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MINIREVIEW

Targeting microRNA-122: Walking on cutting edge of hepatitis C virus infection therapy

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Summary. – Hepatitis C virus (HCV) infection, with an estimated 170 million carriers worldwide, remains a major cause of chronic liver disease. Current anti-HCV treatments have significant side effects and have met with only partial success. Therefore, a more effective therapeutic modality for HCV infection is needed. The stability and propagation of HCV is dependent on the interaction between its genome and a highly abundant liver microRNA (miRNA), known as microRNA-122 (miR-122). As a conserved host factor that would not be expected to evolve resistance mutations, miR-122 makes an attractive antiviral target. In this review we will discuss how targeting miR-122, using antisense oligonucleotides (ASOs), can be a new anti-HCV treatment.

Keywords: hepatitis C virus; microRNA; locked nucleic acid therapy; microRNA-122; human

Contents:

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Abbreviations: ASO = santisense oligonucleotides; HCV = hepatitis C virus; HNF = hepatocyte nuclear factor; LNA =locked nucleic acid; miRNA = microRNA; miR-122 = microRNA-122

1. Introduction

HCV is a major health problem worldwide, infecting an estimated 170 million people (Alavian *et al*., 2007). The current HCV antiviral therapy with interferon/ribavirin is successful in approximately half of the genotype 1 cases. Although addition of protease inhibitors, boceprevir (BOC) and telaprevir (TPV), improved the sustained virologic response rate to 70%, novel therapeutic approaches are still required.

miRNAs are endogenous, small non-coding RNAs involved in the regulation of gene expression in a sequence-specific manner. miR-122, which is the most abundant miRNA in the liver, promotes HCV replication through an interaction with

the 5' UTR of the viral genome (Jopling *et al.*, 2005; Jangra *et al.*, 2010). Different studies indicated that functional inhibition of miR-122 using ASOs leads to effective reduction in abundance of viral RNA, implicating miR-122 as a potential target for anti-HCV therapeutic. The most advanced inhibitor of miR-122 is a locked nucleic acid (LNA)-modified ASO known as miravirsen. Miravirsen is the first and to date only miRNA-targeted drug to enter human clinical trials. In phase 2 human study, the use of miravirsen in patients with chronic HCV genotype 2 infection showed prolonged dose-dependent reductions in HCV RNA levels without evidence of viral resistance (Janssen *et al.*, 2013).

2. Identification of miRNA

miRNAs are short (21nt–23nt), evolutionary conserved, non-coding, single-stranded RNAs of endogenous origin that post-transcriptionally regulate gene expression in animals and plants. They function by binding to the target mRNA with subsequent degradation or translation inhibition. Genes that encode miRNAs are transcribed by RNA polymerase II (pol II) in all eukaryotes or by RNA polymerase III (pol III) in some viruses. These primary transcripts, known as pri-miRNAs, are spliced and have a 5′-7-methylguanosine cap (m7G) and a poly A tail. Pri-miRNA folds into a hairpin that becomes the substrate for a nuclear ribonuclease III (RNase III) enzyme called Drosha. Drosha cleaves pri-miRNAs and yields ~70nt hairpin-like structures, called precursor miRNAs (pre-miRNAs). Once released from the pri-miRNA, the pre-miRNA is exported to the cytoplasm by exportin-5 (Exp5), where it is cleaved by cytoplasmic RNase III, called Dicer, to generate the 21nt–23nt double stranded miRNA. Ultimately, this RNA duplex is unwound and the single strand mature miRNA is completed. The mature miRNA fragment is then incorporated into the RNA-induced silencing complex (RISC) to function as a guide, directing the silencing of target mRNA. Generally, animal miRNAs repress gene expression by interaction with partially complementary target sites in the 3' UTR of target (Eulalio *et al.*, 2008). In contrast, almost all plant miRNAs regulate their targets by directing mRNA cleavage in the coding regions. Growing evidence has demonstrated that miRNAs regulate a wide range of biological processes including development, differentiation, cell proliferation, apoptosis, and immune responses (Ghildiyal and Zamore, 2009; Moazed, 2009).

3. Identification of miR-122

miR-122 was originally identified when the tissue-specific distribution of different miRNAs in mammalian species was characterized by cloning of various mouse tissue samples (Lagos-Quintana *et al.*, 2002). This uncovered that miR-122 dominates the miRNA content of the liver. The exact sequence of miR-122 is conserved among all species in which it has been detected, suggesting that the entire sequence of this miRNA is important for its function (Wienholds *et al.*, 2005).

miR-122 derives from a single genomic locus on chromosome 18, marked 18q21.31. Mammalian miRNA genes are found as single or clustered transcription units, which are located in introns of protein-coding mRNAs, or in both introns and exons of non-coding mRNAs, or they have their own independent transcription units (Rodriguez *et al.*, 2004; Saini *et al.*, 2007). The human miR-122 locus is in a non-coding RNA exon and is not part of a cluster (Rodriguez *et al.*, 2004). Its gene is initially transcribed as a 7.5 kb transcript that is spliced to yield the 4.5 kb pri-miR-122, from which the pre-miRNA is cleaved by the Drosha. Pre-mir-122 is a 66-nt hairpin-shaped precursor molecule from which the endonuclease Dicer cleaves the mature 22-nt miR-122.

Different studies using *in vivo* gene silencing, *in vitro* experimentation, and transcriptome profiling demonstrated that miR-122 regulates networks of genes that control lipid metabolism, cell differentiation, hepatic circadian regulation, HCV replication, and systemic iron homeostasis (Jopling *et al.*, 2005; Esau *et al.*, 2006; Gatfield *et al.*, 2009; Castoldi *et al.*, 2011; Kim *et al.*, 2011). Moreover, pathogenic repression of miR-122 has been observed in nonalcoholic steatohepatitis, liver cirrhosis, and HCC (Budhu *et al.*, 2008; Tsai *et al.*, 2009; Burchard *et al.*, 2010; Castoldi *et al.*, 2011). Different roles of miR-122 in hepatic function and liver pathology will be discussed in subsequent sections.

3.1 miR-122 in liver development

As the most abundant miRNA in liver, miR-122 expression increases during embryogenesis until it constitutes 72% of total miRNA in adult human liver (approximately 66,000 copies per cell) (Chang *et al.*, 2004). It suggests that miR-122 may play an essential role in the regulation of hepatocyte differentiation and liver development. The possible involvement of miR-122 in liver development was first suggested by a study on mice that showed that four liver-enriched transcription factors including hepatocyte nuclear factor (HNF) 1α, HNF3β, HNF4α and CCAAT/enhancer-binding protein α (C/EBPα) activate miR-122 expression by binding to its promoter (Coulouarn *et al.*, 2009; Xu *et al.*, 2010). In line with this report, increased miR-122 was shown to downregulate its target CUTL1, which is a transcriptional repressor of genes regulating the terminal proliferation and differentiation in hepatocytes (Xu *et al.*, 2010). A subsequent study further showed that HNF6 and its paralog Onecut2 strongly stimulate expression of miR-122. Significantly, by forming a positive feedback loop, miR-122 stimulates the expression

of hepatocyte-specific genes and most LETFs, including HNF6 (Laudadio *et al.*, 2012). Together, these observations raise the possibility that LETFs positive feedback on the expression of miR-122 constitutes a regulatory mechanism driving progression of hepatocyte differentiation.

3.2 miR-122 in lipid metabolism

Evidence from sequestrating miR-122 *in vivo* suggests that this miRNA modulates the expression of genes involved in hepatic lipid and cholesterol metabolism.

Different groups of studies have reported that the antagonism of miR-122 in the liver results in sustained decrease in plasma cholesterol levels in both mice and non-human primates (Krützfeldt *et al.*, 2005; Esau *et al.*, 2006; Elmen *et al.*, 2008; Lanford *et al.*, 2010). Also, several genes involved in fatty acid synthesis and oxidation including fatty acid synthase (FAS) enzyme, acetyl-CoA carboxylase 1 (ACC1), and ACC2, were altered in mice treated with anti-miR-122 (Elmén *et al.*, 2008). Despite these achievements in studies of miR-122-mediated lipid metabolism, the molecular mechanisms underlying miR-122-regulated cholesterol biosynthesis remain elusive and still await further investigation.

3.3 miR-122 in HCC

Loss of miR-122 expression in HCC, which is associated with poor prognosis, metastasis and cancer progression, has been confirmed by several studies (Coulouarn *et al.*, 2009; Wu *et al.*, 2009). Several target genes of miR-122 have been identified to be involved in hepatocarcinogenesis. Currently identified targets include Bcl-w (an anti-apoptotic Bcl-2 family member), serum response factor (SRF), peroxiredoxin 2 (an antioxidant protein), cyclin G1, ADAM10 (a distintegrin and metalloprotease family 10), insulin-like growth factor 1 receptor (IGF1R), Wnt1, pyruvate kinase muscle isozyme2 (PKM2), cationic amino acid transporter (CAT-1) and protein kinase interferon-inducible double-stranded RNA-dependent activator (PRKRA) (Chang *et al.*, 2004; Lin *et al.*, 2008; Bai *et al.*, 2009; Fornari *et al.*, 2009; Tsai *et al.*, 2009; Zeng *et al.*, 2010; Jung *et al.*, 2011; Li *et al.*, 2012; Xu *et al.*, 2012).

3.4 miR-122 in HCV infection

MiR-122 has been shown to be essential for replication and amplification of HCV RNA (Jopling *et al.*, 2005), thus plays a role independent of viral entry or assembly and release. HCV is currently classified into six major genotypes and numerous subtypes. It is a hepatotropic, positive-sense, single-stranded RNA virus with a 9.6 kb genome that establishes persistent infection in the liver, eventually leading to cirrhosis and carcinoma. The HCV genome contains a single open reading frame (ORF) encoding the viral polyprotein, which is subsequently cleaved into at least 11 viral proteins by host and viral proteases. This ORF is flanked by 5' and 3' non-coding regions that contain important RNA elements for viral replication and translation.

The first indication that miR-122 is involved in regulating HCV RNA abundance was demonstrated in a study that showed the inhibition of endogenous miR-122 leads to a dramatic reduction in HCV RNA replication in transfected Huh-7 cells, while overexpression of miR-122 leads to increased HCV RNA levels (Jopling *et al.*, 2005).

It has been demonstrated that miR-122 binds to two adjacent sites in 5' UTR, upstream of the HCV-IRES, which is conserved across all six HCV genotypes (Jopling *et al.*, 2005; Jopling, 2008). The binding sites include the 5' proximal S1 site, located about 22nt from the 5'end of the genomic RNA, and the nearby S2 site, located only 16nt downstream. A third potential seed sequence-binding site exists within the 3' UTR, but it has yet to be linked functionally to miR-122. Mutational analysis demonstrated that the replication of the infectious virus is highly dependent on direct interactions of miR-122 with both the S1 and S2 sites (Jangra *et al.*, 2010). The S1 site seems to play a more crucial role in increasing the effect of miR-122 upon the viral replication.

Although different studies have shown the importance of the interactions of miR-122 with HCV RNA in viral RNA amplification, the mechanisms underlying this dependence remain deeply controversial.

Different lines of evidence suggest that the stimulation of HCV translation is conferred by direct interaction of miR-122 with two target sites (S1 and S2) located upstream of the IRES, which accelerate the association of the small ribosomal subunit with the HCV RNA (Henke *et al.*, 2008). IRES allows for cap-independent assembly of the 48S ribosomal complex on viral RNA (Lukavsky *et al.*, 2003; Jangra *et al.*, 2010). Binding of miR-122 may lead to conformational changes in HCV-IRES, which results in promotion of translation. It is also proposed that miR-122 binding to the 5' UTR increases HCV translation by inhibiting interactions between 5' UTR sequence, nt 24–38, and bases 428–442 within the core-coding region (Kim *et al.*, 2003; Díaz-Toledano *et al.*, 2009). This inhibition downregulates cap-independent HCV translation by constraining the IRES in a closed conformation. miR-122 binding could change the IRES conformation from a less active "closed" conformation to a more active "open" conformation. However, as miR-122 is able to enhance the amplification of subgenomic replicons that lack the core-coding sequence involved in this interaction, this putative conformational switch cannot explain how miR-122 stimulates translation (Henke *et al.*, 2008).

The HCV genome does not contain a cap structure at the 5′ end, which normally promotes the cellular RNA stability

by shielding the single-stranded 5′ end from exonuclease digestion. Indeed, it is also suggested that miR-122 protect the HCV genome against degradation by cytoplasmic 5′ to 3′ exonuclease, Xrn1, in much the same manner as a cap or cytoplasmic sensors of viral RNA that induce innate immune responses (Machlin *et al.*, 2011). However, even in the absence of cytoplasmic exonuclease Xrn1, viral mutant defective in miR-122 binding site cannot replicate, indicating that miR-122 has additional yet uncharacterized function(s) in the viral life cycle.

4. miR-122 inhibition as an anti-HCV therapeutic

It is estimated that about 3% of the world's population has been infected with HCV. Treatment of HCV using combination of interferon plus ribavirin leads to sustained clearance in only about half of the HCV-infected patients and the treatment is frequently associated with severe side effects. With the addition of the viral protease inhibitors, boceprevir and telaprevir, to interferon and ribavirin, the rate of sustained virologic, response has improved to 70%, still leaving an unmet clinical need (Kiser *et al.*, 2012; Motavaf *et al.*, 2012). Different new approaches targeting different viral components are currently being developed (Motavaf *et al.*, 2012). However, due to the rapid evolution of HCV genomes through the error-prone activity of the RNA-dependent RNA polymerase development of resistance is frequently associated with the direct-acting antiviral agents. Thus, using a host factor as a target has the potential to avoid such problems. As a conserved and essential host factor for HCV that would not be expected to evolve resistance mutations, inhibiting miR-122 function presents an attractive target for anti-HCV therapy.

Currently, three approaches are used in miRNA loss-of-function studies: genetic knockouts, miRNA sponges and ASOs. A widely employed approach in miRNA inhibition is to use chemically modified ASO, termed antimiRs. AntimiRs are short, single-stranded oligonucleotides designed to specifically bind to and inhibit endogenous miRNA molecules. This strategy, therefore, has much potential for therapy.

4.1 Successful inhibition of miR-122 in animals infected with HCV

Inhibition of miR-122 was first accomplished in mice by intravenous administration of a 3′ cholesterol-conjugated 2'-O-methyl (2'-O-Me) oligonucleotides with terminal phosphorothioate linkage, termed antagomirs (Krützfeldt *et al.*, 2005). This miR122 inhibitor showed to be specific, efficient and long-lasting, which makes it a powerful therapeutic strategy. Similar results were obtained by using a phosphorothioate 2'-O-methoxyethyl (2'-MOE) (Esau *et al.*, 2006). Both of these studies reported that the silencing of miR-122 in mice was achieved by degradation of this targeted miRNA after binding to antagomir.

Inhibition of miR-122 in mice was also achieved by systemically administrated 16nt unconjugated and fully phosphorothiolated LNA-antimiR complementary to the nucleotides 1–16 in the mature miR-122, called SPC3649 (Elmen *et al.*, 2008). In contrast to previous reports, this study implied that the LNA-antimiR binds stably to the miR-122, thereby antagonizing its function. Monitoring of treated mice indicated that the antagonism of miR-122 by LNA-antimiR is reversible. The doses required were much lower than in the antagomir experiments, so an LNA-based strategy seems the most promising approach to take for future therapeutics.

LNA-antimiRs were then tested in a non-human primate, the African green monkey (Elmén *et al.*, 2008). The results obtained were very similar to those in mice, with formation of stable heteroduplexes between the LNA-antimiR and miR-122.

Encouragingly, the animals in these studies did not show any evidence of liver toxicity, but did experience a substantial reduction in plasma cholesterol. The results of these studies were encouraging for therapeutic development, first because the inhibitor shows similar effects and similar lack of toxicity in primates as in mice, implying that it could also be effective in humans, and second, because the effects of the inhibitor are sustained and reversible, which are desirable properties for a drug.

The therapeutic potential of SPC3649, has been investigated in chimpanzees chronically infected with HCV (Lanford *et al.*, 2010). The chimpanzees were treated with weekly intravenous injection over a 12-week period. Two animals received high dose (5 mg/kg) and two animals received a low dose (1 mg/kg). Excitingly, the result showed substantial reduction in viral titer in the two animals given a higher dose of the inhibitor, and in one of the two low dose animals. However the animal that responded to the low dose had a reduction in liver HCV RNA only 1.3 orders of magnitude, compared to 2.3 orders of magnitude in HCV RNA in the liver and 2.6 in the serum of the high dose animals. The reduced viral load was maintained over several weeks of therapy and was not accompanied by any acquisition of viral escape mutations or liver toxicity. The fourth animal, which was one of two animals that received a low dose, had fluctuations in the viral load throughout the study, which made the evaluation of the degree of suppression difficult. The reason for the reduced response in the low dose group was not clear since at the end of dosing, no miR-122 was detected in both dosing regimes. Because previous reports indicated that even markedly low levels of miR-122 can support HCV replication (Sarasin-Filipowicz *et al.*, 2009), it is possible that undetectable levels of miR-122 remaining after the low dose therapy were sufficient to

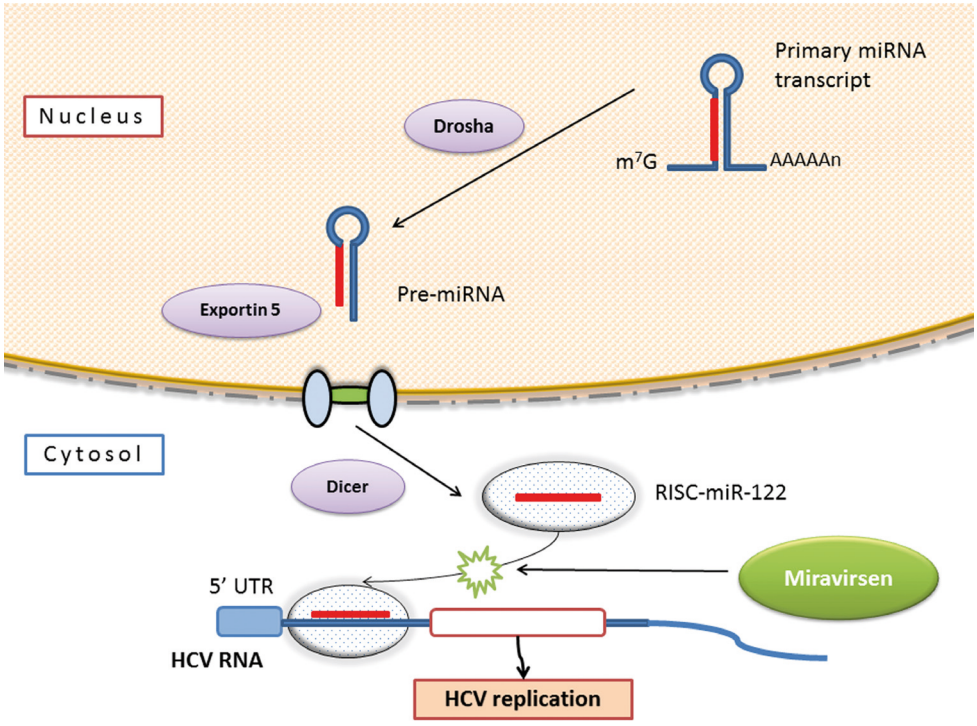

Fig. 1
A model for interaction of miR-122 with the 5' UTR of the HCV RNA and miravirsen's mechanism of action: miR-122 is encoded as a long primary mRNA transcript that in turn produces mature miR-122 through a series of endonucleolytic maturation steps
Mature miR-122 interacts with 5′ UTR of the HCV RNA and promotes its replication. Miravirsen is a high-affinity LNA-modified antisense oligonucleotide that acts by sequestering mature miR-122, leading to the inhibition of miR-122 function and thereby suppression of HCV.

support viral replication. Importantly, deep sequencing of the miR-122 binding region of HCV RNA in samples taken from the high dose animals before, during and after therapy showed no adaptive mutations in this region. This is consistent with the fact that both miR-122 sites are conserved in all HCV genotypes and miR-122 cannot be replaced by a different miRNA (Jopling *et al.*, 2008). The lack of viral resistance during SPC3649 therapy is in contrast with the rapid evolution of mutations to most drugs that directly target HCV. Consistent with previous findings, an increase in the levels of liver mRNAs with miR-122 seed match sites in the 3' UTR was observed in both high dose animals and the responding low dose animal. Furthermore, similar to observations in mice and African green monkeys, SPC3649 led to a significant reduction in total plasma cholesterol in the high dose group. However, in contrast to African green monkeys where high-density lipoprotein (HDL) and its apolipoprotein, Apo-A1 were reduced (Elmén *et al.*, 2008; Lanford *et al.*, 2010), the decreases in low-density lipoprotein (LDL) and apolipoprotein Apo-B in chimpanzees were more pronounced. As infected chimpanzees are better representative of human patients, it is possible that the cholesterol lowering effect of miR-122 antagonism in chimpanzees reflects the expected response in human. These results suggest that miR-122 may also be an attractive therapeutic target to reduce cholesterol levels, as high LDL levels are strongly associated with cardiovascular disease. Another interesting result of this study was the association between reduction in viremia and downregulation of most interferon-regulated genes (IRGs) in the high dose animals and the responding low dose animal. This is encouraging as it suggests that the inhibition of miR-122, even if it does not fully eliminate infection, might be an added beneficial effect due to the restoration of the interferon responsiveness in the liver of non-responding patients.

Encouragingly, the *in vivo* half-life of SPC3649 was shown to be about 20 days in the high dose animals, which presents the possibility of longer periods between administrations. In addition, improved liver histology was observed in both high dose animals after treatment, suggesting that damages induced by HCV infection might be reparable. Evaluation of the liver toxicity indicators did not show SPC3649-related abnormality. Results of the inhibition of miR-122 in preclinical studies provided a very exciting novel approach to treating HCV-infected patients.

4.2 SPC3649 (miravirsen): Novel treatment for HCV-infected patients

SPC3649 (miravirsen in the clinic) is the first and to date only miRNA-targeted drug to have entered human clinical trials. Data from a phase 1 single-ascending dose safety study in healthy volunteers did not show any adverse events. Miravirsen showed to be well tolerated, with an attractive pharmacokinetic profile and a clear dose-dependent pharmacology (Hildebrandt-Eriksen *et al*., 2009).

Santaris Pharma, a clinical-stage biopharmaceutical company focusing on the discovery and the development of RNA-targeted therapies, initiated a phase 2a study to assess the safety and the antiviral activity of miravirsen in treatment-naive HCV-infected patients (Janssen *et al*., 2013). In this study, 36 patients with chronic HCV genotype 1 infection were randomly assigned to three cohorts with nine miravirsen-treated (in doses of 3 mg/kg, 5 mg/kg, or 7 mg/kg) and three placebo-treated subjects. Miravirsen was given as a total of 5 weekly subcutaneous injections over 29 days. It provided a dose-dependent and long-lasting antiviral activity with a mean HCV RNA level decrease of 2–3 logs (log10 IU/ml) from the baseline. Data from this study demonstrated that in one patient who received 5 mg/kg dosage and in four patients who received 7 mg/kg, HCV RNA became undetectable with just four weeks of dosing, suggesting that miravirsen eventually might be appropriate as monotherapy in some patients. However, four of those five patients showed a rebound in viral levels after the discontinuation of miravirsen, which indicates that five weekly injections were not sufficient to induce a sustained virologic response. There were no dose limiting toxicities and no resistance-associated mutations in the two miR-122 seed sites of the HCV genome in any of the patients. The reported adverse events were infrequent and mostly mild and did not warrant the discontinuation of the treatment. Biochemical safety evaluation of miravirsen indicated a sustained decrease in levels of serum alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transpeptidase. There were no clinically significant changes in hemoglobin levels, total white-cell counts, prothrombin time, or activated partial thromboplastin time. Consistent with data from previous studies, a gradual and prolonged non–dose-dependent reduction in cholesterol levels was observed, which was expected because miR-122 is known to antagonize cholesterol homeostasis. However, no change in the ratio of LDL cholesterol to HDL cholesterol was observed.

5. Potential advantages of miravirsen

Several lines of studies revealed different features of miravirsen as a potential treatment for HCV infection. The conservation of the miR-122 binding sites across all HCV genotypes implied that the antiviral effect of miravirsen on HCV would be genotype independent (Li *et al*., 2011). Moreover, in contrast with the rapid evolution of mutations to most drugs that directly target HCV, no escape mutations in the miR-122 binding sites was observed over the course of miravirsen therapy of primates or humans, indicating a high genetic barrier to resistance (Lanford *et al*., 2010). In addition, in some patients receiving short-term therapy alone, HCV RNA was decreased to undetectable levels, suggesting that miravirsen eventually might be appropriate as monotherapy. Furthermore, the pharmacokinetic profile of miravirsen, with a gradual increase in trough levels representing hepatic accumulation and a prolonged tissue clearance half-life, shortens treatment duration, which is an important factor for patient compliance, and reduces the risk for viral escape mutations even further. Unlike the currently approved protease inhibitors, miravirsen is not metabolized via P450 system and is therefore not expected to have significant drug interactions.

Inhibition of miR-122 in nonhuman primate indicated a reduction in expression of IRGs genes in parallel with HCV titers (Lanford *et al*., 2010), suggesting that there might be an added beneficial effect due to restoring of the interferon, which may convert the interferon non-responders to responders by reducing the viral load. Observation of improved liver histology suggests that damage induced by HCV infection might be reparable upon prolonged suppression of viremia and normalization of the interferon pathway. In addition, the liver specificity of miR-122 expression means that side effects of drug delivery to other tissues are not likely to be a problem.

6. Possible problems associated with miravirsen

There are potential problems in targeting an endogenous miRNA as this will result in changes in targets that may potentially have damaging consequences. Although the liver specificity of this miRNA eliminates the issue of drug delivery to other tissues, the very high abundance of this miRNA in the liver implies its important functions. Another issue about miR-122 inhibition is the cancer concern. Different studies have shown that miR-122 is downregulated in HCC, suggesting that it is a potential tumor suppressor of HCC. Thus, loss of miR-122 might be associated with tumor invasiveness and cancer progression (Bai *et al*., 2009; Coulouarn *et al*., 2009; Wu *et al*., 2009). It is suggested that miR-122 likely mediates this effect via deregulation of a variety of target genes that are important in cell cycle regulation and hepatocyte differentiation. However, HCV-induced HCC have been reported to be associated with upregulation of miR-122 (Varnholt *et al*., 2008), suggesting that the role of miR-122

in HCV-derived HCC could be different from that in HCC not associated with HCV. In addition, as miR-122 inhibition in human studies was associated with the decrease in HDL cholesterol concentrations, such function may increase the cardio-vascular events.

Even though different measures of liver toxicity over the course of the studies did not show any apparent therapy-induced toxicity, problems might arise over longer treatment courses or sometime after treatment. Thus, follow-up of the treated patients will be important.

7. Conclusion

The liver-specific miRNA, miR-122, has been shown to be required for the replication of all HCV genotypes. Unlike many other HCV therapies that directly target the virus, miravirsen works by removing this helper molecule. Taken together, it is indicated that miravirsen provides long-lasting suppression of viremia, has a high barrier to viral resistance, and is well tolerated in patients with chronic HCV infection. Though miravirsen appears to be promising in HCV infection, larger studies are necessary to establish its benefits and lack of significant adverse effects, before it can become available as an anti-HCV treatment.

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