MINI REVIEW

Impact of microRNAs for pathogenesis and treatment of hepatitis C virus infection

L’impact des microARN dans la pathogenèse et le traitement de l’infection par le virus de l’hépatite C

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Available online 2 June 2010

Summary The discovery of RNA interference (RNAi), and of all related RNA silencing processes, was one of the major breakthroughs and is currently changing our understanding of liver physiology and pathogenesis of liver disease. Furthermore, recent studies indicate that microRNAs (miRNAs) are a promising therapeutic target. Plant and insect organisms use RNAi as a major antiviral pathway, whereas mammalian viruses interfere with or even usurp the cellular miRNA repertoire. One remarkable example of such usurpation is provided by hepatitis C virus (HCV), which recruits the liver-specific miR-122 to enhance its abundance. In the HCV-infected patient, the impact of miRNAs for pathogenesis is more complex: whereas miR-122 expression shows no correlation with viral load, decreased pretreatment miR-122 levels are associated with nonresponse during IFN therapy. Following-up on these investigations, miRNA-122 has recently been shown to be a target for antiviral intervention. Treatment of chronically HCV-infected chimpanzees with a novel miR-122 antagonist leads to suppression of HCV viremia. The prolonged virological response to miRNA-based treatment holds promise of a new antiviral therapy with a potentially higher barrier to resistance. This review summarizes recent key discoveries of the impact of miRNAs for pathogenesis and treatment of HCV infection.

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**Introduction**

The importance of noncoding RNAs has risen exponentially since the observations that the expression of a gene could be regulated post-transcriptionally in a sequence-specific manner [6,24]. The discovery of RNA interference, and of all related RNA silencing processes, was one of the major breakthroughs and is changing our understanding of physiology and pathogenesis of disease including liver disease and viral hepatitis. It has become rapidly evident that RNA silencing is a pan-eukaryotic process that relies, with few exceptions, on double-stranded (ds) RNA as a key trigger molecule. This ds RNA can be of various origins and give rise to small RNA molecules ranging in size from 19 to 30 nucleotides [22]. miRNAs represent one of the most important and most studied classes of small RNAs across kingdoms. Seven hundred and twenty-one human miRNA genes are currently listed in the miRBase depository, and many additional such molecules likely remain to be discovered owing to their exquisite tissue specificity or stress-induced expression. The biogenesis of miRNAs successively engages a nuclear (Drosha) and a cytoplasmic (Dicer) RNase III-like enzyme to process a long primary transcript and yield a small RNA duplex. Upon unwinding, one of the two strands of this duplex is assembled into the final effector ribonucleoprotein (miRISC). Once loaded with the single-stranded, mature miRNA, the miRISC interacts with mRNA targets to modulate their expression or their stability [1]. Plant and insect organisms use RNAi as a major antiviral pathway, whereas mammalian viruses interfere with or even usurp the cellular miRNA repertoire [1] (Fig. 1).

Viral miRNAs may directly regulate viral and/or host cell gene expression to benefit the virus [31—32]. Furthermore, viruses may use cellular miRNAs for their replication, and in some cases, the expression of cellular miRNAs may be induced or inhibited to reshape the cellular gene expression environment to the benefit of the virus. Indeed, a recent study using multiplexed high-throughput sequencing to characterize changes in small RNA populations that occur during viral infection demonstrated that host miRNAs are most likely an important component of the interplay between human viruses and their hosts [27]. Finally, the expression of certain cellular miRNAs may be disadvantageous to the virus, through their interaction with viral miRNAs or because of their cellular function [7]. Regardless of whether interactions with cellular miRNAs enhance or limit virus replication, cellular miRNAs may have exerted a significant effect on viral genome evolution and certainly have the potential to regulate the tissue tropism of viruses in vivo [7].

### Impact of microRNAs for pathogenesis of hepatitis C virus infection

HCV is a small-enveloped positive-strand RNA virus that has been classified in the genus Hepacivirus of the Flaviviridae family [37]. HCV infection is a major cause of liver cirrhosis and hepatocellular carcinoma worldwide. Recent advancements in the understanding of virus-host interactions have demonstrated that HCV requires multiple host factors for establishment and propagation of HCV infection [2,16,36]. Among these cellular factors is miR-122 which has been identified as key host factor for viral abundance. Using HCV replicons, the Sarnow laboratory has elegantly demonstrated that HCV recruits the liver-specific miR-122 to enhance its replication [14—15]. miR-122 exerts a positive effect on the virus replication in cell culture after imperfect binding in the viral 5' UTR [15]. More recent studies addressing the mechanism of these findings demonstrated that miR-122 contributes to the liver tropism of HCV by accelerating the binding of ribosomes to the viral RNA and thereby stimulating HCV translation [8,25]. Since liver-specific miR-122 enhances the replication of HCV in nonhepatic cells, it is likely that miR-122 is a host factor which contributes to the liver tropism of HCV [3]. Overexpression of other miRNAs such as miR-199a and miR-196 has been shown to modulate HCV replication and expression in cell culture models suggesting other hepatocyte miRNAs are involved in regulation of HCV infection [10,23].

Although the HCV genome itself does not encode for miRNAs [27,32], HCV infection has been shown to modulate the miRNA expression profile in HCV-infected liver-derived cells in vitro and [20,27] in vivo [35]. These observations suggest that HCV not only usurps miRNAs to establish persistent infection but changes the cellular miRNA profile which most likely has further not yet uncovered implications for the viral life cycle and pathogenesis of infection such as viral escape or persistence as shown for other viruses [7].

To address the functional relevance of miRNAs for pathogenesis of HCV infection and the antiviral activity of interferon-alpha (IFN-α) in HCV infection in vivo, Sarasin-Filipowicz et al. analyzed liver biopsies from subjects with chronic hepatitis C undergoing IFN therapy. A number of

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**Figure 1** Clinical impact of hepatitis C virus (HCV)-miRNAs interactions. MicroRNAs (miRNAs) have been identified as host factors for HCV infection as shown for liver-derived miR-122 [15]. Targeting miR-122 by antagonists has been shown to be a promising therapeutic approach of HCV infection in a clinically relevant animal models of HCV infection [18]. On the other hand, HCV infection has been shown to modulate expression of miRNAs infected hepatoma cells in cell culture models and the infected liver [19,27] in vivo [35]. These changes may be of important impact for pathogenesis of liver disease (such as escape and persistence — Gottwein et Cullen [7]) and serve as biomarker for outcome of infection as shown previously for miR-122 [33]. The clinical relevance of HCV-miRNA interactions are highlighted in red and key concepts of miRNAs in HCV pathogenesis is highlighted in blue.
miRNAs including miR-122 were quantified by qRT-PCR [33] and compared between patients nonresponding to treatment or patients showing an early virological response. Using this approach, Sarasin-Filipowicz et al. demonstrated that miR-122 levels were significantly lower in patients with non-response compared to patients showing an early virological response. This correlation was genotype-independent. Furthermore, the authors demonstrated that the level of HCV RNA in liver and serum of the HCV-infected patients did not correlate with the abundance of miR-122. These findings suggest that the impact of miR-122 for HCV replication may be less prominent in the HCV-infected liver in vivo than in HCV cell culture model systems. In summary, the findings of this study provide a marked advancement in the understanding of the role of miRNAs in HCV infection and the antiviral activity of IFN in vivo. The absent modulation of miRNA levels in the human liver in vivo following administration of interferon suggests that it is unlikely that the antiviral effects of IFN during therapy can be predominantly explained by changes in the levels of the investigated miRNAs as suggested by a previous study performed in an HCV cell culture model [28]. A second very important observation of the study is the absence of a correlation between intrahepatic miR-122 and HCV RNA levels, since one would expect that a reduction in miR-122 should decrease viral replication and increase the effects of the therapy. Thus, the findings of Sarasin-Filipowicz et al. suggest that the role of miR-122 for pathogenesis of HCV infection is more complex compared to observations made in cell culture models and further studies are needed to address the impact of miRNAs for HCV infection in the HCV-infected patient. Finally, the finding that miR-122 expression is significantly lower in nonresponder subjects suggests a potential role of miRNA-122 in treatment response and liver miR-122 may serve as biomarker for predicting the outcome of IFN therapy in combination with other markers [29,32].

**MicroRNAs as targets for treatment of hepatitis C virus infection**

Key limitations of current antiviral treatment consisting of pegylated IFN-α and ribavirin are resistance, adverse effects and high costs [34]. Although the clinical development of novel antivirals targeting HCV protein processing has been shown to markedly improve sustained virological response, toxicity of the individual compounds and development of viral resistance remain major challenges [9,21]. Thus, novel antiviral strategies are urgently needed. Since miR-122 has been shown to be an important co-host factor in cell culture models [15,25] and miR-122 levels are associated with outcome interferon-based treatment in HCV-infected patients [32], miR-122 has become a key target for the development of novel antivirals.

To evaluate the importance of miRNAs as a therapeutic target, chemically modified antisense oligonucleotides are used. These oligonucleotides are complementary to the miRNA and preventing its interaction with the target RNA. Two approaches have been used to modify the antisense oligonucleotide, increase its stability and delivery to its target cell: 2’O methylated oligonucleotides, usually coupled to a cholesterol group [17]; and oligonucleotides containing locked-nucleic acid (LNA) residues. Approaches antagonizing miRNA function were first established in vitro [22], followed by proof-of-concept studies in mouse models [17]. Interestingly, these animal studies enabled the identification of cellular targets of miR-122, most of which involved in the cholesterol biogenesis pathway [4–5].

Following-up on these preclinical studies of miR-122 antagonists in animal models, a recent study elegantly showed that the inhibition of miR-122 leads to long-lasting suppression of HCV viremia in chimpanzees [18]. Administration of an LNA antisense oligonucleotide binding to the 5’ part of miR-122 to chronically HCV-infected animals resulted in a marked suppression of HCV RNA. Interestingly, no adaptive mutations were found in the miR-122 binding sites and no rebound in viremia was observed during the treatment [18]. As expected, the antagonism of miR-122 resulted in a strong decrease in serum cholesterol. Since recent studies have demonstrated that loss of HCV RNA cannot be restored by isoprenoid intermediate metabolites [26], miR-122 and its antagonist modulate viral RNA abundance most likely independently of its effect on cholesterol metabolism [18].

Taken together, this proof-of-concept study demonstrates that targeting of miR-122 leads to marked suppression of viremia in chronically HCV-infected chimpanzees and improvement of HCV-induced liver pathology. These findings suggest that targeting miRNA-122 by antagonists holds promise as a novel antiviral therapy. A potential advantage compared to therapeutic strategies targeting viral factors may be the high barrier to resistance, as demonstrated by the lack of rebound in viremia during the treatment and the lack of adaptive mutations in the two miR-122 seed sites of HCV 5’ NCR [18]. Furthermore, conservation of both miR-122 seed sites in all HCV genotypes and subtypes suggests that such therapy will most likely be genotype-independent. A potential limitation of the therapeutic approach for the HCV-infected patient may be the delivery of the oligonucleotide, which needs frequent intravenous injections (for comment see Pfeffer and Baumert [30]). Another potential concern for an approach that targets a host factor could be safety. Although an extensive analysis of clinical symptoms, clinical chemistry and histopathology in the four treated chimpanzees did not reveal any evidence for serious adverse effects, more detailed investigations in humans are required to rule out adverse effects which are not yet uncovered [30]. The discovery of other miRNAs potentially involved as regulators of the HCV life cycle [10,23] may further broaden the scope of miRNAs as antiviral targets.

**Summary and perspectives**

In conclusion, miRNAs play a key role in virus host interactions and pathogenesis of virus-induced disease. Furthermore, miR-122 has been identified as an important liver-derived host factor for HCV infection and target for antiviral therapy. These discoveries underline the functional relevance of miRNAs for pathogenesis of HCV infection as well as the development of novel antivirals overcoming resistance. Clinical trials are clearly the next step to demonstrate the efficacy and safety of miR-122 antagonists in the HCV-infected patient.
An increasing number of recent publications have reported that miRNAs can be quantified in clinical body fluids and tissues and can be used as biomarkers for outcome of disease [11–13]. Furthermore, liver miR-122 have been shown to be associated with treatment response [32]. Thus, it is likely that miRNAs may be of interest as a biomarker for disease progression of HCV-induced liver disease and treatment outcome.

Further studies are needed to:

- better understand the functional role of miR-122 for HCV pathogenesis;
- investigate the role of other miRNAs for the viral life cycle;
- understand the impact of HCV-induced modulation of liver miRNA profiles for pathogenesis of HCV-induced liver disease.

As shown for miR-122, the understanding of these mechanisms will not only advance the understanding of the biology of HCV infection but ultimately lead to novel and innovative treatment approaches targeting HCV infection and identify useful biomarkers for outcome of disease.

Financial support: This work was supported by Inserm and CNRS, France, the EU (ERC — 2008-AdG-233130-HEPCENT, EU Interreg-IV-2009 Hepato-Regio-Net), Agence nationale de la recherche (ANR-05-CEXC-008) (ANRS), France (2008/354), Interreg-IV-2009 Hepato-Regio-Net), Agence nationale de la recherche (ANR-05-CEXC-008) (ANRS), France (2008/354), Paris and the Else-Kröner-Fresenius Stiftung, Bad Homburg (P17/07//A83/06).

Conflicts of interest statement

The authors have no conflicts of interest to declare.

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