, Croce CM. MicroRNA involvement in hepatocellular carcinoma. Anticancer Agents Med Chem 2011;11:500–521.
[55] Imbeaud S, Ladeiro Y, Zucman-Rossi J. Identification of novel oncogenes and tumor suppressors in hepatocellular carcinoma. Semin Liver Dis 2010;30:75–86.
[56] Zeisel MB, Lupberger J, Fofana I, Baumert TF. Host-targeting agents for prevention and treatment of chronic hepatitis C – Perspectives and challenges. J Hepatol 2013;58:375–384.
[57] Lohmann V. Hepatitis C virus RNA replication. Curr Top Microbiol Immunol 2013;369:167–198.
[58] Lindenbach BD. Virion assembly and release. Curr Top Microbiol Immunol 2013;369:199–218.
[59] Grimm D, Thimme R, Blum HE. HBV life cycle and novel drug targets. Hepatol Int 2011;5:644–653.
[60] Baumert TF, Meredith L, Ni Y, Felmlee DJ, McKeating JA, Urban S. Entry of hepatitis B and C viruses – Recent progress and future impact. Curr Opin Virol 2014;4:58–65.
[61] Van der Ree MH, de Bruijne J, Kootstra NA, Jansen PL, Reesink HW. MicroRNAs: role and therapeutic targets in viral hepatitis. Antivir Ther 2014. http://dx.doi.org/10.3851/IMP2766. [Epub ahead of print].
[62] Liu W-H, Yeh S-H, Chen P-J. Role of microRNAs in hepatitis B virus replication and pathogenesis. Biochim Biophys Acta 2011;1809:678–685.
[63] Shrivastava S, Mukherjee A, Ray RB. Hepatitis C virus infection, microRNA and liver disease progression. World J Hepatol 2013;5:479–486.
[64] Ghosh Z, Mallick B, Chakrabarti J. Cellular versus viral microRNAs in host-virus interaction. Nucleic Acids Res 2009;37:1035–1048.
[65] Roberts APE, Lewis AP, Jopling CL. The role of microRNAs in viral infection. Prog Mol Biol Transl Sci 2011;102:101–139.
[66] Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science 2005;309:1577–1581.
[67] Villanueva RA, Jangra RK, Yi M, Pyles R, Bourne N, Lemon SM. MiR-122 does not modulate the elongation phase of hepatitis C virus RNA synthesis in isolated replicase complexes. Antiviral Res 2010;88:119–123.
[68] Murakami Y, Aly HH, Tajima A, Inoue I, Shimotohno K. Regulation of the hepatitis C virus genome replication by miR-199a. J Hepatol 2009;50:453–460.
[69] Chen Y, Shen A, Rider PJ, Yu Y, Wu K, Mu Y, et al. A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. FASEB J 2011;25:4511–4521.

[70] Zhang G, Li Y, Zheng S, Liu M, Li X, Tang H. Suppression of hepatitis B virus replication by microRNA-199a-3p and microRNA-210. Antiviral Res 2010;88:169–175.

[71] Bogerd HP, Skalsky RL, Kennedy EM, Furuse Y, Whisnant AW, Flores O, et al. Replication of many human viruses is refractory to inhibition by endogenous cellular microRNAs. J Virol 2014;88:8065–8076.

[72] Whisnant AW, Bogerd HP, Flores O, Ho P, Powers JG, Sharova N, et al. In-depth analysis of the interaction of HIV-1 with cellular microRNA biogenesis and effector mechanisms. MBio 2013;4:e000193.

[73] Pedersen IM, Cheng G, Wieland S, Volinia S, Croce CM, Chisari FV, et al. Interferon modulation of cellular microRNAs as an antiviral mechanism. Nature 2007;449:919–922.

[74] Hu W, Wang X, Ding X, Li Y, Zhang X, Xie P, et al. MicroRNA-141 represses HBV replication by targeting PPARA. PLoS One 2012;7:e34165.

[75] Zhang X, Zhang E, Ma Z, Pei R, Jiang M, Schlaak JF, et al. Modulation of hepatitis B virus replication and hepatocyte differentiation by MicroRNA-1. Hepatology 2011;53:1476–1485.

[76] Ishida H, Tatsumi T, Hosui A, Nawa T, Kodama T, Shimizu S, et al. Alterations in microRNA expression profile in HCV-infected hepatoma cells: Involvement of miR-491 in regulation of HCV replication via the PI3 kinase/Akt pathway. Biochem Biophys Res Commun 2011;412:92–97.

[77] Bhanja Chowdhury J, Shrivastava S, Steele R, Di Bisceglie AM, Ray R, Ray RB. Hepatitis C virus infection modulates expression of interferon stimulatory gene IFITM1 by upregulating miR-130A. J Virol 2012;86:10221–10225.

[78] Chen Y, Chen J, Wang H, Shi J, Wu K, Liu S, et al. HCV-induced miR-21 contributes to evasion of host immune system by targeting MyD88 and IRAK1. PLoS Pathog 2013;9:e1003248.

[79] Wang Y, Lu Y, Toh ST, Sung W-K, Tan P, Chow P, et al. Lethal-7 is down-regulated by the hepatitis B virus x protein and targets signal transducer and activator of transcription 3. J Hepatol 2010;53:57–66.

[80] Dai X, Zhang W, Zhang H, Sun S, Yu H, Guo Y, et al. Modulation of HBV replication by microRNA-15b through targeting hepatocyte nuclear factor 1α. Nucleic Acids Res 2014;42:6578–6590.

[81] Cazalla D, Yario T, Steitz JA, Steitz J. Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA. Science 2010;328:1563–1566.

[82] Marcinowski L, Tanguy M, Krmpotic A, Rädle B, Lisnić VJ, Tuddenham L, et al. Degradation of cellular mir-27 by a novel, highly abundant viral transcript is important for efficient virus replication in vivo. PLoS Pathog 2012;8:e1002510.

[83] Lu S, Cullen BR. Adenovirus VA1 noncoding RNA can inhibit small interfering RNA and MicroRNA biogenesis. J Virol 2004;78:12868–12876.

[84] Cullen BR. Viruses and microRNAs: RISCy interactions with serious consequences. Genes Dev 2011;25:1881–1894.

[85] Kincaid RP, Burke JM, Sullivan CS. RNA virus microRNA that mimics a B-cell oncomiR. Proc Natl Acad Sci U S A 2012;109:3077–3082.

[86] Kincaid RP, Sullivan CS. Virus-encoded microRNAs: an overview and a look to the future. PLoS Pathog 2012;8:e1003018.

[87] Jin W-B, Wu F-L, Kong D, Guo A-G. HBV-encoded microRNA candidate and its target. Comput Biol Chem 2007;31:124–126.

[88] Lindenbach BD, Thiel HJ, Rice CM. Flaviviridae: the viruses and their replication. Fields Virol., vol. 1. 5th edition, 2007; 1101–52.

[89] Jopling CL, Schütz S, Sarnow P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. Cell Host Microbe 2008;4:77–85.

[90] Jangra RK, Yi M, Lemon SM. Regulation of hepatitis C virus translation and infectious virus production by the microRNA miR-122. J Virol 2010;84:6615–6625.

[91] Machlin ES, Sarnow P, Sagan SM. Masking the 5′ terminal nucleotides of the hepatitis C virus genome by an unconventional microRNA-target RNA complex. Proc Natl Acad Sci U S A 2011;108:3193–3198.

[92] Shimakami T, Yamane D, Welsch C, Hensley L, Jangra RK, Lemon SM. Base pairing between hepatitis C virus RNA and microRNA 122 3′ of its seed sequence is essential for genome stabilization and production of infectious virus. J Virol 2012;86:7372–7383.

[93] Henke JI, Goergen D, Zheng J, Song Y, Schüttler CG, Fehr C, et al. MicroRNA-122 stimulates translation of hepatitis C virus RNA. EMBO J 2008;27:3300–3310.

[94] Goergen D, Niepmann M. Stimulation of Hepatitis C Virus RNA translation by microRNA-122 occurs under different conditions in vivo and in vitro. Virus Res 2012;167:343–352.

[95] Wilson JA, Zhang C, Huys A, Richardson CD. Human Ago2 is required for efficient microRNA 122 regulation of hepatitis C virus RNA accumulation and translation. J Virol 2011;85:2342–2350.

[96] Conrad KD, Giering F, Erfurth C, Neumann A, Fehr C, Meister G, et al. MicroRNA-122 dependent binding of Ago2 protein to hepatitis C virus RNA is associated with enhanced RNA stability and translation stimulation. PLoS One 2013;8:e56272.

[97] Li Y, Masaki T, Yamane D, McGivern DR, Lemon SM. Competing and noncompeting activities of miR-122 and the 5′ exonuclease Xrn1 in regulation of hepatitis C virus replication. Proc Natl Acad Sci U S A 2013;110:1881–1886.

[98] Narbus CM, Israelow B, Sourisseau M, Michta ML, Hopcraft SE, Zeiner GM, et al. HepG2 cells expressing microRNA miR-122 support the entire hepatitis C virus life cycle. J Virol 2011;85:12087–12092.

[99] Kambara H, Fukuhara T, Shiokawa M, Ono C, Ohara Y, Kamitani W, et al. Establishment of a novel permissive cell line for the propagation of hepatitis C virus by expression of microRNA miR122. J Virol 2012;86:1382–1393.

[100] Chang J, Guo J-T, Jiang D, Guo H, Taylor JM, Block TM. Liver-specific microRNA miR-122 enhances the replication of hepatitis C virus in nonhepatic cells. J Virol 2008;82:8215–8223.

[101] Costa DD, Turek M, Felmlee DJ, Girardi E, Pfeffer S, Long G, et al. Reconstitution of the entire hepatitis C virus life cycle in nonhepatic cells. J Virol 2012;86:11919–11925.

[102] Fukuhara T, Kambara H, Shiokawa M, Ono C, Katoh H, Morita E, et al. Expression of microRNA miR-122 facilitates an efficient replication in nonhepatic cells upon infection with hepatitis C virus. J Virol 2012;86:7918–7933.

[103] Hueging K, Doepke M, Vieyres G, Bankwitz D, Frentzen A, Doerrbecker J, et al. Apolipoprotein E codetermines tissue tropism of hepatitis C virus and is crucial for viral cell-to-cell transmission by contributing to a postenvelopment step of assembly. J Virol 2014;88:1433–1446.

[104] Shan Y, Zheng J, Lambrecht RW, Bonkovsky HL. Reciprocal effects of micro-RNA-122 on expression of heme oxygenase-1 and hepatitis C virus genes in human hepatocytes. Gastroenterology 2007;133:1166–1174.

[105] Hou W, Bukong TN, Kodys K, Szabo G. Alcohol facilitates HCV RNA replication via up-regulation of miR-122 expression and inhibition of cyclin G1 in human hepatoma cells. Alcohol Clin Exp Res 2013;37:599–608.

[106] Bukong TN, Hou W, Kodys K, Szabo G. Ethanol facilitates hepatitis C virus replication via up-regulation of GW182 and heat shock protein 90 in human hepatoma cells. Hepatology 2013;57:70–80.

[107] Sarasin-Filipowicz M, Krol J, Markiewicz I, Heim MH, Filipowicz W. Decreased levels of microRNA miR-122 in individuals with hepatitis C responding poorly to interferon therapy. Nat Med 2009;15:31–33.

[108] Su T-H, Liu C-H, Liu C-J, Chen C-L, Ting T-T, Tseng T-C, et al. Serum microRNA-122 level correlates with virologic responses to pegylated interferon therapy in chronic hepatitis C. Proc Natl Acad Sci U S A 2013;110:7844–7849.

[109] Kamo Y, Ichikawa T, Miyaaki H, Uchida S, Yamaguchi T, Shibata H, et al. Significance of miRNA-122 in chronic hepatitis C patients with serotype 1 on interferon therapy. Hepatol Res 2014. http://dx.doi.org/10.1111/hepr.12317. [Epub ahead of print].

[110] Estrabaud E, Lapalus M, Broët P, Appourchaux K, De Muynck S, Lada O, et al. Reduction of microRNA 122 expression in IFNL3 CT/TT carriers and during progression of fibrosis in patients with chronic hepatitis C. J Virol 2014;88:6394–6402.

[111] Hildebrandt-Eriksen ES, Aarup V, Persson R, Hansen HF, Munk ME, Ørum H. A locked nucleic acid oligonucleotide targeting microRNA 122 is well-tolerated in cynomolgus monkeys. Nucleic Acid Ther 2012;22:152–161.

[112] Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 2010;327:198–201.

[113] Chung RT, Baumert TF. Curing chronic hepatitis C – The arc of a medical triumph. N Engl J Med 2014;370:1576–1578.

[114] Trépo C, Chan HLY, Lok A. Hepatitis B virus infection. Lancet 2014;S0140-6736:60220–60228.

[115] Wang S, Qiu L, Yan X, Jin W, Wang Y, Chen L, et al. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1)-modulated P53 activity. Hepatology 2012;55:730–741.

[116] Qiu L, Fan H, Jin W, Zhao B, Wang Y, Ju Y, et al. MiR-122-induced down-regulation of HO-1 negatively affects miR-122-mediated suppression of HBV. Biochem Biophys Res Commun 2010;398:771–777.

[117] Li C, Wang Y, Wang S, Wu B, Hao J, Fan H, et al. Hepatitis B virus mRNA-mediated miR-122 inhibition upregulates PTTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. J Virol 2013;87:2193–2205.

[118] Song K, Han C, Zhang J, Lu D, Dash S, Feitelson M, et al. Epigenetic regulation of MicroRNA-122 by peroxisome proliferator activated receptor-

gamma and hepatitis b virus X protein in hepatocellular carcinoma cells. Hepatology 2013;58:1681–1692.
[119] Peng F, Xiao X, Jiang Y, Luo K, Tian Y, Peng M, et al. HBx down-regulated Gld2 plays a critical role in HBV-related dysregulation of miR-122. PLoS One 2014;9:e92998.
[120] Fan C-G, Wang C-M, Tian C, Wang Y, Li L, Sun W-S, et al. MiR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. Oncol Rep 2011;26:1281–1286.
[121] Wen W, Ding J, Sun W, Fu J, Chen Y, Wu K, et al. Cyclin G1–mediated epithelial-mesenchymal transition via phosphoinositide 3-kinase/Akt signaling facilitates liver cancer progression. Hepatology 2012;55:1787–1798.
[122] Forner A, Gilabert M, Bruix J, Raoul J-L. Treatment of intermediate-stage hepatocellular carcinoma. Nat Rev Clin Oncol 2014;11:525–535.
[123] van Rooij E, Kauppinen S. Development of microRNA therapeutics is coming of age. EMBO Mol Med 2014:e201100899.
[124] Lecellier C-H, Dunoyer P, Arar K, Lehmann-Che J, Eyquem S, Himber C, et al. A cellular microRNA mediates antiviral defense in human cells. Science 2005;308:557–560.
[125] Lagos D, Pollara G, Henderson S, Gratrix F, Fabani M, Milne RSB, et al. MiR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. Nat Cell Biol 2010;12:513–519.
[126] Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Nature 2008;454:780–783.