MicroRNA and its Potential Use for the Treatment of Hepatitis C Virus Infection

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Hepatitis C Virus and Treatment Regimen

Hepatitis C virus (HCV) is a world-wide health problem with about 150 million people (3% of the world population) (www.who.int) chronically infected. HCV is a single, positive-strand RNA virus that belongs to Flaviviridae family [1]. The full length of HCV genome contains 9.6 kb and encodes a polyprotein precursor of about 3000 amino acids that is then cleaved by host and viral proteases into three structural proteins (core, E1, and E2) and seven nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [2,3].

Chronic HCV infection frequently results in progressive inflammation, fibrosis, cirrhosis, and an increased risk of hepatocellular carcinoma [4]. There are 6 major genotypes of HCV and genotype 1 is the most prevalent in many countries and also is the hardest to treat [5–9]. Standard treatment for HCV infection involves a combination therapy with pegylated interferon alpha (IFN) and ribavirin (RBV). However, only 40–50% patients infected with HCV genotype 1 and 80% in patients infected with HCV genotypes 2 or 3 achieve a sustained virologic response (SVR) [10,11]. Most recently, a direct acting antiviral agent (DAA)-based “triple therapy”, combining a HCV NS3/4A protease inhibitor with IFN/RBV, has been proposed to be the standard-of-care for genotype 1 infection and this triple therapy improved the SVR up to 75% [9,12]. Two NS3/4A protease inhibitors, Telaprevir and Boceprevir, have been approved by the Food and Drug Administration (FDA) [13] Although the adoption of triple therapy has improved response rates, the high cost, drug-drug interactions and severe side effects limit its use, especially in many developing countries where the treatment need most [13,14]. Therefore, it is necessary to explore other treatment options.

MicroRNAs and their Mimics or Anti-microRNAs

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression after transcription [15–18]. Mature miRNAs are transcribed from introns and exons of protein-coding genes or intergenic regions [19]. Transcription of miRNA genes forms primary transcripts (pri-miRNA) that usually contain a hairpin structure consisting of a double-stranded stem and a terminal loop [20]. The stem-loop structure is then cleaved by an RNaseIII-like enzyme called drossha, together with its binding partner DGCGR8 (DiGeorge syndrome critical region 8), to produce a ~70 nt precursor miRNA (pre-miRNA) [14,21–23]. The pre-miRNA is then transferred from the nucleus to the cytoplasm with the help of exportin-5 and its co-factor Ran-GTP (GTP bound form of cofactor Ras-related nuclear protein), where it is processed into a duplex structure by RNA polymerase III dicer [24]. Subsequently, one strand named “guide strand” binds to an Argonaute protein and is integrated into an RISC (RNA-induced silencing complex) that recognizes and binds to the target miRNA; the other strand is degraded and nonfunctional [15,25]. The binding of miRNA and its target results in degradation of the target mRNA or suppression of mRNA translation [26,27].

More than 1500 miRNAs (www.mirbase.org, released 2012) have been identified in human genome and they are involved in almost every cell process, including development, differentiation, proliferation, death, disease pathology, and antiviral defence [28–31]. As a major host factor, miRNA represents an interesting field for studying the host-HCV interaction, ranging from HCV infection to the new targets for antiviral therapy [32]. In recent years, some oligonucleotide compounds were designed to mimic or sequester miRNAs as potential drug candidates and achieved promising results. For the miRNA mimic, the “guide strand” is identical to the interested miRNA and the “nonfunction strand” is modified and typically linked to a molecule such as cholesterol for enhanced cellular uptake [33]. Anti-miRs are modified antisense oligonucleotides harboring the full or partial complementary reverse sequence of a mature miRNA that can reduce the endogenous levels of an miRNA [33]. Two approaches to produce anti-miRs have been reported: one is 2-O-methyl-group (OMe)-modified oligonucleotides and the other is locked nucleic acid (LNA)-modified oligonucleotides [34,35].

In this review, we will focus on miRNAs that have been shown to have promising therapeutic effect for the treatment of HCV infection.

Treatment of HCV by T targeting miR-122

Of all the human miRNAs, miR-122 is one of the most studied. As a liver-specific and the most abundant miRNA (~70% of the liver total miRNAs), miR-122 plays a pivotal role in regulating hepatic functions [36]. Previous studies demonstrated that miR-122 is essential for HCV replication, which makes it an attractive host target for anti–HCV therapy [37]. miR-122 protects HCV from host immune attack by binding to two adjacent target sites in 5’untranslated region (5’ UTR) of HCV RNA [38,39]. Miravirsen (SPC3649, Santaris Pharma, Hørsholm, Denmark), an anti-miR-122 agent complementary to miR-122, has been shown to have significant anti-HCV activity both in animal model and Phase I and IIa studies [40–42]. Treatment of HCV chronically infected chimpanzees with a LNA-modified oligonucleotide (SPC3649) achieved a prolonged and marked suppression of HCV viremia with no evidence for viral resistance or side effects in the treated animals [41]. Most recently, a randomized, doubleblind, placebo-controlled,
ascending multiple dose-ranging Phase IIa study was finished in patients with chronic HCV genotype 1 infection [42]. The results showed that four-week injection of miraviren provided significantly dose-dependent anti-HCV activity with a mean reduction of 2 to 3 logs from baseline in HCV RNA and 5 patients even had an undetectable HCV RNA levels after the treatment period [42]. However, considering miR-122 is an important host cellular factor involving in hepatocytes metabolism, although current data from the clinical study was promising, further investigation with long period of treatment and large sample size are needed to further clarify these concerns [32,43].

Other miRNAs that have Potential Use for the Treatment of HCV Infection

Besides miR-122, many other miRNAs were identified to be involved in HCV replication and infection. Similar to miR-122, miR-196 and miR-199a were reported to have direct interaction with HCV RNA by binding to NSSA-coding region and 5'UTR IRES respectively, which make them the most promising therapeutic targets [44,45]. miR-130a was reported to regulate HCV replication in vitro [46,47]. Chowdhury et al. identified IFITM1, which can inhibit HCV replication, as a potential miR-130a target [46]. From this study, it is reasonable to conclude that miR-130a is a positive regulator for HCV replication. However, most recently, Li et al found that the replication of HCV RNA was significantly decreased after overexpression of miR-196 and miR-199a were reported to have direct interaction between miR-130a and HCV and miR-130a as the potential target for HCV treatment. Although all these miRNAs are promising targets for HCV treatments, further studies are needed to uncover their cellular targets and their mechanism of action.

Future Directions

The advent of anti-miR-122 drugs is opening an exciting new era for hepatitis C treatment although more comprehensive and long-time effects should be pursued. Many other miRNAs have been reported to regulate HCV replication and they have great potential as therapeutic targets for HCV infection.

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