MicroRNAs in Hepatitis C infection

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Résumé

Les microRNAs sont actuellement un vaste champ d'investigation, ce qui est sans surprise suite à l'intérêt croissant que ces derniers ont généré depuis leur découverte en 1993. Les microRNAs se sont montrés être impliqués dans un grand nombre de mécanismes physiologiques, et plus récemment dans certaines pathologies virales. Dans le cadre de l'infection par le virus de l'hépatite C (VHC), le rôle des microRNAs est particulièrement intéressant puisque le virus est capable de modifier le profil d'expression des microRNAs de l'hôte afin d'établir un environnement de réplication plus favorable. Voyant leur expression profondément modifiée par le VHCV, les microRNAs contribuent également potentiellement au développement de différents symptômes pathologiques liés à l'infection par le VHC. Les microRNAs de l'hôte peuvent cependant avoir un effet négatif sur le virus, antagonisant différentes étapes de son cycle de réplication. Les mécanismes sous-jacents à une telle inter-régulation n'ont été que partiellement dévoilés jusqu'à présent, les protéines virales ainsi que le génome du HCV jouant un rôle clé. D'un point de vue thérapeutique, il est maintenant envisagé d'utiliser les microRNAs circulant comme biomarqueurs permettant un suivi de l'évolution de la pathogénèse liée à l'infection par le VHC, ou comme une base pour le développement de nouvelles thérapies. Cette monographie a pour but de mettre en avant les connaissances actuelles dans ce domaine, discutant les différents aspects de la relation VHC-microRNAs de l'hôte et présentant quelques thérapies innovantes.

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0. Abbreviations (alphabetic ordered):

a.a. : amino acids

AAV : Adeno-Associated Virus Vector

ANGPTL3 : Angiopoietin-like protein 3

AGO : Argonaut Proteins family

ARF : Alternative Reading Frame

CHC : Chronic Hepatitis C

DGCR8 : DiGeorge Syndrom Critical Region 8

ER : Endoplasmic Reticulum

GAGs : Glycosaminoglycans

HCC : Hepatocellular carcinoma

HCV : Hepatitis C Virus

Huh7 cells : human hepatoma cells

IFN : Interferon

IL6 : Interleukin-6 (signaling cascade)

JFH-1 : Japanese Fulminant Hepatitis isolate-1

LNA : Locked Nucleic Acid

miRNA : Micro-RNA

mRNA : messenger RNA

ORF : Open Reading Frame

PTEN : Phosphatase and Tensin Homologue deleted on chromosome 10

RdRp : RNA-dependant RNA polymerase

RISC : RNA Induced Silencing Complex

RNAi : RNA interference

SLs : Stem-Loops

UTRs : Untranslated Regions

1. Introduction

Discovered two decades ago (Lee et al., 1993), microRNAs have since became a vast and important field of research and have shown to be involved in a lot of different virus-related diseases, among which hepatitis C virus infection (Gottwein and Cullen, 2008; Liu et al., 2010). It is now well-known that HCV is able to take control over various host factors such as miRNAs in order to increase its fitness (Bhanja Chowdhury et al., 2012). By inducing profound changes in its replicating environment through host miRNAs modulation, it is not surprising that this aberrant regulation of host miRNAs could be linked to different HCV-related pathogenesis. How HCV regulates host miRNAs and *vice et versa* is still under investigation, but few clues have emerged recently showing both direct implication of HCV RNA genome and indirect regulation by HCV proteins. The purpose of this work is to explore and bring a global review of the complex interplay between HCV and host miRNAs and the possible repercussions on host physiology and HCV lifecycle.

1.1 The Hepatitis C Virus

a. History of research

The history of hepatitis C Virus (HCV) began in 1975 with the discovery of a viral agent causing a transfusion associated hepatitis, which was neither type A nor type B (Feinstone et al., 1975). Thereafter, it was only in 1989 that the Michael Houghton's group succeeded in cloning and in identifying the genome of HCV by screening a cDNA library with serum from a patient suffering from nonA-nonB hepatitis (Choo et al., 1989). This crucial step allowed the classification of HCV among the members of the *flaviviridae* family as a *hepacivirus*, as well as its characterization at the molecular level. Despite the fact that it was not possible for many years to study the virus *in vitro* using infectious cell culture models, the recent advances over the past 20 years have enabled to strengthen through experimental systems (as replication assays) our understanding of the lifecycle, the virology and the biochemical features of HCV (Lindenbach et al., 2005; Zhong et al., 2005).

b. Virion structure

According to filtration and electron microscopy experiments, HCV viral particles would measure 40-80 nm in diameter (Wakita et al., 2005). HCV genomic RNA, envelope proteins E1,E2 and the core protein are the main components of the HCV viral particles (Bartenschlager et al., 2011). An icosaheadral nucleocapsid, which encapsidates the viral RNA genome (Bartenschlager et al., 2011; Penin et al., 2004) is surrounded by an host cell-derived double-layer lipid envelope in which the E1 and E2 glycoproteins are anchored (Bartenschlager et al., 2011). It has been shown that HCV viral particles circulate in the serum of the infected host under various architecture, so that the virions can be associated with a wide variety of components such as lipids (cholesterol, triglycerides, phospholipids), lipoproteins (apoE, apoB, APOC1, apoC2 and APOC3) and immunoglobulins (Andre et al., 2002; Nielsen et al., 2008; Penin et al., 2004). Generally, it is believed that circulating virions associated with very-low-density lipoproteins and low-density lipoproteins correspond to the infectious fraction, however, as mentioned above, were also observed circulating virions bound to Igs and freevirions (Andre et al., 2002). Circulating virions with non-enveloped HCV nucleocapside properties have also been identified in plasma (Maillard et al., 2001).

c. Genome variability

As mentioned previously, HCV belongs to the *hepacivirus* genus in the *flaviviridae* family, therefore, the viral genome is a positive-polarity single-strand RNA, that is characteristic of this type of virus (Penin et al., 2004). Due to the high rate of replication (production and clearance of 10 ^ 12 virions / day in a given individual) and lack of proof-reading function of the viral NS5B RNA dependent RNA polymerase (RdRp), HCV genome exhibits a high genetic variability (Moradpour et al., 2007). The term "quasispecies" is used to express the HCV genomic variability within a single infected host (Simmonds et al., 2005). Moreover, HCV isolates are generally classified by genotypes and subtypes. Thus, there are seven distinct genotypes (numbered 1-7) varying from 31% to 33% in nucleotide sequences, within which there are subtypes (designated a, b, c, and so on) varying from 20% to 25% (Simmonds et al., 2005). Although HCV shows remarkable sequence variability, the genome contains many relatively invariant regions in sequence and size between different genotypes, subtypes or quasispecies. These invariant segments often correspond to regions conferring a significant survival benefit or occupying a vital function for the life cycle of the virus (Moradpour et al., 2007; Tang and Grisé, 2009).

d. Genome structure

The HCV genome is 9.6 kb long, and consists of a single continuous open reading frame (ORF) encoding a 3000 amino-acid (a.a.) polyprotein, surrounded by 3' and 5' untranslated regions (UTRs) containing structural cis-regulation elements essential for RNA replication and translation (Moradpour et al., 2007; Tang and Grisé, 2009). The 5'UTR, which is 341 nucleotides in length, is involves 6 secondary structure domains (numbered I to VI) containing highly structured RNA elements named stemloops (SLs) (Brown et al., 1992).The SLII, III, IV constitute the internal ribosome entry site (IRES) needed for initiation of cap-less translation of HCV RNA genome, which requires both viral and cellular proteins such as eukaryotic initiation factor (eIF) to finally results in the production of a single viral polyprotein (Kim and Chang, 2013). It has been shown that the 5'UTR also possess some key sequences required for the RNA replication (Friebe et al., 2001). Recently, two tandem binding sites for a specific liver-abundant microRNA (miRNA), miR-122, were discovered downstream the SL1 in the 5'UTR, and found to regulate the viral replication and translation in presence of miR-122 (Henke et al., 2008; Jopling et al., 2005). *(This point will be discussed later on, see 2.3.a).* The 3'UTR consists of three distinct regions, a short one of about 40 nucleotides that vary between genotypes, a poly (U / UC) tract of variable length and finally an invariant region of 98 nucleotides called the "X-tail" containing SLs structures (Kolykhalov et al., 1996; Tanaka et al., 1996). The conserved 3'-UTR sequences and structures, such as the X-tail carrying SLs, or also a minimal poly-U tract of 26 nucleotides are necessary for viral replication in cell culture (Friebe and Bartenschlager, 2002; Yi and Lemon, 2003) and in vivo (Yanagi et al., 1999). In addition, an essential cis-acting replication element (CRE) called 5B-SL3 has been found in the coding ORF, precisely in the NS5B RdRp C-terminal sequence, including stem-and-loop structures (You et al., 2004). Apparently, a structure that is thought to be essential for replication named « kissing-loop » is formed at the 3'-end of the viral genome by the interaction between one of the 5B-SLs, 5B-SL3.2, with another SLs located in the X'-tail, the 3'-SLII (Diviney et al., 2008; Friebe et al., 2005; You et al., 2004). Moreover, the 5B-SL3.2 can interact with another upstream RNA sequence in the NS5B to form another complex structure called a « pseudoknot » also involved in replication (Diviney et al., 2008).

e. HCV proteins

As we said before, the HCV genome contains an ORF of 9024 to 9111 nucleotides, coding for a 3000 a.a. polyprotein, which is post- and co-translationally processed by both cellular and viral proteases to generate 10 proteins divided into 2 categories : structural proteins and non-structural proteins (Kim and Chang, 2013). The processing of non-structural proteins is carried out by 2 viral proteases, the NS2-3 and the NS3-4A serine proteases, while structural proteins and p7 are cleaved by the endoplasmic reticulum (ER) signal peptidase (Moradpour et al., 2007). The structural proteins corresponding to the N-terminus of the polyproteins which include Core,

E1 and E2 proteins, are separated by cleavage signal peptides (situated between Core|E1, E1|E2, E2|P7, and P7|NS2) targeted by the ER signal peptidase (Tang and Grisé, 2009). In addition, another cleavage of core protein is performed by a signal peptide peptidase, which produce a 21 kd mature protein apparently necessary for viral particle assembly (Ait-Goughoulte et al., 2006).

Structural and non-structural proteins have various activities within the viral life cycle, and these will be briefly summarized here [reviewed in (Moradpour et al., 2007; Tang and Grisé, 2009)]. Core (21-23 kd) as nucleocapside protein has a lot of functionalities important for the assembly of viral particles, including RNA binding, homo-oligomerization and association with lipid droplets. E1 (31-35 kd) and E2 (70 kd) are highly glycosylated type I transmembrane proteins forming a heterodimer, which is the main component of the viral envelope. Thus, such as Core, E1 and E2 are key actors for viral particle formation. Moreover, E1 and E2 as viral surface proteins are involved in HCV entry into target cell by specific receptor binding (Tang and Grisé, 2009). P7 (7 kd) a hydrophobic polypeptide with two transmembrane (TM) domains, has been shown to form hydrophobic pores by oligomerization in the ER membrane, which have an ion channel activity (Griffin et al., 2003) . P7 is essential for viral assembly and release (Steinmann et al., 2007). The non-structural proteins are principally involved in viral replication and polyprotein processing. NS2 (21-23 kd) contains a C-terminal protease domain forming an active protease with the N-terminal protease domain of NS3 (69 kd) dedicated only to cleave the NS2-NS3 junction (Tang and Grisé, 2009). NS3 N-terminal serine-protease domain in association with its non-covalent NS4A co-factor is then responsible for cleavage of the remaining NS junctions (NS3/ NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B). In addition, the NS3-NS4 serine protease can cleave different host proteins such as Toll/interleukin-1 receptor domain-containing adaptor protein inducing IFN-β (TIRF), mitochondrial antiviral-signalling protein (MAVS), CARD adapter inducing interferon beta (CARDIF) and virus-induced-signaling adapter (VISA), reducing the host cell antiviral response (Tang and Grisé, 2009). On the other hand, the C-terminal part of NS3 has helicase and NTPase activities, fundamental for viral assembly (Ma et al., 2008) and also probably required for RNA replication. NS4A (6 kd) protein is as mentioned above, an important co-factor of NS3 serine protease (Tang and Grisé, 2009). NS4B (27 kd) is an integral membrane protein, and its best-known function is to induce the formation of specific membrane structure named “membranous web”, which is the site where the replication complex assemble to perform RNA replication cycles (Egger et al., 2002). NS5A can be found in two forms, basally phosphorylated (56 kd) or hyperphosphorylated (58 kd). This phosphorylation is conserved in *flaviviridae* and *hepaciviruses*, suggesting that it is important for HCV lifecycle (Reed et al., 1998). Furthermore, some studies provided some evidences that NS5A phosphorylation is important for RNA replication (Appel et al., 2005). NS5A appear to be involved in viral assembly as well (Tellinghuisen et al., 2008). NS5B (68 kd) is the RdRp, the main enzyme of the RNA replication process. Using genomic positive single strand RNA, the RdRp first synthesises complementary negative strand RNA as an intermediate for genome replication, and then proceeds to produce positive single strand genomic RNA using the negative strand as a template (Moradpour et al., 2007). NS5B contains some domains shared by the majority of RdRps, such as the thumb, fingers (forming the active site) and palm domains, present in typical right-hand RdRp shape (Bressanelli et al., 1999). NS5B also shows some other interesting features as the ability to bind membrane with its C-terminal TM domain, which seems to be critical for RNA replication (Moradpour et al., 2004), the presence of a GTP-binding site, a β-hairpin loop, and the capacity to homo-oligomerise (Tang and Grisé, 2009; Wang et al., 2002). HCV also possesses an alternative reading frame (ARF) overlapping the core gene, which is generated by ribosome phase shifting. The expression of this ARF results in production of the so-called F protein, which is a core-related protein of unknown functions (Branch et al., 2005).

f. HCV Lifecycle

Our understanding of the virus lifecycle has increased significantly, considering recent development of an efficient HCV culture system (using Huh-7.5 cell line) established in 2005 and based on an unique isolate from a Japanese patient suffering of a fulminant hepatitis (JFH-1). This culture system allowed the *in vitro* study of the complete viral lifecycle for the first time, accelerating our understanding of entry, replication, assembly and egress steps (Lindenbach et al., 2005; Wakita et al., 2005; Zhong et al., 2005). Here is a brief summary of the HCV lifecycle. To enter into hepatocytes which are the main site of viral replication, HCV uses the receptor-mediated endocytosis (Burlone and Budkowska, 2009). Six cell-surface molecules are implicated in the internalization of HCV by this multi-step process. First, glycosaminoglycans (GAGs) and low-density lipoprotein receptor (LDLR) are required as attachment factors to establish an initial virus-host cell interaction (Burlone and Budkowska, 2009). Then, Tetraspanin CD81 cell-receptor and human Scavenger receptor class B site I (SR-BI) form a complex which allows the transfer of HCV to the tight junctions, where junction molecules Claudin-1 (CLDN1) and Occludin (OCLN) trigger the entry of HCV by Clathrin-mediated endocytosis (Blanchard et al., 2006; Evans et al., 2007; Liu et al., 2009). Recently, it has also been shown that the epidermal growth factor receptor (EGFR) and Ephrin receptor A2 (EphA2) play a role as host co-factors for viral entry (Lupberger et al., 2011). Following the internalization, low-pH in endosomes induces uncoating of the HCV particles by a pH-dependent fusion process (viral envelope/endosome membrane), leading to the release of the HCV genomic RNA into the cytosol (Burlone and Budkowska, 2009). The HCV genomic RNA is then used for replication or translated at the rough endoplasmic reticulum (rER) to produce the polyprotein. Afterwards, the polyprotein is processed co- or post-translationally to yield viral mature proteins (Bartenschlager et al., 2011). The amplification of viral genomic RNA takes place into the membranous web, where generated copies will be used for replication, polyprotein translation, or encapsidation (Bartenschlager et al., 2011). Assembly of new virions occurs at the surface of lipid droplets, partially due to specific interactions between viral proteins and lipids (Miyanari et al., 2007). Newly produced virions bud into the ER lumen, and are then released outside the host cell along the VLDL secretion pathway, completing the lifecycle (Huang et al., 2007). (*Figure 1.)*

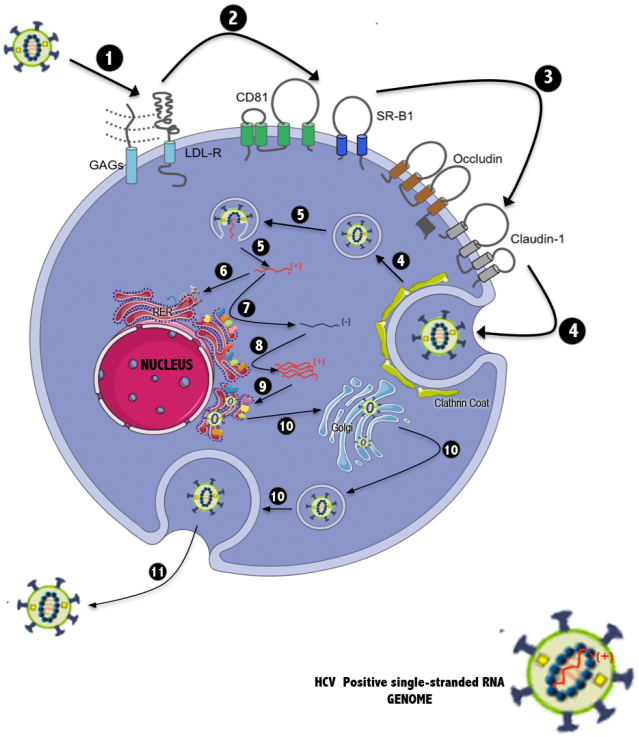

Figure 1. HCV Life Cycle

1) Interaction of HCV with glycosaminoglycans (GAGs) and LDL-receptor (LDLR).
2 + 3) Formation of a complex between Tetraspanin receptor (CD81) and Scavenger-Receptor class B site 1 SR-B1, allowing the transfer of HCV to the tight junctions.
4) Clathrin-mediated endocytosis of HCV triggered by tight junction proteins Claudin-1 (CLDN1) and Occludin (OCLN).
5) Uncoating pH-dependent (fusion Viral envelop-endosome) and release of HCV RNA (+) in cytosol.
6) Translation of HCV RNA at the ER surface and production of a polyprotein.
7) Polyprotein processing leading to 7 mature viral proteins, which are required for HCV RNA replication at the membraneous web.
8+9) Amplification of HCV RNA followed by viral particles formation at lipid-droplets surface and budding into ER lumen.
10) Secretion of HCV particles through the VLDL pathway.
11) Egress of HCV particles.

g. HCV Hepatopathogenesis

Approximately 3% of the population is infected by HCV worldwide, which is responsible of chronic and acute liver diseases (Ke and Chen, 2012). During the first 6 months after infection, (acute phase) only a minority (5-20 %) of patients spontaneously eliminate the virus, whereas the majority (80-95%) become chronically infected by HCV and develop chronic hepatitis C (Tang and Grisé, 2009; Zoulim et al., 2003). In major cases, chronic hepatitis gradually turns to various liver affections such as hepatosteatosis (lipid accumulation), liver fibrosis, cirrhosis and at last hepatocellular carcinoma (HCC) (in 3-5% of infected people) (Zoulim et al., 2003). These HCV-associated liver pathologies might be the long-term consequences of various cellular responses triggered by the viral infection. Recent studies provide results indicating that HCV infection is able to activate diverse cellular responses such as ER stress/unfolded protein response (UPR), autophagy, apoptosis, and cell cycle arrest (Ke and Chen, 2012).There are now evidence showing that these HCV-triggered responses could alter the cell metabolism by changing lipid metabolism, interfering with cell growth and/or proliferation, or activating oncogenic pathways (Ke and Chen, 2012). Thus, these responses coupled with other factors could lead to various HCV-associated hepatopathologies. Investigations are currently focusing on direct effects of HCV proteins on chronic hepatitis C progression, which could be liver steatosis development, increase of oxidative stress, cirrhosis and fibrosis development, and finally facilitating HCC carcinogenesis (Tang and Grisé, 2009).

1.2 MicroARNs

a. History

MicroRNAs (miRNAs) are a family of small non-coding transcripts involved in translational and post-translational sequence-specific regulation of gene expression in plants and animals. MiRNAs were first discovered in 1993, when Lee *et al.* (1993) noticed that an essential gene for temporal regulation of C. elegans larval development does not encode proteins, but rather produces two small RNAs transcripts. However, miRNAs were not immediately recognized as regulators of gene expression, and it was only recently that research turned to this field, especially thanks to the team of C.C. Mello who was the first to introduce and demonstrate RNA interference (RNAi) in 1998 (Fire et al., 1998). Thereafter, miRNAs have been found to have a lot of negative effects on gene expression, but also potential positive effects.
Until today the number of known human miRNAs has increased drastically (more than 1500, according to http://www.mirbase.org/), and *in silico* studies suggest that an important amount (60%) of mammalian protein-encoding genes are subject to regulation by these miRNAs (Friedman et al., 2009). Furthermore, several studies have highlighted the fact that a single miRNA is able to target hundreds of distinct mRNAs and that one single mRNA can reciprocally be targeted by numerous miRNAs (Selbach et al., 2008). All of these interactions result in complex regulatory networks, which are likely to be essential for most developmental and physiological processes (He and Hannon, 2004). By the same token, investigations on the role of miRNAs in pathologies and possible therapies emerged in the last few years.

b. Biogenesis

As previously said, miRNAs are small single-stranded non-coding RNA of about 18-22 nucleotides playing a role in regulation of gene expression. MiRNAs biogenesis is carried out in a series of sequential stages, beginning in the nucleus with the transcription of miR genes by specific polymerases, the RNA polymerase III and II (Winter et al., 2009). This step leads to production of primary-microRNAs (pri-miRNAs), further processed in the nucleus by the Drosha (Rnase III)-DGCR8 microprocessor complex and resulting in hairpin precursor-miRNAs (pre-miRNAs) of about 70 nucleotides (He and Hannon, 2004; Winter et al., 2009). The pre-miRNAs are then exported into the cytoplasm by the Exportin-5-Ran-GTP nucleo-cytoplasmic shuttle, where the hairpin is cleaved by the RNase III enzyme Dicer (part of the RNA induced silencing complex (RISC) loading complex) to produce mature miRNAs duplex of about 22 nucleotides (Winter et al., 2009). The duplex is later dissociated, and even though both strands of the duplex are able to be chosen to be part of the RISC, only one of the two strands, the functional guide strand, is generally incorporated therein, while the other, the passenger strand, is degraded (Filipowicz et al., 2008; Schwarz et al., 2003; Winter et al., 2009). In addition to the incorporation of the functional guide strand of the miRNA, Argonaute proteins (AGO) are assembled into RISC as the main key effector proteins (Filipowicz et al., 2008; Liu et al., 2004; Winter et al., 2009). When the RISC is fully assembled, the functional strand of the miRNA is used to recognize target mRNAs, which are then silenced by RISC through mRNA degradation, destabilisation or translational repression (Bartel, 2009; Winter et al., 2009). Here we have succinctly described the linear canonical pathway of miRNA biogenesis, which has been seen for a long time as linear and universal for all mammalian miRNAs. However, it has recently been shown that there are a lot of different specificities related to different miRNAs [reviewed in (Winter et al., 2009)].

c. Gene targeting and regulation of transcription

Generally, base-pairing interactions between the “seed” region, a specific 2-8 nucleotides sequence located in the 5’ end of miRNAs, and some sequences elements in the 3’UTR of target mRNAs, are required to process the post-transcriptional repression (Bartel, 2009; Filipowicz et al., 2008). Possible base-pairing within 5’UTR or coding region of mRNA is also possible

according to some studies, but will not be discussed here (Filipowicz et al., 2008). Uncommonly, when the base-pairing complementarity between both miRNA and mRNA sequences is almost perfect, the target mRNA can undergo a cleavage directed by ago proteins, leading to direct mRNA degradation similar to the RNAi pathway (Hutvágner and Zamore, 2002; Yekta et al., 2004). However, more frequently, miRNAs proceed to target gene silencing by mRNA destabilisation or translational repression (Filipowicz et al., 2008). These processes generally involve various mechanisms, such as inducing decapping and degradation of target mRNAs by deadenylation, blocking the translation initiation at the cap recognition step or at the 60S joining, post-initiation slowing of translation or ribosome "drop-off", and finally a proteolytic cleavage of nascent peptides has also been suggested (Eulalio et al., 2008; Filipowicz et al., 2008).

d. Implication in Viral pathogenesis and host defenses

As miRNAs are implicated in many physiological and pathological processes, it is not surprising that these small molecules play an important role in various viral infections. Different options are possible regarding the regulation of viral infection, pathogenesis or immunity by both viral and cellular miRNAs. Since it has been shown that several virus families encode their own miRNAs, it is likely that they regulate host gene expression or eventually viral gene expression to promote/favor viral replication (Gottwein and Cullen, 2008; Kumar, 2011). Otherwise, host miRNAs might benefit the viral replication through their cellular functions, thus to establish a more favorable environment for replication. Viruses may also alter the cellular gene expression profile by modifying negatively or positively the expression of several host miRNAs (Gottwein and Cullen, 2008; Kumar, 2011; Roberts et al., 2011a). Host miRNAs might also interact directly with viral RNAs, resulting in positive or negative effects on viral replication, such as miR-122 an endogenous hepatocyte miRNA which promote replication of HCV (Gottwein and Cullen, 2008; Roberts et al., 2011a). The effect of this particular miRNA will be further discussed in more details. Finally, in part due to contrasting host miRNAs expression between different cellular types, cellular miRNAs are presumably involved in viral tissue tropism *in vivo* (Gottwein and Cullen, 2008).

2. HCV regulation of host cellular MicroARNs

Host miRNAs have been found to be modulated directly or indirectly by HCV. Our goal in this short review is to highlight the relationship between host miRNAs and HCV infection. We will summarize above the different possible ways by which a virus can interact with host miRNAs, with a particular focus on the possible effects of HCV infection on host miRNAs expression and *vice versa*.

2.1 Host miRNAs modulation by HCV infection: Pathogenesis outcome

Interestingly, Liu *et al.* (2010) discovered some significantly correlated modifications in both miRNAs and mRNAs expression profiles in hepatoma cells during HCV infection *in vitro*, revealing a possible implication of miRNA regulatory networks in gene expression response to HCV infection. Among the HCV-regulated miRNAs, many of them are known to be involved in various cellular functions, indicating a possible role in HCV-related pathogenesis or antiviral mechanisms within liver cells (Liu et al., 2010). Here we will summarize some interrelation between various HCV-induced liver disorders and some HCV-regulated miRNAs.

Table 1: miRNAs associated with liver disease progression

miRNA	Regulation by HCV	Target	Function	Reference
miR-24	Down-regulation	TGF-β signaling activated	Promotes liver fibrosis and viral abundance	(Liu et al., 2010)
miR-491, miR-320	Down-regulation	Akt/PI3Kinase pathway	Favors hepatocarcinogenis	(Ishida et al., 2011)
miR-155	Up-regulated	Wnt Pathway (APC, β-catenin, Cyclin D1)	Hepatocyte proliferation, tumorigenesis and inflammation	(Zhang et al., 2012b).
miR-141	Up-regulated	Tumor suppressor DLC-1	Favors early development of hepatocarcinogenesis.	(Banaudha et al., 2011).
miR-449a	Down-regulated	NOTCH1 for translational silencing resulting in Up-regulation of NOTCH1 and NFKB pro-inflammatory pathways (TNFalpha, YKL40, NOTCH1)	Favor liver fibrosis and HCC.	(Sarma et al., 2012)
miR-21	Up-regulated	SMAD7 / TGFβ pathway	Favor fibrogenesis in HCV infected liver.	(Marquez et al., 2010)
miR-27(a&b)	Up-regulated	PPAR-alpha, ANGPTL3	Modulation of triglycerides homeostasis and lipid accumulation, leading to possible induction of steatosis. Possible implication with HCV-triggered IFN response.	(Singaravelu et al., 2014)
miR-107 & miR-449a	Down-regulated	IL6 mediated signaling cascade	HCV induction of inflammatory response and fibrosis.	(Sarma et al., 2014)
miR-152	Down-regulated	Wnt1 pathway	HCV-infected hepatocyte proliferation and HCC.	(Huang et al., 2014)
miR-29	Down-regulated	ECM components	Possibly favors HCC and fibrosis	(Bandyopadhyay et al., 2011)
miR-93 & miR-345	Up-regulated	$p21^{Waf1/Cip1}$ gene expression	Repress $p21^{Waf1/Cip1}$ gene expression thus favoring development of HCC during chronic hepatitis C.	(Shiu et al., 2013)

a. Immune system, inflammation and liver fibrosis

For example, HCV is able to induce a down-regulation of the expression of miR-24, a miRNA responsible for silencing the TGF-β pathway and decreasing the HCV replication, suggesting that in the context of HCV infection, the repression of the TGF-β pathway could be disrupted, contributing to further development of liver fibrosis and increase of viral abundance (Liu et al., 2010). HCV down-regulates also two other important miRNAs, miR-107 and miR-449a, which are normally inhibiting the expression of the inflammatory chemokine CCL2 (Chemokine ligand 2) involved in several liver diseases, by inhibiting the IL6 signaling pathway (Sarma et al., 2014). The regulation of these two miRNAs, upon HCV infection, could lead thus potentially to inflammation and fibrosis (Sarma et al., 2014). Following the same idea, deregulation of miR-449a upon HCV-infection can lead to up-regulation of a pro-inflammatory pathway involving TNFα, YKL40 and NOTCH1*, (*constituents of the NOTCH signaling pathway*)* (Sarma et al., 2012)*.* More precisely, the loss of transcriptional repression of NOTCH1 by miR-449a following its downregulation is responsible for activating the expression of YLK40, a known inflammatory marker of liver fibrosis progression (Pungpapong et al., 2008; Sarma et al., 2012). In addition, HCV proteins have been found to modulate negatively the immune system, using NS5A and NS3/4A complex which can increase expression of miR-21 gene by interacting with PKCε/JNK/c-Jun and PKCα/ERK/c-Fos pathways respectively (Chen et al., 2013). This leads to the formation of the heterodimeric AP-1 transcription factor (formed by c-Jun and c-Fos) activating the miR-21 promoter (Chen et al., 2013). The outcome of miR-21 overexpression is the downregulation of MyD88 (Myeloid differentiation factor 88) and IRAK1 (Interleukine-1 receptor associated kinase 1), two proteins shown to participate in this case to type I IFN-based immune response against HCV (Chen et al., 2013).(*Figure 2.)* (*Table 1.)*

b. Hepatocellular carcinoma

To underline the possible implication of HCV-regulated host miRNAs in development of carcinomas, several studies demonstrated that the expression of a subset of endogenous miRNAs may be differentially expressed during the development of HCV-related HCCs (Ura et al., 2009; Varnholt et al., 2008). Several mechanisms by which the dysregulation of miRNAs may lead to hepatocarcinogenesis have been proposed. The down-regulated miRNAs could be possible tumor suppressor and up-regulated ones might be used as oncogenic deregulators by HCV, possibly to favor the viral process (Varnholt et al., 2008) . For instance, miR-141 has been shown to regulate negatively a potential tumor suppressor DLC-1 (a Rho-GTPase activating protein) at the translational level, suggesting a crucial role in the early development of hepatocarcinogenesis (Banaudha et al., 2011). Through their computational study to identify miRNA-mRNA regulatory modules associated with HCV infection, Peng *et al*. (2009) also reported some miRNAs as negative regulators of some presumed tumor suppressor genes such as Smad4, CD82 and PTEN (Phosphatase and tensin homologue deleted on chromosome 10), and thus again emphasizing the idea that a chronic HCV infection may lead to liver-cancer through modulation by miRNAs. In other cases, more complicated pathways have been suggested. Several differentially expressed host miRNAs in HCV-infected liver are involved in immune response, lipid metabolism, cell-cycle, proteasome, antigen presentation, akt/PI3 kinase pathway showing a profound influence on host physiology through a complex network of pathways (Gottwein and Cullen, 2008; Ishida et al., 2011; Liu et al., 2010; Peng et al., 2009; Ura et al., 2009). A good illustration of the complexity of such processes is the regulation of multiple cellular functions by miR-449a, a down-regulated miRNA found within hepatocytes of patients infected by HCV (Sarma et al., 2012). It appears that miR-449 promotes both cell cycle arrest and apoptosis (Lizé et al., 2010; Noonan et al., 2010), and according to Sarma *et al*. (Sarma et al., 2012) , being down-regulated during HCV infection, it may promote the development of HCC in patients chronically infected. The implication of the Wnt pathway has also emerged recently. Indeed, miR-152, a miRNA which normally repress the Wnt1 pathway (possibly by targeting the 3'-UTR of Wnt1), appears to be downregulated by HCV core protein in HepG2 cells, promoting an abnormal cell proliferation (Huang et al., 2014). In regard to these findings, miR-152 could

act as a tumor-suppressor down-regulated upon HCV infection and participate to HCC development (Huang et al., 2014). On the contrary, miR-155, which is upregulated upon HCV infection, has been shown to be implicated in tumorigenesis and inflammation by promoting the Wnt pathway (Zhang et al., 2012b). Another case where HCV core proteins induce changes in host gene expression using cellular miRNAs, is brought by Shiu *et al*. (2013), in which mature or truncated core proteins transfected in human hepatoma cells subsequently upregulate two miRNAs, miR-93 and miR-345, which repress $p21^{Waf1/Cip1}$ gene expression, which possibly favor HCC development during chronic hepatitis . (*Figure 2.)* (*Table 1.)*

c. Steatosis

Some miRNAs deregulated in the course of HCV infection may also be involved in the mechanisms leading to steatosis. It has been established that genotype 3a (but not 1b) core proteins transfected in hepatoma cells *in vitro* exploit a miRNAs process (still unknown) to post-transcriptionally inhibit the translation of PTEN mRNA, an essential factor found to be down-regulated in steatosis in HCV genotype 3a-infected patients (Clément et al., 2011). Recently, following the same idea, the role of the liver-abundant miR-27 in HCV-related steatosis has been investigated. Indeed, HCV appeared to be able to enhance, in a genotype independent-manner, miR-27 expression both *in vivo* and *in vitro* (Shirasaki et al., 2013; Singaravelu et al., 2014). MiR-27 was found to regulate lipid metabolism by acting on the synthetic lipid transcription factor Retinoid X receptor alpha **(**RXRα), as well as repressing/downregulating a lot of other lipid metabolism-related genes/transcription factors such as ApoE3, ApoB100, ApoA1, lipid transporter ATP-binding cassette subfamily A member 1 (ABAC1), Angiopoietin-like protein 3 (ANGPTL3) or Peroxisome proliferator-activated receptor alpha (PPAR-α) (Shirasaki et al., 2013; Singaravelu et al., 2014). Such a modulation of Lipid-related factors is likely to participate to both lipid accumulation and growth of lipid droplets following up-regulation of miR-27 (Singaravelu et al., 2014). In regard of the interaction between HCV and miR-27, the role of HCV proteins has been investigated/mentioned, and overexpression of Core and NS4B proteins appears to independently up-regulate the expression of miR-27 (Singaravelu et al., 2014).

Thus, by altering the lipid metabolism through miR-27, HCV is likely to induce development of steatosis and favor a chronic infection (Shirasaki et al., 2013; Singaravelu et al., 2014). (*Figure 2.)* (*Table 1.)*

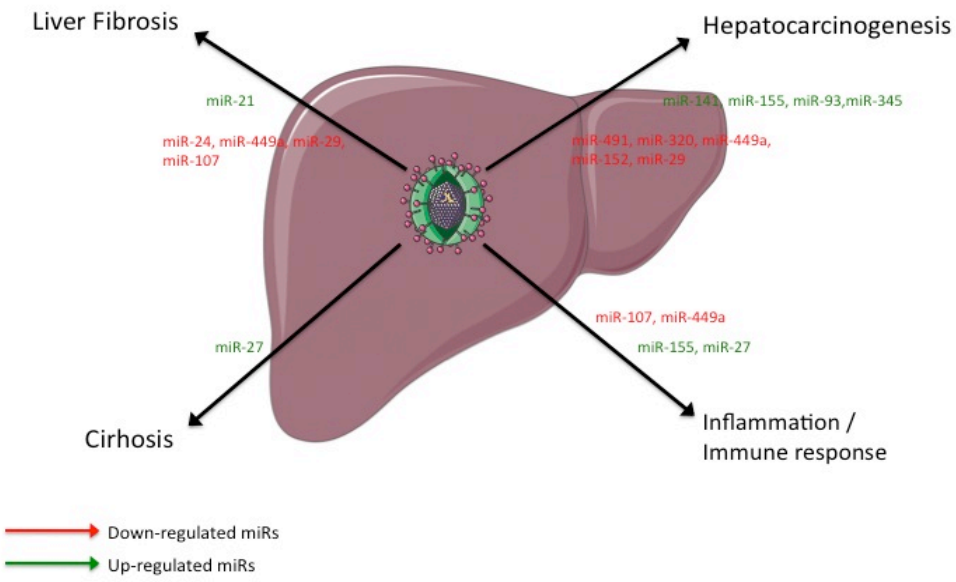

Figure 2. HCV-related miRNAs : pathogenesis outcome

Here are represented the relation between various HCV modulated miRNAs, which favor development of liver diseases upon HCV infection.

2.2 Host miRNAs modulation by HCV infection: effects on HCV Life cycle

In addition of being involved in the development of HCV-related liver diseases, certain miRNAs whose expression is altered in the course of HCV infection may have an impact on the HCV life cycle itself (Ishida et al., 2011). Three miRNAs, miR-192, miR-215 and miR-491, showing an affected expression in Huh-7 hepatoma cells infected by HCV, were found to be regulators of HCV replication (Ishida et al., 2011). After further functional investigations, the group discovered that miR-491 interferes with the PI3k/Akt pathway, implying possibly the suppression of this pathway in the non-genotype specific enhancement of HCV replication (Ishida et al., 2011). It appears that host miRNAs are able to control the virus to a large extent, considering that some miRNAs such as miR-24, miR-149, miR-638 and miR-1181 clearly influence the entry of the virus, its propagation, and finally its genome replication (Liu et al., 2010). In a similar way, miR-141 up-regulation in HCV-infected primary hepatocytes, is believed to increase HCV replication (Banaudha et al., 2011). A further coherent example of how HCV triggers the modulation of the host miRNA profile to establish a favorable environment for its replication is the case of miR-130a, which is up-regulated by HCV *in vivo* and *in vitro* and ostensibly promotes the establishment of a persistent infection (Bhanja Chowdhury et al., 2012). To briefly summarize, miR-130a has been shown to down-regulate Interferon-induced transmembrane protein 1 (IFITM1), a protein, which has been shown to operate against infection by viruses (Bhanja Chowdhury et al., 2012; Wilkins et al., 2013). In the same manner, HCV infection causes the overexpression of miR-21, a host miRNA which down-regulates IFN type I-dependent immune response against HCV, and thus promotes viral spread (Chen et al., 2013). Unsurprisingly, HCV infection seems not only to up-regulate some favorable cellular miRNAs, but also to down-regulate the most prejudicial for its lifecycle. Thus, because it was shown to be one of the downregulated miRNAs in HCV-infected liver cells, miR-29 possessing a putative anti-viral activity is a good example to illustrate this concept as it is repressed by HCV possibly in order to facilitate its replication (Bandyopadhyay et al., 2011). On the contrary, some miRNAs modulated by HCV infection appear to act against viral replication (Yang et al., 2014). For instance, miR-942 is down-regulated by HCV which leads to the induction of apoptosis through ISG12a/TRAIL-mediated pathway, potentially impairing the viral replication (Yang et al., 2014). *(Table 2.)*

Table 2: miRNAs that regulate HCV replication

miR	Regulation by HCV	Target	Function	Reference
miR-130a	Up-regulated	IFITM1, a interferon-stimulated gene	Inhibits INF signaling and promotes HCV replication	(Bhanja Chowdhury et al., 2012; Wilkins et al., 2013)
miR-192, miR-215 and miR-491	Down-regulated	PI3K/Akt pathway	Favor replication of HCV in Huh7 hepatoma cell line.	(Ishida et al., 2011)
miR-24, miR-149, miR-638 and miR-1181	Up-regulated or Down-regulated (see table in Liu et al. 2010)	Different pathways (see table in Liu et al. 2010).	Potentially influence the entry of the virus, its propagation, and finally its genome replication	(Liu et al., 2010)
miR-141	Up-regulated (upon genotype 1a, 1b & 2a transfection)	Tumor suppressor gene DLC-1	Believed to increase HCV replication HCV-infected primary hepatocytes	(Banaudha et al., 2011)
miR-21	Up-regulated	Myeloid differentiation factor 88 (MyD88) and Interleukin-I receptor associated kinase 1(IRAK1) (through PKCε/JNK/c-Jun and PKCα/ERK/c-Fos pathways)	Represses IFN type-I dependent immune response and promotes viral spread.	(Chen et al., 2013).
miR-29	Down-regulated	Not investigated	Putative anti-viral function by inducing a decrease in viral RNA.	(Bandyopadhyay et al., 2011)
miR-27	Up-regulated	PPAR-α, ANGPLT3,	Potential anti-viral effects.	(Shirasaki et al., 2013; Singaravelu et al., 2014)
miR-942	Down-regulated	ISG12a / TRAIL-mediated-pathway	Trigger apoptosis in response to HCV infection	(Yang et al., 2014)

2.3 Direct interactions between HCV genome and miRNAs

Although HCV proteins may have an important role in the intercommunication between HCV and host cells through miRNAs, some studies have also reported a direct interaction between the HCV RNA genome and cellular miRNAs, leading to a variety of different effects, affecting both the viral cycle and the host defenses (Jopling, 2008; Pedersen et al., 2007).

a. Positive regulation of HCV by direct RNA genome binding by miR-122

The most famous liver-abundant miRNA that has been found to interact with HCV RNA genome is miR-122, which is able to bind two tandem sites, S1 (7-nts : ACACUCC) and S2 (6-nts : CACUCC) within the 5'UTR of HCV RNA genome through its complementary seed sequence (Conrad et al., 2013; Jangra et al., 2010; Jopling et al., 2005, 2008). These contiguous binding sites are highly conserved among the 6 HCV genotypes, and the binding of miR-122 was shown to be essential for HCV replication in cell culture systems (Berger and Randall, 2009; Jangra et al., 2010; Jopling, 2012; Jopling et al., 2005). As stated in several reports, miR-122 positively affects HCV life cycle by potentially upregulating viral polyprotein translation, augmenting both infectious HCV production and RNA abundance, and apparently also by protecting HCV RNA from degradation (Henke et al., 2008; Jangra et al., 2010; Jopling et al., 2005; Machlin et al., 2011). Another conserved potential binding site (7-nts : ACACUCC) that is complementary to miR-122 seed sequence is situated in the 3'UTR of HCV RNA genome, but no functions in translation stimulation by miR-122 were found (Henke et al., 2008; Jopling et al., 2005). (*Figure 3)*

It was proposed that miR-122 is capable of enhancing the HCV RNA translation at two stages, first during the early initiation phase, when it amplifies the rate of ribosomal 48S initiation complex formation by augmenting the association of the small ribosomal subunit with HCV RNA, and secondly at later stage when it increases polysome formation (Henke et al., 2008). Some recent studies showed that some HCV genome features are important for miR-122 enhancement of translation (Jangra et al., 2010). For instance, miR-122 direct interaction with both S1 and S2 homologous sites located upstream the IRES in the 5'UTR of HCV genome is required for this enhancement of IRES-directed translation (Jangra et al., 2010). It has been shown that besides the HCV IRES, the uncapped status of HCV RNA (m^7G 5'-cap is able to counteract the positive translation effect of miR-122) is a necessary feature in HCV genome for miR-122 binding, (Roberts et al., 2011b).

In addition to HCV genome features, some host factors such as Ago proteins (Ago-2 /1) may take part in 5'UTR-dependent upregulation of HCV RNA translation by miR-122, as shown by some studies demonstrating that knockdown of Ago proteins in Huh7/Huh 7.5 cells resulted in the inability of miR-122 to activate translation (Roberts et al., 2011b; Wilson et al., 2011). Wilson et al. (2011) also discovered that Ago2 is essential for translation stimulation by binding of miR-122 to S1 and S2, suggesting that miR-122 recruits Ago2 to the 5'-UTR, forming a RISC-like protein complex that activates translation. This hypothesis was supported in a recent study showing, notably by immunoprecipitation assays and by assays where miR-122 binding sites where masked by secondary structures, that base-pairing between miR-122 seed sequence and complementary binding sites (S1,S2) within a single-stranded section of HCV 5'UTR is necessary for miR-122 to directly recruit Ago2 to the HCV 5'-UTR (Conrad et al., 2013). In Conrad *et al.* (2013) study, it is also demonstrated that Ago2 accumulates on HCV 5'UTR with time and that stable attachment of the Ago2 and miR-122 containing complex to the 5'UTR is crucial to promote RNA translation. Apparently, DICER and Trans-activation-responsive RNA-binding protein (TRBP) processing of pre-miR-122 yielding to mature duplex precursor is needed for such an activation of HCV RNA translation and accumulation (Zhang et al., 2012a). Besides the involvement of Ago proteins in HCV translation stimulation by miR-122, it is likely that other factors also participate, as shown by Roberts *et al.* (2013) who demonstrated that the P-body protein Lsm1 is specifically involved in IRES-dependent activation of HCV RNA translation by miR-122.

Even more interesting, besides its ability to increase efficiency of HCV translation, it was noted that Ago2-miR-122-containing complex has also a protective and stabilizing role when attached to the HCN RNA 5'UTR (Conrad et al., 2013; Shimakami et al., 2012a, 2012b). It appears that the 3'-end of the miR-122 bound to S1 in 5'UTR of HCV genome is making additional base-pairing with the 5'-end of HCV RNA, forming a 3'-overhang, which is suggested to have a possible function in masking the 5'-termini, and thus potentially avoiding recognition by antiviral cytoplasmic RNA-sensors and sheltering it from degradation by 5'-exonucleases (Machlin et al., 2011). For instance, the formation of a stable ternary structure in the HCV RNA 5 'UTR resulting in part of extensive base-pairing outside the seed sequence and high-order interactions between two miR-122 and HCV 5'-UTR (Mortimer and Doudna, 2013) may confer resistance to Xrn-1 degradation, the principal pathway leading to HCV RNA decay within infected cells (Li et al., 2013). (*Figure 3.)*

However, even if miR-122 coupled to other elements has been found to have a role in HCV RNA stabilization, accumulation or enhancement of HCV proteins translation, several reports cited here suggest other predominant functions of miR-122 within various stages of HCV replication (Li et al., 2013; Mortimer and Doudna, 2013; Roberts et al., 2013). Therefore, the mechanisms by which miR-122 favor HCV replication are still mainly unknown, and remain to be investigated further.

b. Negative regulation of HCV through direct interaction with miRNAs

In contrast to miR-122 that promotes HCV viral cycle, some miRNAs have proven the ability to affect the viral life cycle of HCV negatively by direct interaction with the RNA genome (Cheng et al., 2012; Murakami et al., 2009; Pedersen et al., 2007). In the first place, it was shown that among a group of eight miRNAs induced by anti-viral IFN-β and predicted to target HCV RNA genome, two of them, miR-196 and miR-448 have the ability to restrict HCV replication by directly binding a region homologue to their seed sequence within the RNA genome (Pedersen et al., 2007). These binding sites have been found in coding regions within both J6CF and JHF1 genome, more precisely into the core sequence for miR-448 and into the NS4A sequence for miR-196 (Pedersen et al., 2007). A study based on mutation that impairs miR-196 binding to HCV RNA in transfected Huh7.5 cells demonstrated that the inhibiting effect of miR-196 is mainly due to direct HCV RNA targeting (Hou et al., 2010). Otherwise, another miRNA, miR-199a has been described to repress HCV replication, independently of IFNs stimulation, by base-pairing at a specific site within the stem-loop II of the IRES located in the 5'-UTR region of the HCV genome (Murakami et al., 2009). Even more interesting, recent investigations carried out by Cheng *et al.* (2012) have shown that the miRNA Let-7b affects HCV life cycle by direct contact with HCV genome implying an ago2-containing complex. Among the three described let-7b binding sites in HCV genome (named MRE1, 2 and 3), two were found in the region encoding the viral protein NS5B, and one in the 5'UTR, all contributing to the suppressive effect of let-7b on HCV replication (Cheng et al., 2012). Moreover, according to Chen *et al.* (2012) the HCV replication inhibition by Let-7b involves mechanisms independent of translation repression, and appears to be prevalent for all HCV genotypes . (*Figure 3.)*

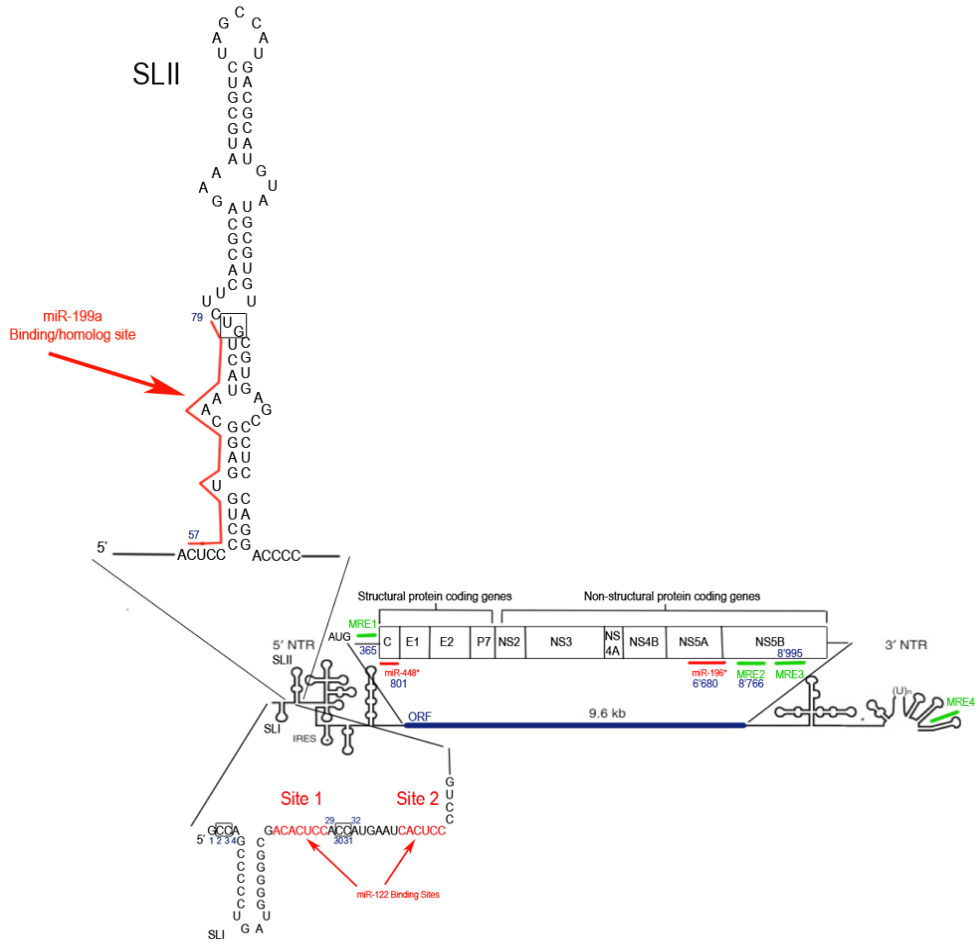

Figure 3. HCV RNA genome & miRNAs binding sites

Here are represented various miRNAs binding sites on HCV RNA genome, site 1 and site 2 represented in red are miR-122 binding sites within the 5'-UTR, miR-199a binding site represented in red is located in the SLII stem-loop within 5'-, miR-448 binding site represented in red is located within the Core protein sequence, miR-196 binding site represented in red is located within the NS5A protein sequence, the 4 binding site for Let-7b (MRE1,2,3 et 4) are indicated in green and located respectively in the 5'UTR close to the AUG codon, in the NS5B protein sequence, and finally in the 3'UTR. The number in blue beside every binding site indicate the position of the first nucleotide homologous to the miRNAs seed-sequence.

2.4 HCV is also able to directly modulate the RNAi machinery

Several reports revealed that HCV proteins can also interact directly with the RNAi machinery for multiple purposes which will be reviewed thereafter (Chen et al., 2008a; Ji et al., 2008; Wang et al., 2006). Wang *et al.* (2006) demonstrated that DICER, a key RNAse within the host RNAi process, can target and cleave HCV genomic double-stranded RNA structure such as the dsRNA replication intermediate or hairpin structures within the IRES, and can reduce HCV replicon replication as well, supporting a possible antagonization of the course of HCV lifecycle (Wang et al., 2006). What is interesting in this idea is that core proteins are able to abolish RNA-silencing by direct interaction with DICER, causing the suppression of RNAi-dependent antiviral response, which might conceivably favor development of a chronic HCV infection and related diseases (Chen et al., 2008a; Wang et al., 2006). More interestingly, knowing that DICER is involved in microRNA maturation [reviewed in (Winter et al., 2009)], both studies also advance that by negatively interacting with DICER, HCV core proteins may be able to deregulate various host miRNAs, likely to produce a range of diverse effects including gene expression modulation (Chen et al., 2008a; Wang et al., 2006). In regard of these findings, Ji *et al.* (2008) in turn, bring the idea that core protein is probably not the only protein that directly regulate miRNA maturation pathways, by demonstrating that E2 envelop protein interacts with Ago-2, an essential catalytic element within RISC (Gregory et al., 2005).

3. Potential use of miRNAs for HCV therapy

3.1. Circulating miRNAs as potential biomarkers for HCV infection and further related liver diseases.

It has been shown that miRNAs are « present and stable » in body fluids, especially in serum and plasma and that their expression profiles are specific to tissues, organs, biological stages or pathologies ((Chen et al., 2008b), Reviewed in [(Etheridge et al., 2011)]). These circulating miRNAs have been reported to be associated with various components (such as proteins, exosomes, macrovesicles, apoptotic bodies or even high density lipoprotein (HDL) and are thought to participate to intercellular communication (reviewed in (Szabo and Bala, 2013)). These features, the possibility of collecting samples in a non-invasive manner and the fact that their levels and profiles (in body fluids) are easily detected and measured by various methods (e.g microarray, q PCR), place them as good potential biomarkers for early detection and monitoring of HCV infection or further HCV-related liver diseases (Etheridge et al., 2011; Shrivastava, 2013).

Recent studies have provided insights into the relation between HCV infection and circulating miRNAs. For instance, a wide miRNA profile study showed that the modulation of several serum miRNAs (miR-134, miR-320c and miR-483-5p) in HCV infected patient could be related to various liver pathogenesis related pathways (Shwetha et al., 2013). The idea that liver-specific miR-122 could work as a biomarker for liver diseases has also been previously raised (Ding, 2012). (*Table 3.)*

Table 3. Circulating miRNAs signatures in HCV and other related diseases.

Circulating miRNAs	Expression level	Potential marker of :	Ref.
miR-122, miR-34a	Increased	Diagnosis and severity of the disease, loss of hepatocytes	(Cermelli et al., 2011)
miR-21	Increased	Necroinflammation activity	(Bihrer et al., 2011a)
miR-155	Increased	Liver damage and necroinflammation	(Bala et al., 2012a, 2012b)
miR-192, miR-125b, miR-146a	Increased	Chronically HCV-infected patients	(Bala et al., 2012b; Cermelli et al., 2011; van der Meer et al., 2013)
miR-92a, miR-20a	Increased	Early HCV infection, HCV-related fibrosis progression	(Shrivastava et al., 2013)
miR-618, miR-650	Increased	Urine marker of HCC development upon HCV infection	(Abdalla and Haj-Ahmad, 2011)

It was demonstrated that increased serum levels of miR-122 and miR-155 are respectively associated with liver damage and inflammation regardless of the etiologies (Bala et al., 2012b). Recent studies suggested that miR-122 circulating levels could reflect damages or loss of hepatocytes in patients infected by HCV (Kumar et al., 2014; Trebicka et al., 2013) and specifically during chronic HCV infection, where high levels of serum miR-122 were found to be linked to liver injury (van der Meer et al., 2013). Not only miR-122 but miR-34a, miR-192, miR-21, miR-125b, miR-146a and miR-155 serum levels were found higher in chronically infected patients compared to healthy controls (Bala et al., 2012a; Bihrer et al., 2011a; Cermelli et al., 2011; van der Meer et al., 2013). MirR-122, miR-21 and miR-34a were reported to be correlated with alanine transaminase (ALT) levels, disease severity and inflammation in chronically infected patient, thus suggesting they could possibly work as diagnosis and severity marker of CHC (Bihrer et al., 2011a, 2011b; Cermelli et al., 2011; Kumar et al., 2014). As miR-21 and miR-122 serum levels are strongly correlated with necroinflammatory activity in chronically infected patients, these miRNAs could be also used as a necroinflammatory marker (Bihrer et al., 2011a, 2011b). The inflammatory related miR-155 was found to be increased in both monocytes and serum in treatment-naïve chronically infected patients but not in monocyte from patients who had cleared the virus after therapy, implying that miR-155 may potentially be used as a marker of HCV infection (Bala et al., 2012a). As miR-92a and miR-20a serum/plasma levels are higher in patients with HCV infection compared to healthy controls, they may be used as markers for early detection of HCV-infection (Shrivastava et al., 2013). MiR-20a was further demonstrated to be highly expressed in serum from early to late stage of fibrosis in HCV-infected patients, but not in non-infected patients, therefore showing the possibility to serve as a biomarker of HCV-related fibrosis progression (Shrivastava et al., 2013). Interestingly, another study using a screening method based on urine as sample source, found a modification in expression levels of 2 miRNAs, miR-618 and miR-650, and reported them as a potential sensitive miRNA signature related to HCC development in HCV infected patients (Abdalla and Haj-Ahmad, 2011). However, only little is known about circulating miRNAs so far and further investigations to confirm their role as useful and sensitive biomarkers in the context of HCV infection are required. (*Table 3.)*

3.2 Miravirsen : the antagomiR based therapy against HCV.

As the classical anti-HCV drugs such pegylated-interferon (IFN), ribavirin or proteases inhibitors (telaprevir, bocaprevir) are showing lack of efficiency, in addition to be subject to viral resistance and cause potential side effects (Drenth, 2013), new approaches to treat HCV are welcome. Even if some recent research has led to the development of really efficient and promising drugs such as sofosbuvir (Jacobson et al., 2013; Lawitz et al., 2013), Miravirsen (antagomiRs-based therapy) is still encouraging. Miravirsen (originally SPC3649) is a Locked nucleic acid modified (LNA-antagomiRs) that target and repress the liver-specific miR-122 (Lindow and Kauppinen, 2012). Here we will succinctly summarize the main points about this developing therapy.

a. How does it work?

MiR-122, as it was reviewed before, is a well-known liver-specific miRNA involved in the HCV lifecycle (Norman and Sarnow, 2010) (reviewed in (Lindow and Kauppinen, 2012)). MiRNAs inhibitors named « AntagomiRs » are synthetic single-stranded chemically-modified oligonucleotides complementary to endogenous miRNAs (Krützfeldt et al., 2005) and as some antagomiRs were already proven to be efficient miR-122 inhibitors with interesting pharmacological properties (long-lasting and dose-dependent effects) (Elmén et al., 2008; Krützfeldt et al., 2005), it was later used to design Miravirsen (SPC3649) (Elmén et al., 2008).

In more detail, SPC3649 structure is composed of :

- A 15-mer oligonucleotide core with some Locked nucleic acid (LNA, a nucleotide analogue locked in the ideal conformation by a 2'-O, 4'-C-methylene bridge added to the ribose ring (http://www.exiqon.com/lna-technology, Kaur et al., 2006; Vester and Wengel, 2004)) modifications (notably at both 5' and 3' ends) which increase the stability of the later formed heteroduplex with miR-122 (Elmén et al., 2008; Kurreck et al., 2002; Lindow and Kauppinen, 2012).
- A phosphorothioate (PS)-backbone improving nuclease resistance and pharmacokinetics properties (Lindow and Kauppinen, 2012).

The fundamental principle of this approach is the ability of SPC3649 to target the 5'- seed region of mature miR-122, resulting in its sequestration in an heteroduplex, thus impairing miR-122 essential function for HCV lifecycle (Janssen et al., 2013; Lindow and Kauppinen, 2012). The targeting of 5'- seed sequence was chosen notably because it is the HCV binding-sequence that hybridizes with 2 strongly conserved miR-122 binding sites within HCV genome, rendering the treatment potentially efficient against all HCV genotypes (Janssen et al., 2013; Lindow and Kauppinen, 2012).

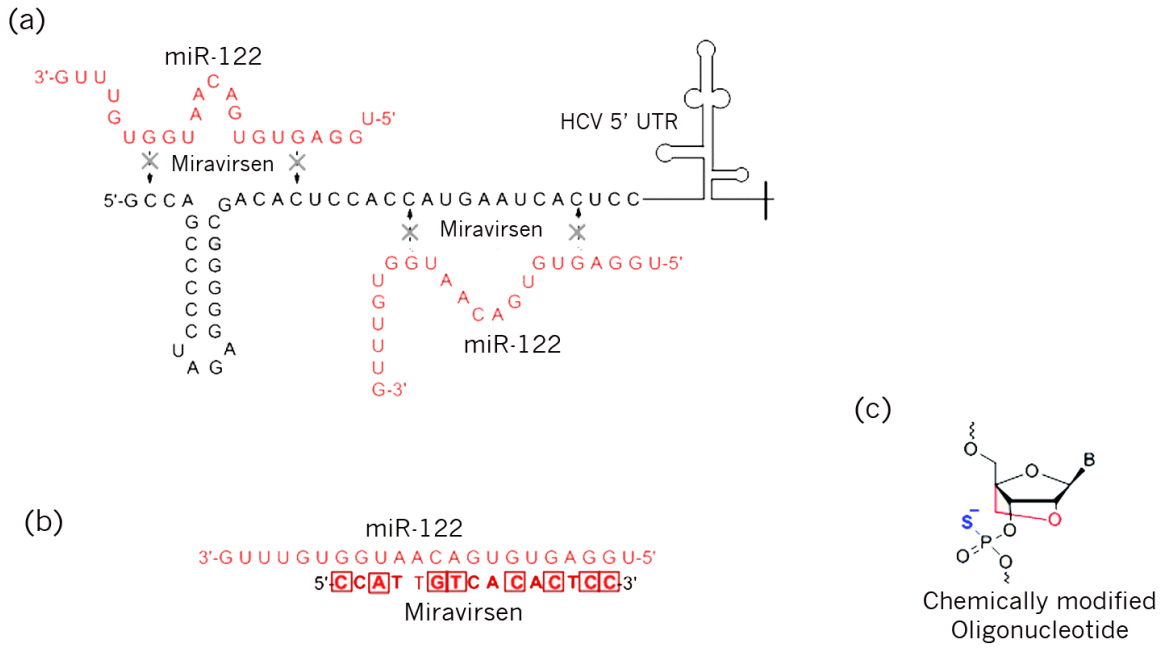

Figure 4. Miravirsen

(a) Scheme representing the inhibition of binding between miR-122 and HCV 5'-UTR by Miravirsen. (b) Heteroduplex formed by miR-122 and Miravirsen, chemically modified oligonucleotides (LNA) are circled by red squares. (c) Chemical structure of a Chemically modified oligonucleotide. (adapted from (Sharma et al., 2014)).

b. Preclinical, clinical studies and advancement

Efficiency and safety were assessed within rodents and primates models. Miravirsen appeared very efficient in antagonizing of miR-122 in mice and African green monkeys, producing a long-lasting and dose-dependent response (Elmén et al., 2008). In addition, neither LNA-associated toxicity (Elmén et al., 2008), negative side effects nor degenerative changes in any organs were observed (Hildebrandt-Eriksen et al., 2012), ensuring the safety of the approach for further clinical studies. Before Miravirsen entered into clinical trials, another study using chronically infected chimpanzee reported Miravirsen efficiency in inhibiting HCV and also the absence of undesired side effects or viral rebond (Lanford et al., 2010).

The first class I clinical trial was conducted in 2008/2009 and showed a dose-dependent pharmacology and a no dose-limiting toxicities (Lindow and Kauppinen, 2012; Qiu and Dai, 2014). Another clinical trial phase I started recently to test the potential positive effect of a co-administration of Miravirsen and Teleprevir (anti-HCV drug, serine-protease NS3/4A inhibitor (Lin et al., 2006)). In 2010, phase II clinical trial involving treatment-naïve patient was conducted (Janssen et al., 2013). The results of this trial indicate that a four- week miravirsen monotherapy provides a dose-dependent long-lasting suppression of HCV RNA and viremia, has a high barrier to viral resistance, and is well tolerated, producing no severe adverse side-effects (Janssen et al., 2013; Lindow and Kauppinen, 2012; Qiu and Dai, 2014). Currently, a second phase II trial is ongoing, evaluating the potential of a miravirsen therapy in chronically infected patient, which did not respond to peg-interferon/ribavirin therapy (Qiu and Dai, 2014).

In conclusion, anti-miR-122 therapy based on antagomiRs (such as Miravirsen) still needs more investigations and development, but really appears as a promising approach to treat HCV infection.

3.3 Use of an artificial inhibitor cluster to treat hepatitis C.

Recently, a new strategy to treat HCV based on RNAi has been developed (Yang et al., 2013). The main idea is to replace five endogenous miRNAs within the cluster 17-92a by 5 artificial anti-HCV sequences complementary to HCV genome (positive strand) and deliver the new cluster to infected hepatocytes using a recombinant adeno-associated virus (AAV) vector. The cluster is then processed through the endogenous miRNA pathway, producing 5 mature anti-HCV miRNAs therefore inhibiting HCV replication, inhibiting viral spread, potentially curing cells and finally preventing emergence of escaped mutants.

This new approach is relevant and promising for several reasons. First the fact to target multiple regions within HCV genome results in a high genetic barrier to resistance and further drug selection. The targeting of conserved sequence allows also the possibility for the anti-HCV miRNAs to target several HCV genotypes. Both *in vitro* and *in vivo* assays are promising, and bring not only the possibility to inhibit viral replication and spread, but also to potentially clear HCV from hepatocytes. Moreover, no liver toxicity was found during *in vivo* assay, and the use of liver promoter improves the safety of the technique. Finally, the authors mention the possibility to use endogenous exosome intercellular pathway for the transfer of anti-HCV miRNAs from cell to cell, thus avoiding the need to transfect all the hepatocytes, which could have undesirable effects. Taking in consideration all this points, this new approach to treat hepatitis C definitively deserves further attention and clinical evaluation is required. (*Figure 5.)*

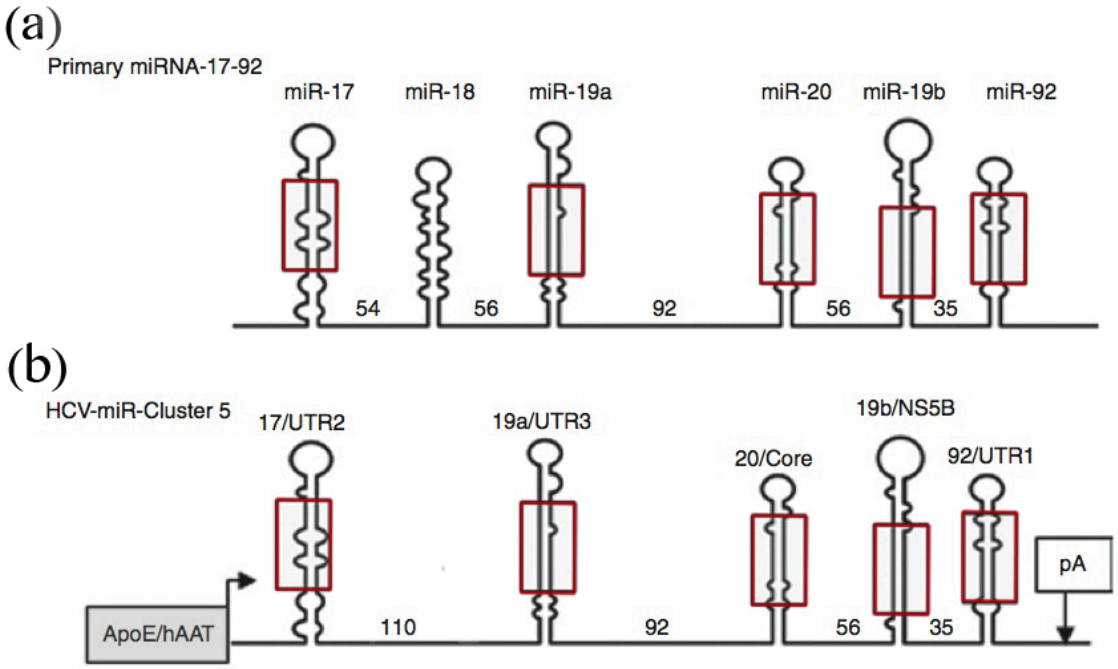

Figure 5. Structure of HCV-AAV-miRNAs-Cluster 5

(a) Five of the six miRNAs of the natural cluster 17-92a were used as scaffold to create the artificial miRNAs that target HCV genome (positive strand). (b) The endogenous sequences of the mature region of the natural miRNAs were replaced by complementary HCV sequences (19-23nts) known to be effective small interfering RNAs and short hairpin RNA. The new miRNAs sequences are squared in red, 3 of them target conserved sequence within 5'UTR of HCV genotype 1b, 1 target a sequence within a structural protein gene (core) and 1 target a sequence within a non-structural protein gene (NS5B). The vector also comprises two regulatory elements, human apolipoprotein E control region combined with Human alpha-one antitrypsin promoter (ApoE/Haat) that ensures liver specific expression. Numbers between pre-miRNAs represents nucleotides. (adapted from (Yang et al., 2013))

4. Conclusion

Taken together, the overall reports reviewed here, raise the idea that HCV-infection may have an important impact on host miRNAs regulation and *vice et versa*, resulting in a wide range of effects on both viral replication and pathogenesis related thereto. Although this field of research is now of great interest, detailed mechanisms deciphering the HCV-host miRNAs interplay are currently only slightly unveiled, and further in depth and specific investigations are required. Indeed, even if some studies mentioned here focused on the mechanistic role of HCV proteins (Core or NS3A/B, NS5A) in the modulation of host miRNAs expression (Shiu et al., 2013; Singaravelu et al., 2014), or reported direct interaction with HCV genome, most reports were only underlining observed correlation between HCV infection, host miRNAs expression profiles and viral lifecycle or pathogenesis.

However, due to the identification of miRNAs differentially expressed and of their target genes in diverse stages of Hepatitis C (acute, chronic, cirrhosis and HCC) and to the flourishing understanding of their functions, it is now in sight to use endogenous miRNAs as biomarkers to help monitoring the evolution of HCV-associated liver disease or even to develop new therapies (Janssen et al., 2013; Shrivastava, 2013; Shrivastava et al., 2013). A good example of the result of the expanding research and knowledge about HCV and miRNAs is the development of promising therapies such as Miravirsen, which is encouraging even in regard of other diseases.

In conclusion, I would say that miRNAs appear now as important modulators of host physiology by profoundly modifying gene expression, and a better understanding of their mechanistic role and implication in some cellular regulatory networks will certainly help to uncover some key aspects of various diseases such as HCV.

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