The role of microRNAs in modulating host innate immunity and HCV infection: implications for HCV pathogenesis and treatment

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Hepatitis C virus (HCV) infection is a major public health problem and there is no effective prophylactic vaccine available at present. The innate immunity is the first line of defense to defend pathogen infection, which is characterized by the induction of type I and III interferons (IFNs), interferon-stimulated genes (ISGs) and pro-inflammatory cytokines to collectively inhibit viral replication and to mediate the adaptive immune response. Meanwhile, HCV has developed multiple strategies to evade and subvert host immunity, thus favoring persistent infection. The close interaction between HCV and the host immune system has been widely reported though the exact mechanisms still need to be unveiled.

MicroRNAs (miRNAs), ～22 nucleotides in length, is a class of short non-coding RNAs involved in eukaryotic post-transcriptional gene regulation. In the past decade the role of miRNAs in HCV infection and host immune responses has become increasingly appreciated. It has been shown that various miRNAs regulate HCV replication and infection by interacting with HCV genome directly or by regulating the host innate immunity to build a non-specific anti-viral or pro-viral microenvironment. miR-122, a liver-specific miRNA, is well studied for its pro- HCV activity through binding to HCV RNA directly to protect it from being degraded. Interestingly, several other miRNAs including miR-196b, miR-448, miR-199a, and let-7b were reported to be able to bind and degrade the HCV genome. Other miRNAs influence HCV infection indirectly either by influencing the expression of type I interferons through targeting members of Toll-like receptor(TLR) signaling pathway or Retinoic acid-inducible gene 1(RIG-I) pathway or by regulating the Jak-stat signaling pathway or by some unknown mechanisms. Understanding the role of miRNAs in immune regulatory network and HCV infection will provide potential drug targets employing various miRNA-specific anti-viral strategies.

Keywords: miRNA; innate immunity; HCV infection

1. MicroRNAs: Biogenesis and Function

MicroRNAs (MiRNAs) are a class of single-stranded small non-coding RNAs, which contain about 22 nucleotides and contribute to regulate target gene expression at posttranscriptional level in order to play critical roles in many biological processes(1-3). Mature miRNAs are processed from much larger primary transcripts (known as pri-miRNAs), which are encoded by the genomic DNA and transcribed by RNA polymerase II in most cases (2, 4). The pri-miRNAs contain 5′ cap and 3′ poly(A) tails as well as hairpin structures, which are cleaved by the nuclear RNase III Drosha (also known as ribonuclease 3) and its binding partner DiGeorge syndrome critical region gene 8 (DGCR8) to form precursor miRNAs (pre-miRNAs) (2, 3). Pre-miRNAs are then transferred from the nucleus to the cytoplasm by a nuclear transport receptor exportin-5(4). In the cytoplasm, pre-miRNAs (~70 nucleotides) are further processed into ~22-nucleotide miRNA/miRNA* duplexes by the cytoplasmic RNase III Dicer (5, 6). After processing, one strand of the duplex (the guide strand, also represents a mature miRNA) is preferentially incorporated into a miRNA-induced silencing complex (miRISC), whereas the other strand (passenger or miRNA*) is released and degraded(7). The thermodynamic stability of the miRNA duplex ends determines which strand is preferentially selected (7). The miRNA binds to its target mRNA leading to inhibition of translation (partially complementary) and/or a decrease in mRNA stability (complete paring) (4, 6, 8). The interplay between miRNAs and their regulating target genes is very complicated as each miRNA may regulate about 400 mRNAs of target genes and each mRNA is likely to be regulated by several miRNAs in mammalian(9). miRNA has been reported to be involved in almost every biological process, and their roles in HCV infection and host immune responses have become increasingly appreciated in the last decade (10-13).

2. MicroRNAs and HCV infection

Hepatitis C virus (HCV) is a hepatotropic, single-stranded positive RNA virus which contains a genome of about 9.6kb (14, 15). It has been shown that various miRNAs regulate HCV replication and infection by interacting with HCV genome directly or by regulating the host innate immunity to build a non-specific anti-viral or pro-viral microenvironment. In addition to binding with HCV RNA directly such as miR-122, most miRNAs influence HCV infection through regulating host immune processes (16).

The host innate immune response which is the first-line of defense against virus infection is characterized by the induction of type I interferons(IFNs), activation of natural killer (NK) cells and production of pro-inflammatory

cytokines, to restrict viral replication and modulate the adaptive immune responses(17). In this chapter, we focus on the miRNAs that are relevant to the type I IFN pathway and HCV infection.

2.1 MicroRNAs involved in type I interferon production and HCV infection

Virus-host interaction has been an essential process in the pathogenesis of many viral infections. Upon virus infections, host cells are equipped with a series of mechanisms to initiate innate immune response to limit the spread of virus. In the meantime, many viruses are able to develop smart strategies to evade the host immune attack. Viral pathogen-associated molecular patterns (PAMPs) are sensed by two classes of cellular receptors: the membrane associated toll-like receptors (TLRs) and the cytosolic resident RNA helicases retinoic acid inducible gene-I (RIG-I) , leading to the activation of the transcription factors NF-κB, IRF3 and IRF7 and induction of type I IFNs (18, 19). To date, 10 functional TLRs (TLR1-10) have been identified in human and 6 have been reported to be involved in HCV infection(20). HCV dsRNAs, an intermediate formed during replication, bind to TLR3 and recruit its adaptor protein TRIF (TIR domain containing adaptor protein-inducing interferon β), which subsequently recruit TRAF6, TRAF3, and TBK-1 and phosphorylates IRF3 (21-24). Except for TLR3, all the other TLRs share a common adaptor molecule, myeloid differentiation factor 88 (MyD88), to trigger inflammatory responses (20, 25, 26). RIG-I senses viral dsRNAs and interacts with mitochondrial antiviral signaling protein (MAVS) on mitochondrial membrane that activates downstream non-canonical kinases of the IB kinase complex, TBK-1 (TANK-binding kinase 1) and IB kinase, to phosphorylate IRF3(27, 28).

miR-146a was the first miRNA identified as a negative regulator of immune system. miR-146a is induced by NF-κB-dependent TLR pathway and inhibits NF-κB activity through targeting IRAK1 and TRAF6(28). In addition to repress type I IFNs production, Tang et al. demonstrated that miR-146a also targets IFN regulatory factor 5 (IRF-5) and STAT-1, thereby attenuating the type I IFN signaling (29). A cell-based assay showed that HCV infection repressed miR-146a expression (30), but whether the regulation of miR-146a on IFN signaling will eventually influence HCV infection is still unknown. miR-155 is also TLR signal-induced and is involved in a negative feedback loop of type I IFN signaling through down-regulation of downstream components in the pathway. One study showed that miR-155 targets MyD88 directly and as such plays an important role in TLR4-induced IFN pathway (31). Moreover, through targeting suppressor of cytokine signaling 1 (SOCS1), a classical negative regulator of type I IFN signaling, miR-155 enhanced type I IFN-mediated anti-VSV effect (32). HCV infection up-regulated miR-155 expression in vivo and vitro (33) though the effect of miR-155 on HCV replication remains further investigation. miR-132 regulates innate antiviral immunity by inhibiting expression of the p300 transcriptional co-activator(34).

Overexpression of miR-130a in Huh7.5.1 cells was found to inhibit HCV replication and to stimulate the expression of type I interferons (IFNα and IFNβ) significantly(35). Considering Huh7.5.1 cell is deficient in both TLR3 and RIG-I, these results imply that miR-130a may inhibit HCV replication probably by restoring host innate immune response, though the detailed target genes and mechanism need to be further identified.

There are limited reports about miRNA regulation of HCV production through impacting type I IFN production which is partially because IFN induction/production is deficient in most of the current HCV cell culture system. It is necessary to identify other HCV permissive cell lines with intact innate antiviral defense system to explore the comprehensive mechanism on how miRNAs regulate HCV infection in collaboration with host IFN signaling pathway.

2.2 MicroRNAs influence HCV infection through Interfering with IFN responses

Type I interferons (IFNα/β) are produced by cells infected with viruses and by key sentinel cells of the innate immune system: macrophages and dendritic cells (DCs). The secreted IFNα/β bind to the IFNα receptor (IFNαR1 and IFNαR2) expressed widely on the cell surface to activate the JAK/STAT pathway. This activates Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2), which then phosphorylate the receptor resulting in the recruitment and phosphorylation of signal transducer and activator of transcription (STAT) proteins. Then phosphorylated STAT1, STAT2 collaborate with IFN-regulatory factor 9 (IRF-9) and form interferon-stimulated gene factor 3 (ISGF3) complex, which is translocate to the nuclear and binds to IFN-stimulated response element (ISRE) sequences of ISGs to activate a few hundred classical antiviral ISG genes (17, 36).

miR-122 is a well-documented miRNA that has a confirmed stimulating role for HCV replication by binding to HCV RNA directly(37). However, the promoting effects of miR-122 on HCV production are far more complicated than this. Yoshikawa et al. found that over-expression of miR-122 suppressed ISRE activity while knockdown enhanced ISRE activity significantly (38). Further investigation showed that silence of miR-122 results in lower expression level of suppressor of cytokine signaling (SOCS3), a negative regulator of IFN signaling, via promoter methylation in mouse liver (38). However, it is interesting that a recent study identified SOCS1 as one of the targets of miR-122, which means higher miR-122 abundance is beneficial to the IFN production against HCV infection due to decreased SOCS1 expression(39). Meanwhile, SOCS1 and SOCS3 were reported to be the targets of miR-221, through which miR-221 boosts IFN's anti-HCV effect (40).

miRNAs can even target ISGs or key components of type I IFN signaling. For example, Chowdhury et al. demonstrated that miR-130a stimulates HCV replication through targeting an anti-viral interferon stimulatory gene

IFITM1 (interferon induced transmembrane protein 1) (41). miR-942 was implicated to regulate HCV-induced apoptosis of human hepatocytes by targeting ISG12a(42). Most recently, miR-373 was identified as a regulator of type I pathway through targeting JAK1 and IRF9 (43).

2.3 MicroRNAs influence HCV infection directly or IFN-independent

As HCV is a RNA virus, some miRNAs can bind to HCV genome through base-pairing. miR-122 is the most abundant liver-specific miRNA, accounting for around 70% of the total miRNA content in mammalian liver tissue(44). The interaction between miR-122 and HCV genome and the requirement of miR-122 for HCV replication were first discovered by Jopling et al. in 2005 and then confirmed by several studies(37, 45-47). miR-122 binds HCV RNA in association with Ago2 mediating the stability of HCV RNA and protecting the viral genome from cleavage by 5′ exonuclease activity of the host mRNA decay machinery(48). miR-141, which can be induced by HCV infection, may also be necessary for efficient HCV replication(49). miR-196 is able to inhibit HCV replicon expression in cell lines, both directly by targeting the HCV RNA and indirectly by targeting Bach1(a basic leucine zipper), which finally increase the expression of anti-oxidant and anti-inflammatory molecule (50, 51). After the inhibitory effect of let-7b on HCV replication through targeting its genome was reported(52), Cheng et al. found that all the let-7 family miRNAs have anti-HCV effect and all the members share the same target: insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1)(53). Over-expression miR-199a has been reported to suppress HCV RNA replication, while inhibition of miR-199a by a specific antisense oligonucleotide upregulated viral replication in two cell lines bearing the replicons HCV-1b or -2a (54). However, a most recent research showed that miR-199a-5p enhanced HCV replication through regulating pro-survival pathways (55). Some known miRNAs involved in HCV infection and their interaction with the innate immune system are summarized in Table 1.

3. Prospective

The importance of miRNAs in regulating host innate immunity to facilitate or to inhibit virus infection has become increasingly clear in recent years. Although the registration of direct acting antivirals (DAAs) demonstrated promising results with more than 90% success rate in clearing the HCV RNA in patients, the long-term consequences remain to be evaluated (16). In addition, the high cost of DAAs makes it difficult to use in many developing countries, where HCV infection is more serious. Therefore the IFN-based therapy will still be critical to HCV infection for some time to come (38). More speculatively, the first miRNA-based anti-HCV drug- Miravirsen, a locked nucleic acid modified antisense oligonucleotide for miR-122, showed long lasting antiviral activity in treatment of chronic HCV genotype 1 infected patients in Phase IIa clinical trials(56). Taken together, understanding the role of miRNAs in immune regulatory network and HCV infection will provide potential drug targets employing various miRNA-specific anti-viral strategies.

Table 1 miRNAs relevant to HCV infection and innate immune response.

microRNA	Targets	Function	Impact on HCV
miR-146a	IRAK1 and TRAF6	Reduce NF-KB activity	No report(28)
	IRF-5and STAT-1	Attenuate I-IFN signaling pathway	No report(29)
miR-155	MyD88	Regulate TLR4 pathway	No report(31)
	SOCS1	Enhance type I IFN pathway	No report(32)
miR-132	Co-activator p300	Impair IFNβ expression	No report(34)
miR-130a	IFITM1	Repress anti-viral protein	Promote HCV replication(41)
	unknown	Restore IFN production	Inhibit HCV replication(35)
miR-373	JAK1 and IRF9	Regulate type I IFN pathway	promotes HCV replication(43)
miR-122	HCV RNA	Stabilize HCV genome	Promote HCV replication(37, 45-47)
	SOCS3 promoter methylation	suppressed ISRE activity	Contribute to HCV replication(38)
	SOCS1	beneficial to the IFN production	Control HCV infection(39)
miR-221	SOCS1 and SOCS3	beneficial to the IFN production	Enhance IFN's anti-HCV effect(40)
miR-942	ISG12a	regulate HCV-induced apoptosis	No report(42)
miR-141	DLC-1		Promote HCV replication(49)
miR-196	Bach1	Increase anti-inflammatory activities	Inhibit HCV replication (50)
	HCV genome		Inhibit HCV replication (51)
miR-448	HCV genome		Inhibit HCV replication (50)
miR-199a	HCV genome		Inhibit HCV replication(54)
miR-199a-5p	————	Activate pro-survival pathways	Promote HCV replication(55)
Let-7b	HCV genome		Inhibit HCV replication(52)
Let-7s	IGF2BP1		Inhibit HCV replication(53)

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