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Review

miR-122, a paradigm for the role of microRNAs in the liver☆

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Recent studies have uncovered profound and unexpected roles for a family of tiny regulatory RNAs, known as microRNAs (miRNAs), in the control of diverse aspects of hepatic function and dysfunction, including hepatocyte growth, stress response, metabolism, viral infection and proliferation, gene expression, and maintenance of hepatic phenotype. In liver cancer, misexpression of specific miRNAs suggests diagnostic and prognostic significance. Here, we review the biology of the most abundant miRNA in human liver, miR-122, and consider the diversity of its roles in the liver. We provide a compilation of all miRNAs expressed in the liver, and consider some possible therapeutic opportunities for exploiting miRNAs in the different settings of liver diseases.

***Keywords*: RNA interference; MicroRNA; Liver; Metabolic disorders; Stress; Hepatocarcinoma; Cholangiocarcinoma; Viral hepatitis; miR-122**

1. Introduction

The RNA interference (RNAi) pathway achieves silencing of gene expression. The best characterized triggers of RNAi are small interfering RNAs (siRNAs) and endogenous double-stranded (ds) RNAs. The predominant form of dsRNA in mammalian cells is derived from endogenous microRNAs (miRNAs), which consist of non-coding RNA molecules of 18–25 nucleotides emerging after a multiple step maturation process [1].

First discovered in *Caenorhabditis elegans* [2], miRNAs have quickly been considered as a fundamental component of the regulatory system of gene expression. miRNAs direct the binding of protein complexes to specific nucleic acid sequences to affect either chromatin structure, or mRNA stability, or translation. In humans, the first clue of miRNA contributing to diseases came from the identification of patients suffering from either DiGeorge syndrome or mental retardation who presented mutations, which resulted in dysfunction of miRNA biogenesis [3]. So far, most efforts have been directed towards the study of alteration of miRNA expression in tumorigenesis [3]. Today, approximately 500 miRNAs genes have been identified in the human genome.

Currently, the biological functions of miRNAs are actively being sought. Some studies have notably uncovered roles for miRNAs in stress resistance, in metabolism, in defence against pathogenic infections, and importantly, in the coordination of cell proliferation and cell death, in tumorigenesis. How a single miRNA regulates multiple-target mRNAs or even entire pathways is partic-

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Abbreviations: RNAi, RNA interference; siRNA, silencing RNA; dsRNA, double-stranded RNA; miRNA, microRNA; shRNA, short hairpin RNA; vmiRNA, viral microRNA; HCC, hepatocellularcarcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; RISC, RNA-induced silencing complex.

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ularly well exemplified by the liver-enriched miRNA miR-122. Thus, miR-122 accounts for a paradigm to review most of the complex influences of microRNAs reported to date in normal liver, and in liver diseases along with their therapeutic or diagnostic potential.

2. miR-122: a liver-specific microRNA

2.1. Discovery and biogenesis of miR-122

One of the first clues of the existence of miRNAs in mammals came from studies on genetic alterations in woodchuck liver tumors. In 1989, a gene rearrangement of *c-myc* and an unusual transcript, named *hcr*, was described in one of these tumors [4]. This transcript was characterized as liver specific, essentially non-coding, specifically nuclear, and processed by endonucleases [4]. Further, *hcr* was proposed to be the precursor for miR-122. In the current understanding, the part of the *hcr* transcript encompassing the so-called "pri-miRNA" is predicted to be processed to form a 66-nt long "pre-miRNA", which presents a hairpin structure with 79% base pairing, and which will ultimately be cleaved by the endonuclease Dicer to form the mature miR-122 [5].

In 2002, systematic cloning and sequencing of small RNAs prepared from different mouse tissues led to the identification of miR-122 as an abundant miRNA in the liver [6]. miR-122 was further characterized as the most frequent miRNA isolated in the adult liver, reaching around 70% of all cloned miRNAs [7]. miR-122 is found in mouse, woodchuck and human livers, in human primary hepatocytes, and in cultured liver-derived cells, such as mouse Hepa 1-6 cells and human Huh7 cells [5,8].

2.2. Evolutionary conservation of miR-122

MicroRNAs are known to be evolutionary conserved across species [9]. Here, we examined genomic DNA for orthologous sequences in ten animal species to assess the conservation of miR-122 across species. The overall conservation of 10 sequences, which we documented as a phylogenetic tree (Fig. 1) is remarkable. *Homo sapiens*, *Bos Taurus*, *Sus scrofa*, *Mus musculus*, and *Rattus norvegicus* are clustered in the same clade. These observations support the hypothesis that an ancient precursor of the miR-122 gene may have been common to the earliest animal lineages. So far, the role and specificity of miR-122 in the liver seem rather conserved although it is known that a conserved miRNA can regulate, in distinct organisms, different genetic pathways and developmental processes [10].

2.3. miR-122 amongst other liver-expressed miRNAs

A variety of experimental approaches has been used to characterize miRNAs and their expression patterns [6,11–14]. Complementary bioinformatics screens and algorithms provide an invaluable source of prediction of hundreds of additional miRNAs [15–17]. A review of the literature reveals limited references in the identification of miRNAs that are specifically expressed in human liver [18–21]. We compiled these miRNAs and their relative levels of expression in Table 1. From this table, overall comparison between the miRNAs that are expressed in adult and/or fetal liver suggests a developmental regulation of miRNAs expression. While miR-122 appears as the most highly expressed miRNA in adult liver, miR-92a and miR-483 seem to be more specifically expressed in the fetal liver (Table 1). Thus, in the

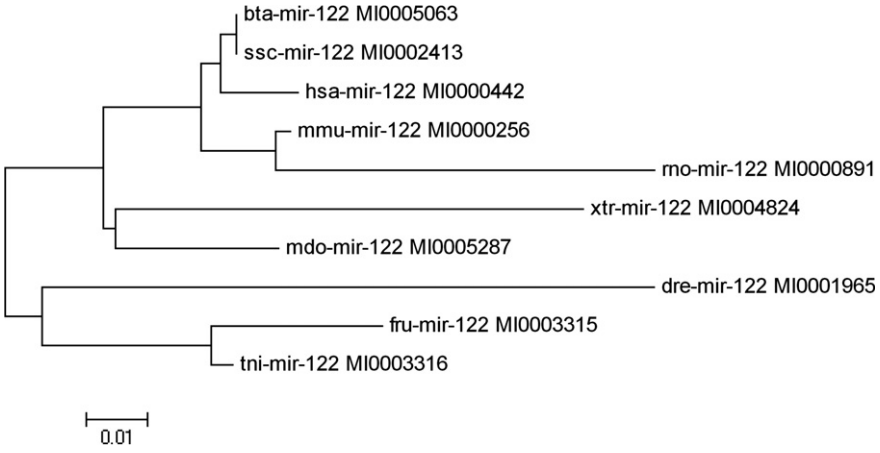

Fig. 1. Phylogenetic tree of miR-122 sequences. For analysis, we selected 10 representative miR-122 RNA precursors amongst 10 species. Sanger accession numbers are indicated for each sequence. The phylogenetic tree is based on a multi-gene analysis using MEGA v4.0 with Neighbor-Joining method. Distance scale is shown below. Full species names corresponding to the miRNAs are as follows: ssc, *Sus scrofa*; bta, *Bos taurus*; mmu, *Mus musculus*; hsa, *Homo sapiens*; gga, *Gallus gallus*; mdo, *Monodelphis domestica*; tni, *Tetraodon nigroviridis*; fru, *Fugu rubripes*; xtr, *Xenopus tropicalis*; rno, *Rattus novergicus*; dre, *Danio rerio*. Despite the short sequences, the major clades are well separated in this phylogenetic tree. Most mammalian vertebrates cluster well in the upper part of the tree.

Table 1
Repertoire of liver miRNAs

	Atlas liver	Adult liver	Fetal liver
hsa-miR-122	+++	+++	++
hsa-miR-126	+++	/	/
hsa-miR-16	++	++	/
hsa-let-7a	++	++	/
hsa-miR-22	++	+++	+
hsa-miR-125b	++	+++	+
hsa-miR-143	++	/	/
hsa-let-7b	++	++	/
hsa-miR-99a	++	++	/
hsa-let-7c	++	++	/
hsa-miR-181a	++	/	/
hsa-miR-194	++	+++	/
hsa-miR-451	++	/	/
hsa-miR-3-d	++	++	/
hsa-miR-15b	++	/	/
hsa-miR-193a-5p	++	/	/
hsa-miR-24	++	+++	++
hsa-miR-29a	++	/	/
hsa-miR-23b	++	+	/
hsa-miR-26b	++	/	/
hsa-miR-27b	++	++	/
hsa-miR-3-a	++	++	/
hsa-miR-92a	++	++	+++
hsa-miR-13-a	++	/	/
hsa-miR-15a	++	++	/
hsa-miR-186	++	/	/
hsa-miR-191	++	++	/
hsa-miR-26a	++	/	++
hsa-miR-28	++	++	/
hsa-miR-1- -	++	/	/
hsa-let-7d	++	/	/
hsa-miR-17	++	/	+
hsa-miR-185	++	/	/
hsa-miR-192	++	++	/
hsa-miR-3-e	++	/	/
hsa-miR-381	++	/	/
hsa-miR-99b	++	/	+
hsa-miR-1-3	++	+	/
hsa-miR-23a	++	/	/
hsa-let-7f	+	/	/
hsa-let-7g	+	/	/
hsa-miR-139-5p	+	+	/
hsa-miR-14-	+	/	/
hsa-miR-142	+	++	/
hsa-miR-144	+	/	/
hsa-miR-151	+	/	+
hsa-miR-154	+	/	/
hsa-miR-193a-3p	+	/	/
hsa-miR-193b	+	/	/
hsa-miR-195	+	/	/
hsa-miR-199a	+	+++	+
hsa-miR-21	+	+++	+
hsa-miR-223	+	/	/
hsa-miR-25	+	++	/
hsa-miR-27a	+	++	/
hsa-miR-29b	+	/	/
hsa-miR-29c	+	/	/
hsa-miR-3-c	+	+++	+
hsa-miR-32-	+	/	/
hsa-miR-377	+	/	/
hsa-miR-378	+	/	/
hsa-miR-424/322	+	/	/

Table 1 (*continued*)

	Atlas liver	Adult liver	Fetal liver
hsa-miR-425	+	/	/
hsa-miR-486__os-5p	+	/	+
hsa-miR-5-5	+	/	/
hsa-miR-874	+	/	/
hsa-miR-885-5p	+	/	/
hsa-miR-1-7	traces	/	/
hsa-let-7e	traces	/	/
hsa-miR-98	traces	/	/
hsa-miR-3-b	–	++	/
hsa-miR-34	–	++	/
hsa-miR-1-6a	–	++	/
hsa-miR-125a	–	++	/
hsa-miR-142	–	++	/
hsa-miR-148	–	++	/
hsa-miR-149	–	++	/
hsa-miR-189	–	++	/
hsa-miR-199b	–	+	/
hsa-miR-21-	–	++	/
hsa-miR-321	–	+++	/
hsa-miR-145	–	++	+
hsa-miR-93	–	+	+
hsa-miR-483	–	/	+++
hsa-miR-484	–	/	+
hsa-miR-485	–	/	+
hsa-miR-487	–	/	+
hsa-miR-2-	–	/	++
hsa-miR-18	–	/	+
hsa-miR-19b	–	/	+
hsa-miR-1-6b	–	/	+
hsa-miR-345	–	/	+
hsa-miR-41-	–	/	+

Notes: The left column corresponds to the most up-to-date compilation in the liver referred to as "atlas liver" [21], the middle column to "adult liver" [20], and the right column to "fetal liver" [19]. We assigned "+" and "−"signs to indicate the levels of expression of the various miRNAs that we assessed from the different studies [19,20]. The minus sign is used to indicate very low to undetectable levels, one plus to three pluses indicate a gradual expression from low levels to very high levels. The slash stands for the miRNAs that were not assayed. Cases outlined in dark grey illustrate the highest levels of expression and cases in light grey the moderate expression but found at least in two studies.*Abbreviation:* hsa-miR: homo sapiens microRNA.

search for miRNA-deregulated pathways involved in liver diseases, miR-122 serves as an interesting candidate.

3. Putative to experimentally validated targets of miR-122

3.1. Contribution of miR-122 in cellular stress response

Using computational tools, some genes, which were proposed as putative miR-122-target genes, were further experimentally confirmed in cultured hepatocytes. Chang et al. used the Lewis-based model of prediction of miRNA targets [22] to predict a binding site for miR-122 in the 3′-untranslated region (UTR) of the cationic amino acid transporter (CAT-1) mRNA [7]. Consistent with this prediction, which would lead to repression of CAT-1 mRNA by miR-122, an inversed pattern of expression of CAT-1

and miR-122 was noted at all stages of liver development. An antisense strategy targeting miR-122 with a 2′-*O*-methoxyethyl (2′-OMe) oligonucleotide in the human Huh7 cells brought up the dynamic evidence that miR-122 inhibited CAT-1 mRNA [23]. Interestingly, miR-122-induced inhibition through the CAT-1 3′UTR was efficiently relieved upon amino acid starvation, which validates CAT-1 as a target of miR-122 and suggests a role for miR-122 in cellular stress response [23].

3.2. Contribution of miR-122 to hepatocarcinogenesis

miR-122 was reported to be significantly and specifically down-regulated in hepatocarcinoma (HCC) in humans as in rodents [24,25]. Amongst the putative target genes of miR-122 that can be predicted using computational tools, at least three are of interest in tumorigenesis: the gene for N-Myc, which is frequently rearranged in woodchuck liver tumors by woodchuck hepatitis virus [26], the gene referred to as "down-regulated in liver malignancy" [27], and the gene for Cyclin G1 [28]. In fact, miR-122 was shown to modulate *cyclin G1* expression in HCC-derived cell lines, and an inverse correlation between miR-122a and *cyclin G1* expression in primary liver carcinomas was further observed [28]. These studies suggest an influence of the down-regulation of miR-122 and the converse expression of cyclin G1 in hepatocarcinogenesis.

3.3. miR-122 targets hepatitis C virus

A striking observation was made by Jopling et al. that replication of the hepatitis C virus (HCV) was dependent on the status of miR-122 expression. Indeed, HCV RNA can replicate in the Huh 7 cells, which express miR-122, but not in HepG2 cells, which do not express miR-122 [8]. To further assess the role of miR-122, silencing of miR-122 was carried out in

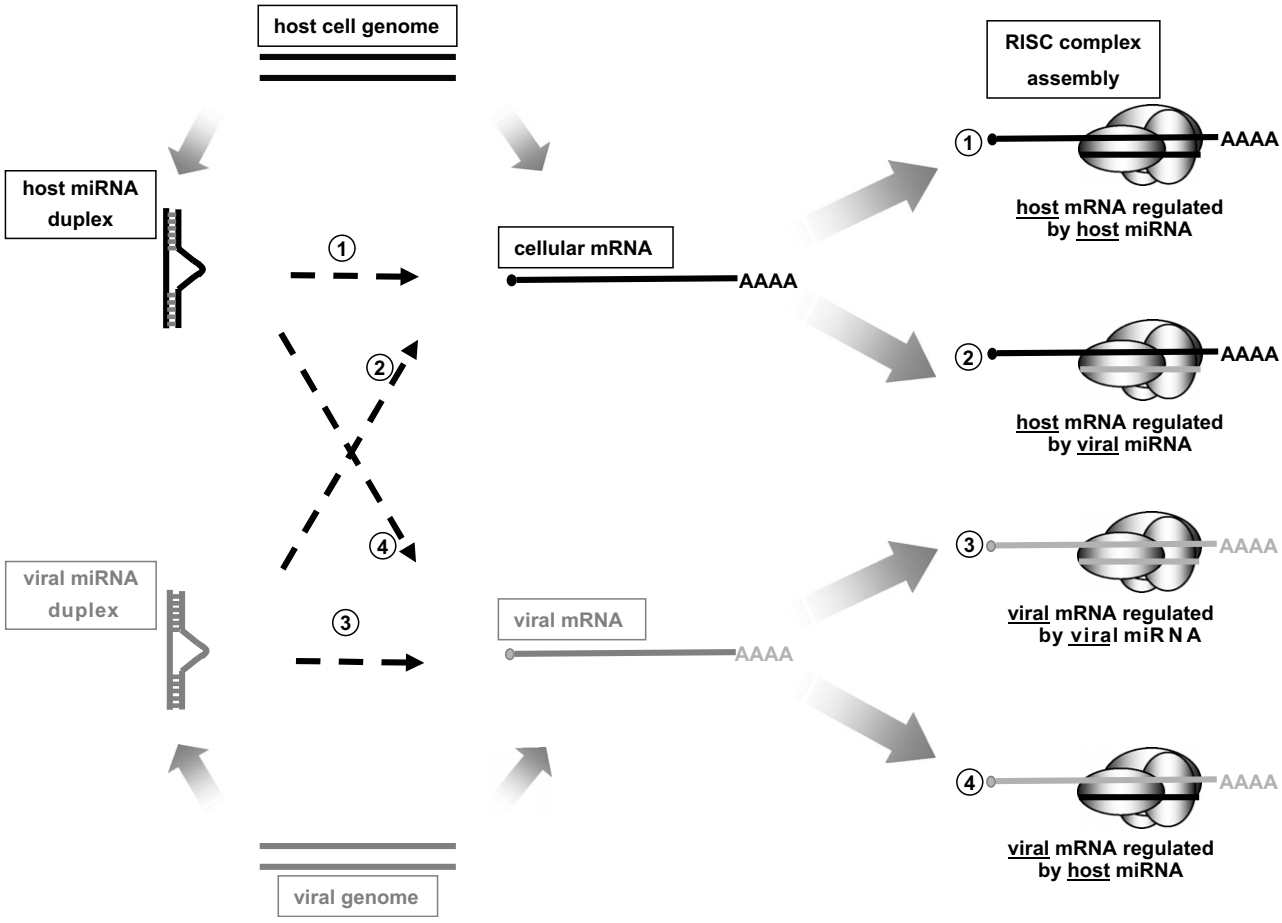

Fig. 2. Complexity of miRNA-mediated host–pathogen interaction. Genes within the host genome as well as the viral genome encode miRNAs and mRNAs. In the liver, known examples of the host–pathogen interaction involve host miRNA in their regulation of either cellular (arrow 1) or viral mRNAs (arrow 4), and viral miRNA in their regulation of cellular (arrow 2) or viral mRNAs (arrow 3). In turn, four situations arise in which host-encoded miRNAs can target host mRNAs (referred to as 1, e.g., miR-122 regulates cytoprotective enzyme heme-oxygenase 1 [29]), or virus-encoded miRNAs may bind to host mRNAs (referred to as 2, e.g., EBV vmiRNAs putatively binds Bcl-2 mRNA [40]), or virus-encoded miRNAs may bind to viral mRNAs (referred to as 3, e.g., HBV vmiRNA possibly targeting HBV genome [43]), or host-encoded miRNAs can target viral mRNAs (referred to as 4, e.g., interferon-beta induced miRNAs can bind sequences in the HCV genome [63]).

Huh7 cells [8], as in two other HCV replicon cell lines [29]. Using distinct antisense strategies, these studies consistently resulted in a marked loss of replicating RNAs from HCV [8] and eventually lowered its production [29,30]. Mutational analysis of a putative miR-122 binding site in the 5′-end of HCV genome provided the evidence of the direct role of miR-122 into HCV replication [8]. Additionally, an indirect effect of miR-122 inhibition on HCV regulation was also characterized at the level of two cellular genes (Fig. 2), via the up-regulation of the cytoprotective enzyme heme-oxygenase 1 (HO-1) and the converse down-regulation of HO-1 repressor Bach1 [25]. Altogether, these studies reveal miR-122 as a potential target for HCV treatment.

3.4. Overall role of miR-122 in adult liver

Using distinct protocols to silence miR-122, Krutzfeldt et al. and Esau et al. found the overall importance of miR-122 in the regulation of metabolism [31,32]. Through an antisense strategy based on a 2′-OMe phosphorothioate-modified oligonucleotide specific to miR-122, Esau et al. observed that several genes that regulate lipid metabolism, specifically the key enzyme phosphomevalonate kinase, were down-regulated [32]. Remarkably, silencing miR-122 in high-fat fed mice resulted in a significant reduction of hepatic steatosis, which was associated with reduced cholesterol synthesis rates and stimulation of hepatic fatty-acid oxidation [32].

Likewise, Krutzfeldt et al. proposed a novel antisense strategy based on a cholesterol-conjugated 2′-OMe oligoribonucleotide complementary to the targeted miR-122, and referred to it as an "antagomir" [31]. Silencing of the miR-122 resulted in increased expression of several hundred genes, which were notably represented as putative miR-122 target mRNAs, including those that are normally repressed in hepatocytes. These results argue for the involvement of miR-122 in maintaining an adult-liver phenotype by suppressing the expression of non-liver genes. At last, in both studies, silencing miR-122 resulted in a notable decrease of plasma cholesterol levels, which was consistently associated with decreased expression of genes involved in cholesterol biosynthesis. These results provided a great source of hope that miRNAs could serve as therapeutic targets [33].

4. Existing rationale for miR-based therapeutic approaches

4.1. miR-122 antagomir as a new agent for liver-specific RNAi

Antisense oligonucleotide approaches and siRNA-like technologies for inhibiting miRNA function are being explored as potential therapeutic agents. The current strategy to constitutively synthesize siRNA molecules is based on viral vectors that express short hairpin RNA (shRNA), which share features of miRNAs [34–36]. A particular caveat of this approach lies in the dosage. Indeed, intravenous infusion of AAV/shRNA vectors in mice results in strong competition of small RNAs for limiting cellular factors required for their processing [35], and a possible side effect in the activation of interferon response though no change was noted in the expression of miR-122 [37].

Actually, the exciting first step towards miRNA therapy in the liver was with miR-122 antagomir [31]. In this study, the pharmacological approach proposed by Krutzfeldt et al. was remarkably efficient, specific and stable. Injection of the antagomir into the tail veins of the mice selectively degraded miR-122 but not other miRNAs from the liver, even after more than 20 days indicating a durable effect [31]. Also, the selectivity of the effect of miR-122 inhibition was provided by the fact that despite distinct antisense protocols, a similar effect on cholesterol rates was observed [31,32]. Thus, in contrast to the challenges raised by gene therapy, the efficacy of intravenous antagomir to target the liver suggests that some issues such as the mode of delivery and the specificity will be more easily alleviated. Taken together, these studies show that while some safety issues still need to be carefully addressed, treatment of hepatic disorders through RNAi is becoming a plausible scenario.

4.2. Hepatitis viruses as adequate targets

Over the past 4 years, strategies based on targeting hepatitis B virus (HBV), and to a lesser extent HCV, by both synthetic and expressed activators of the RNAi pathway have proved efficient to inhibit viral replication both *in vitro* as *in vivo* [38]. Recently, a number of reports shed new light on the role of miRNAs as critical effectors in the intricate host–pathogen interaction networks, which involves three levels: (i) the RNAi pathway, (ii) viral miRNAs, and (iii) cellular miRNAs, as schematized in Fig. 2 and summarized in Table 2.

Through a systematic approach of silencing host factors, Randall et al. demonstrated the requirement of different proteins of the RNAi pathway, in particular Dicer, for optimal HCV replication [30]. In contrast, a previous study revealed the ability of Dicer to inhibit the replication of subgenomic HCV replicons [39]. Together, these results indicate that Dicer interferes with HCV replication either as an antiviral or a facilitating factor, which then likely implicates other cellular factors.

One breakthrough came from the discovery that a number of viruses, including hepatotropic ones, encode miRNAs. The first evidence of viral miRNA (vmiRNA) came from a study by Pfeffer et al. [40], who discovered

Table 2
Experimental characterization of miRNAs in the liver

	Characterization	miRNA	References
Metabolism	Regulation of lipid metabolism (cholesterol biosynthesis) Maintenance of adult liver phenotype	miR-122	[31,32]
Stress	Inversed pattern expression miR-122/CAT-1 in liver Alteration of cellular miRNA in response to nutrient or toxic stress response	miR-122	[23,59]
	Response to hepatotoxicants	miR-298, miR-370	[60]
Cancer	Regulation of NF2, modulation by Stat-3 Involved in survival signaling by interleukin-6	let-7a	[54]
	Regulation of c-myc, regulated by Wy-14,643	let-7c	Review [52]
	Overexpression in HCC	miR-195, miR-199a, miR-92, miR-20, miR-18, miR-18 precursor	[24,28,55]
	Overexpression in HCC, modulation of cell proliferation	LIN28B	[51]
	Overexpression in HCC, modulation of cell proliferation, migration and invasion Overexpression in malignant cholangiocytes	miR-21	[25]
	Response of cholangiocarcinoma cells to chemotherapy	miR-21	[47]
	Epigenetic regulation by interleukin-6	miR-370	[61]
	Inverse correlation with cyclin G1 expression in primary liver carcinoma Regulation of cyclin G1 in HCC-derived cell lines Low expression in HCC	miR-122	[28]
	High expression in response to tamoxifen and methyl-deficient diet	miR-34	[62,24]
Infection	*Viral miRNA*		
	Putative vmiRNA within HBV genome	vmiR	[43]
	Cellular miRNA		
	Inhibition of HCV replication	*Dicer*	[39]
	Permissive of HCV replication, cytoprotection through modulation of heme-oxygenase 1	miR-122	[8,29,30]
	Targeting viral genomes and induced by interferon beta	miR-1, miR-30, miR-128, miR-196, miR-296, miR-351, miR-431, miR-448	[63]

Abbreviations: miRNA, microRNA; vmiR, viral microRNA.

five vmiRNAs in cells infected by Epstein–Barr virus (EBV) that interfere with EBV latency and also with host cell genes expression [40] (Fig. 2). Since, vmiRNAs have been cloned in Kaposi's sarcoma-associated herpesvirus (KSHV), human cytomegalovirus (HCMV), mouse gammaherpesvirus 68 (MHV68), herpes simplex virus 1 (HSV1), and simian viruses (SV40) [41]. By contrast, no vmiRNAs have been cloned in RNA viruses such as hepatitis delta virus (HDV) [5], nor HCV, nor human inmmunodeficiency virus 1 (HIV1) [42]. Beyond the negative cloning data on HCV, the existence of vmiRNAs is unlikely in most RNA viruses that are cytoplasmic-restricted due to the need for pre-miRNA to be processed in the cytoplasm. Early on, it has been demonstrated that HDV RNA is resistant to Dicer action [5]. More recently, extensive searches on hepatitis viruses led to scan a single putative vmiRNA within HBV genome [43]. Surprisingly, the only potential target of this miRNA was not found within the human genome but within its own genome (Fig. 2). This interesting finding suggests a novel mechanism of vmiRNA action and opens new means to target hepatitis viruses.

Experimentally, the recent study by Pedersen et al. provided great insights into validating sequence-predicted targets of cellular miRNAs within the HCV genome (Fig. 2). Using results of miRNA microarray analysis in response to the current standard treatment for chronic HCV infection, i.e., interferon, they could demonstrate that cellular miRNAs, including miR-122, were specifically regulated by interferon displayed antiviral activity against HCV. Thus, as discussed above, miRNAs, whether cellular or viral, have emerged as viral regulators of host and/or viral gene expression. As a consequence, the range of interactions possible through miRNA–mRNA crosstalk at the host–pathogen interface becomes considerable.

4.3. Interests converging on miRNAs in liver cancer treatment

There are different means by which miRNAs give rise to strong interests in cancer (Table 1). One is the intriguing link between fragile sites and the genomic location of miRNAs [44], exemplified by miR-122 embedded in the

hcr locus [4]. Other studies noted that hot genomic regions often involved in HCC – either by deletion or by viral insertion – tend to encompass miRNA-containing regions. In particular, the common deletion found in 13q31 in human colocalizes with the cluster miR-17-92, which is rather referred to as an oncogene [45]. Likewise, it was suggested that HBV integration nearby miR-200a, at the fragile FRA1A site in the genome, could promote HCC through silencing miR-200a expression, which is known to be decreased in HCC [46]. Another observation concerns the integration of AAV in mice, which was shown to be associated with HCC, and was repeatedly localized in the same 6-kb region of chromosome 12, which encompasses no fewer than 34 miRNAs [35]. Conversely, it was noted by Meng et al. that miR-141, which showed strong overexpression in malignant cholangiocytes, was specifically localized in 12p, a region of known chromosomal aberration in biliary tract cancers [47].

Another reason for interest in miRNAs in cancer lies in their putative role as master regulators of cellular processes involved in tumorigenesis [48–50]. Specifically in the liver, few miRNAs have been shown to modulate important targets of proliferation such as miR-122 targeting cyclin G1, let-7 targeting LIN28B [51] and c-myc in response to PPARalpha [52], or miR-141 targeting CLOCK, which can act as a tumor-suppressor [47]. Importantly, as demonstrated by Meng et al. miR-21 targets PTEN [47], and results in further modulation of HCC cell migration and invasion, through modulating the phosphorylation of focal adhesion kinase and expression of matrix metalloproteinases 2 and 9 [25]. These data raise further the potential interest in decreasing miR-21 to limit HCC growth and metastasis.

The important breakthrough in the field of hepatocarcinogenesis came from the accurate correlation of alterations in miRNAs with tumor proliferation and differentiation. Notably in HCC, this alteration seems to take place at the expression level rather than at the sequence levels [53]. So far, there has been very limited insight into the characterization of this modulation, though the influence of interleukin-6 in malignant cholangiocytes was noted [54]. Using miRNA microarray analysis in patient-derived paired samples from the tumoral tissue and the non-tumoral adjacent tissues, Murakami et al. [55] found miR-92, miR-20, miR-18, and miR-18 precursor, which were inversely correlated with the degree of HCC differentiation. Consistent with different studies, miR-199a and miR-21 were, respectively, found lowered and overexpressed [24,25,28,55]. Therefore, it appears that a limited number of miRNAs, which could stand as an miRNA signature, could help in future molecular profiling of HCC [55,56]. These observations in HCC are all the more interesting because diagnostic and prognostic significance of any tested markers has proved limited due to their high variability. Furthermore, Meng et al. showed that inhibition of miR-21 sensitized the response of cholangiocarcinoma cell lines to chemotherapy [47]. This observation gives rise to significant hope that miR-21 could serve as a biomarker for drug response in cholangicarcinoma.

4.4. Therapeutic opportunities and challenges

In the near future, the distinctive signature patterns of miRNA expression associated with liver cancer should allow classification of different stages in tumor progression. Further, creating artificial miRNAs with salutary effects by promoting the expression of beneficial gene products (e.g., tumor-suppressor proteins) or targeting viral genomes (e.g., molecules designed to specifically target HCV-genome sequences) may become part of our patient management and complement chemotherapy and antiviral treatments.

As for miR-122, its role in regulating cholesterol biosynthesis, in maintaining the adult-liver phenotype, its association with hepatocarcinogenesis and its role in HCV replication make it an invaluable target to expand our knowledge in the pathophysiology of diverse liver diseases. One potential therapeutic application comes from the effect of miR-122 antagomir in high-fat fed mice to reduce hepatic steatosis [31], which may provide an interesting opportunity to treat patients with non-alcoholic steatohepatitis. Another interesting application of miR-122 antagomir consists in taking advantage of its effect on the down-regulation of adult-liver genes expression [31] to generate *in vitro* a new attractive expandable cell source for hepatocyte transplantation that would feature stem/progenitor cell phenotype.

It has been less than 5 years since the discovery that natural miRNAs were also functional in humans [57]. So far, the fast pace of discovery in this field is providing increasing rationale for manipulating miRNAs therapeutically. As such the number of recent patent applications is growing quickly [58]. Given the broad effects of miR-122 in the liver, and the number of miRNAs, it is quite certain that many new and unanticipated roles of miRNAs in the control of normal and abnormal liver function are awaiting discovery.

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