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RESEARCH HIGHLIGHT

Hepatitis C virus' Achilles' heel – dependence on liver-specific microRNA miR-122

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Hepatitis C virus (HCV) is an important human liver pathogen that has infected over 170 million people worldwide, often leading to liver cirrhosis and hepatocellular carcinoma [1]. While the efficacies of novel inhibitors of the viral polymerase and protease enzymes are being explored in numerous clinical trials, current therapies against HCV infections are limited to a combined administration of pegylated interferon-α and ribavirin. However, this combination therapy has considerable side effects, which include influenza-like symptoms, depression and anemia [2]. Moreover, even after 48 weeks of therapy, sustained virologic response rates are observed in only half of the patients infected with the prevalent genotype 1 HCV [2]. Thus, there is a need for exploring novel therapeutic approaches that target both viral and host factors that are essential for viral growth.

It has been known that the RNA genome of HCV forms an unusual interaction with a liver-specific microRNA, miR-122 [3, 4]. Assembly of the microRNA-HCV RNA complex is essential to maintain viral RNA abundance, while disassembly of the oligomeric complex by modified, antisense oligonucleotides, that sequester miR-122, leads to rapid loss of viral RNA in cultured cells [3, 4]. The recent study by Lanford and colleagues shows that intravenous injections of HCV-infected chimpanzees with locked nucleic acids (LNA), that sequester and inactivate miR-122 in the liver of the animals, results in a dramatic reduction in HCV yield without emergence of viral escape mutants and adverse effects to liver functions of the host [5]. Thus, these findings suggest that targeting these small host RNAs may be a potential novel antiviral approach.

To appreciate the impact of the findings made by Lanford *et al.* [5], it is important to emphasize the known roles for microRNAs in cellular and HCV gene expression. MicroRNAs are transcribed from cellular genes as long RNA precursors, which are processed by nuclear and cytoplasmic nucleases to approximately 22 nucleotide RNAs. These small RNAs ultimately regulate gene expression through binding to complementary sequences in mRNAs, and cause either degradation or translational repression of target mRNAs [6-8]. Importantly, specific microRNA abundances are altered in several diseases, including cancer, suggesting that microRNAs regulate cellular growth and differentiation. In addition, a few virus-encoded microRNAs can also target viral or host mRNAs, thereby downregulating innate immune responses of the host or suppressing the onset of apoptosis during latent herpes virus infections [9]. In contrast, it was discovered that miR-122 binds at two adjacent sites at the very 5' end of the HCV RNA genome and that formation of these microRNA-viral RNA complexes is essential to maintain viral RNA genome abundances in cultured liver cells [3, 4] (Figure 1). Both miR-122 binding sites are highly conserved among all HCV genotypes, an important observation given the diversity of the quasispecies that is found in HCV patients.

To explore the feasibility of using miR-122 as a potential antiviral target, it was of course necessary to understand its normal function in the liver. Several studies have characterized the effects of miR-122 inhibition in mice and African green monkeys [10-13]. It was found that antisense miR-122 administration consistently resulted in a reduction in cholesterol and fatty acid biosynthesis, accompanied by lower plasma cholesterol and triglyceride levels (Figure 1). Liver histology and function were not affected by antisense miR-122 treatment. In fact, mice with fatty livers that were given anti-miR-122 demonstrated an improvement in liver histology, presumably due to a reduction in lipid biosynthesis.

To examine whether antisense miR-122 molecules affected HCV yield in a primate model, Lanford *et al.* [5] administered LNAs, designated SPC3649, intravenously, and without any carrier formulations, to chimpanzees that had been chronically infected with HCV. SPC3649 is a 15-nucleotide long molecule that is complementary to the 5' end

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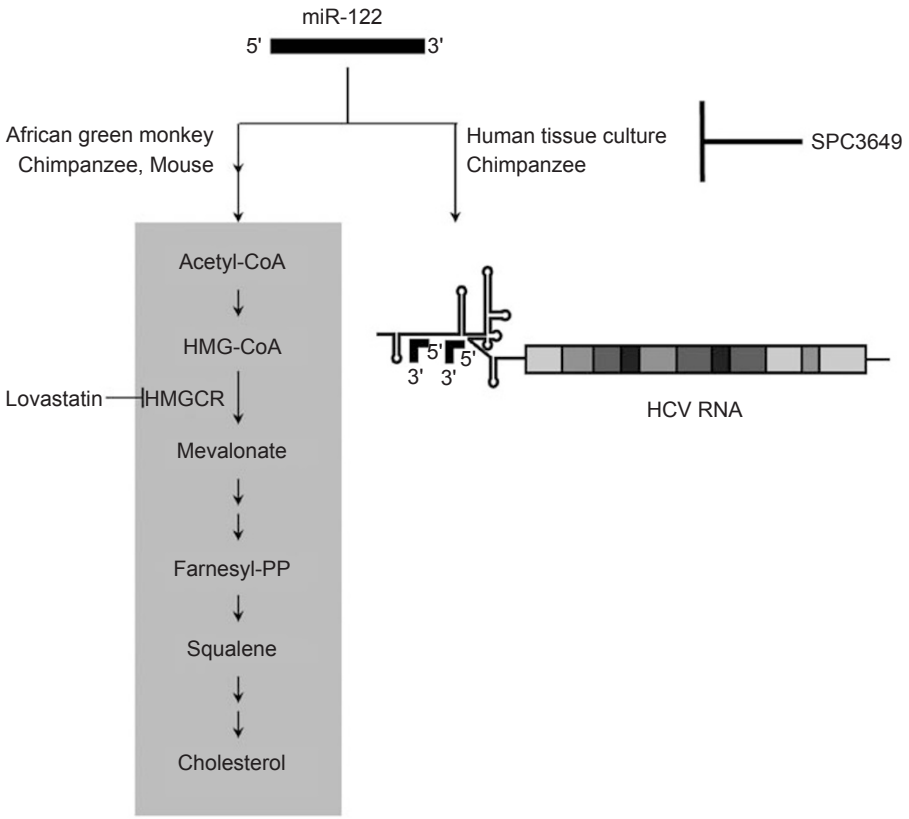

Figure 1 Effects of microRNA miR-122 on cholesterol biosynthesis and Hepatitis C virus. Stimulation of the cholesterol biosynthesis pathway by miR-122 is shown on the left. The Lovastatin target and the rate limiting enzyme of the pathway, HMGCR, is highlighted. Interaction of miR-122 with HCV RNA via two complementary binding sites in the 5' nontranslated region of the viral genome is shown on the right. The novel miR-122 inhibitor, an antisense locked nucleic acid (LNA) oligonucleotide, termed SPC3649, is shown. HMGCR, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase. PP, pyrophosphate.

of miR-122 (Figure 1). Four chimpanzees were tested in total, two at a high dose of 5 mg/kg SPC3649 and two at a lower dose of 1 mg/kg, given once per week for twelve weeks. SPC3649 effectively sequestered miR-122 into heteroduplexes for up to 8 weeks after the last treatment in the high dose animals. As found previously, serum cholesterol levels dropped as much as 44 percent. No SPC3649-related toxicity was detected. Importantly, treatment with SPC3649 led to a pronounced reduction in HCV yield. High dose SPC3649-treated animals demonstrated a maximum of 2.6 log reduction in serum HCV. One of the low dose-treated animals showed a 1.3 log reduction, while the other low dose-treated chimpanzee had variable HCV levels. Interestingly, all four chimpanzees showed SPC3649-miR-122 heteroduplex formation, detected in northern blots. Lanford *et al.* suggest this may indicate that very low levels of uncomplexed, undetectable miR-122 in the low dose SPC3649-treated animals can sustain HCV RNA abundance. It is also possible that the high doses of SPC3649 have additional as-of-yet uncharacterized effects on HCV, independent of miR-122 sequestration. An alternative explanation for the increased effects in high dose-treated chimpanzees could be that these two animals started with higher baseline HCV titers.

Remarkably, there was no evidence that SPC3649-resistant virus emerged during treatment, because deep-sequencing analyses of viral RNA revealed no specific outgrowth of virus with mutations in either miR-122 binding site. This is astonishing, because one would expect that point mutations in the viral genome, that could recruit another microRNA, might have been selected. As a point of comparison, Chen *et al.* reported that treatment with a polymerase inhibitor led to mutations in the polymerase gene and emergence of resistant HCV [14]. Therefore, miR-122 forms most likely a distinct oligomeric complex with the HCV RNA that involves multiple interactions between miR-122 and HCV.

Finally, liver transcriptome analysis confirmed that, as found in other animal models, SPC3649 treatment led to an increase in the abundance of cellular mRNAs that contain the nucleotide motif complementary to the "seed"

sequence of miR-122 (i.e. nucleotides 2 to 8 of miR-122) which is an important component of target mRNA recognition [6]. Furthermore, SPC3649-treated animals displayed diminished abundances of interferon-regulated mRNAs, reduced levels of the liver distress marker alanine aminotransferase and improved liver histology.

This is the first demonstration that miR-122 inhibition by antisense LNA oligonucleotides results in loss of HCV in a non-human primate. The lack of associated toxicities and resistant virus suggests promise to using SPC3649 as a novel anti-HCV therapeutic. Whether SPC3649 is safe and efficacious in humans is unknown as-of-yet. Of course, miR-122 is predicted to target hundreds of cellular mRNAs, some of which encode proto-oncogenes. Thus, there needs to be caution in employing long-term treatment with antisense miR-122 molecules. However, ongoing clinical trials will reveal whether short-term treatments with SPC3649 in combination with interferon- or non-interferon-based antiviral compounds may cause a more significant reduction in viral yield, especially in patients that do not respond to interferon-based therapy alone. On a broader scale, these findings argue that microRNA-targeted therapeutics may be possible, at least in the liver.

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