Review

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Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma

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Summary

MicroRNAs (miRNAs) are evolutionary conserved small non-coding RNAs that regulate gene expression by mediating post-transcriptional silencing of target genes. Since miRNAs are involved in fine-tuning of physiological responses, they have become of interest for diagnosis and therapy of a number of diseases. Moreover, the role of dysregulated miRNAs in maintaining the malignant phenotype has profound implications for cancer therapy. We will review the best defined cellular miRNAs and changes in their expression profile in hepatocellular carcinoma (HCC). Cellular miRNAs can also be released into the circulation, and these miRNAs are detected in most body fluids. Circulating miRNAs are associated with HCC and are possible biomarkers. Finally, by affecting several clinically relevant targets, artificially increasing or decreasing the expression level of a given miRNA offers fascinating therapeutic perspectives. We will therefore highlight recent developments in miRNA-based gene therapy with a focus on their therapeutic potential for HCC.

Introduction

Hepatocellular carcinoma (HCC) is the major primary liver cancer, which is the fifth most common cause of cancer worldwide [1], with about 750,000 patients globally reported each year (International Agency for Research on Cancer, IARC). An estimated 80–90% of all HCCs arise from the cirrhotic liver. Major risk factors are chronic viral hepatitis B (HBV) or C (HCV), which account for 80–90% of all HCCs worldwide [2], and alcoholic and non-alcoholic steatohepatitis-associated liver cirrhosis. Liver cancer is the third cancer-related cause of death, with an annual mortality of about 700,000 persons globally. Low survival is attributed to late diagnosis, resistance to treatment, tumor recurrence, and metastasis, hence stressing the need for novel diagnostics and therapeutics. Specific miRNAs have been shown to be involved in various biological processes, including development, cellular proliferation, apoptosis, and oncogenesis [3]. The finding that individual miRNAs may target several hundred genes, and that a large percentage of mRNAs may be subject to regulation by miRNAs, further underscores the emerging importance of miRNA-mediated regulation [4,5]. Here, we review miRNA biogenesis and its alterations as well as miRNA polymorphisms linked to HCC, miRNA detection methods, the association of cellular and circulating miRNAs expression patterns with HCC, their predicted target genes, and discuss the diagnostic and therapeutic potential of some miRNAs.

Key Points 1

- miRNAs are ~22-nt long RNAs implicated in post-transcriptional regulation of various biological processes including development, cellular proliferation, apoptosis, and oncogenesis
- miRNA biogenesis involves multiple steps requiring nucleases Drosha and Dicer to process the initial 1-4 kb transcript called pri-miRNA, with intermediate pre-miRNA of ~70-nt long, into the mature miRNA ~22-nt long RNA

miRNA expression profiles associated with HCC

miRNA biogenesis and mechanism of action

miRNAs are endogenous ~22-nt long single stranded RNAs. There are currently 1492 human miRNA sequences registered in the miRBase database (http://www.mirbase.org). miRNAs are non-coding but are implicated in post-transcriptional regulation of genes involved in fundamental cell processes and in diseases [3]. The miRNA gene is usually transcribed by RNA polymerase II in the nucleus into a primary transcript called pri-miRNA (Fig. 1A) of approximately 1–4 kb [6]. These transcripts can be

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Abbreviations: miRNA, microRNA; RNAi, RNA interference; HCC, hepatocellular carcinoma; UTR, untranslated region; AHL, adjacent healthy liver; HL, healthy liver; CLD, chronic liver disease; HCV, hepatitis C virus; HBV, hepatitis B virus; TACE, transarterial chemoembolization; SNP, single nucleotide polymorphism; AFP, α-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive AFP; DCP, des-γ-carboxyprothrombin.

either monocistronic – a single miRNA gene behind a promoter – or polycistronic – expressed from one transcript as a cluster containing several miRNA gene products e.g. the miR-17∼92 miRNA polycistron [7]. Depending on their genome position, globally about 50% of all miRNA genes are intragenic, the so-called mirtrons – likely to be regulated through their host gene – but they can also be located in intergenic regions i.e. likely to be independent transcriptional units [8]. The pri-miRNA is then cleaved by the microprocessor complex which consists of the nuclease Drosha and the double-stranded RNA-binding protein DiGeorge syndrome critical region gene 8 (DGCR8) into a precursor miRNA (pre-miRNA) (Fig. 1B). This ∼70-nt long pre-miRNA is exported to the cytoplasm via Exportin-5 (Fig. 1C) where it will be cleaved by another nuclease, Dicer, into an imperfect miRNA–miRNA* duplex (Fig. 1D) of ∼18–25 nucleotides [9]. While the passenger strand (miRNA*, in black in Fig. 1) is commonly degraded, the mature miRNA guide strand (in red in Fig. 1) is loaded into the RNA-induced silencing complex (RISC; Fig. 1E) where further regulations will be undertaken, depending on the level of complementarity between the miRNA and its target in the 3′ untranslated region (3′ UTR) of the messenger RNA (mRNA). In case of perfect complementarity, the mRNA will be cleaved by RISC and degraded; in case of imperfect complementarity, translation will be repressed [10]. In mammals, decreased mRNA levels were shown to be preceding protein decrease in 84% cases [11]. Functional target sites within the mRNA usually consist of a 6–7-nt long sequence which is complementary to the miRNA sequence, followed by an adenosine, the so-called miRNA "seed" sequence. Target mRNAs end up in cytoplasmic processing-bodies (P-bodies) where they are degraded [12]. Interestingly, other recently discovered classes of non-coding RNAs can also participate in regulation of gene expression and/or have been associated with HCC. Ender *et al.* showed that the small nucleolar RNA (snoRNA) ACA45 is processed by Dicer into RNAs of miRNA-like length (20- to 25-nt long) that will bind to Argonaute proteins (Ago), moreover they demonstrated the miRNA-like function of ACA45 by luciferase reporter assays [13]. Yang *et al.* identified a long non-coding RNA (lncRNA) named High Expression in Hepatocellular Carcinoma (lncRNA-HEIH) that is differentially expressed in HCC and whose expression level is positively associated with tumor recurrence and negatively correlated with survival. In addition, they showed that shRNA-mediated down-regulation of lncRNA-HEIH significantly inhibited the growth of tumors in a xenograft mouse model [14].

Regulation of miRNA processing in association with HCC

Mature miRNA processing involves multiple steps, and each can potentially be affected, having an impact on the resulting net amount of produced mature miRNA. The process starts with the pri-miRNA transcription, which can be regulated by transcription factors or genes that are dysregulated in HCC, and that bind to the corresponding sequence located upstream in the promoter region. For example, the oncogenic transcription factor c-Myc binds upstream of miR-17 and upregulates the transcription of the miR-17–92 tumor-promoting polycistron [15]. Interestingly, c-Myc similarly binds upstream of other miRNAs, e.g. let-7 and miR-26a, this time by repressing their transcription, indicating that cellular miRNAs can have opposite functions in cancer development [16]. In a similar fashion, the tumor-suppressor gene p53 upregulates the transcription of miR-34, resulting in cell cycle arrest and apoptosis [17–20]. Epigenetic mechanisms e.g. histone deacetylation and DNA methylation can result in miRNA silencing. For instance, Furuta *et al.* showed that methylation of miR-124 and miR-203 genes in HCC cell lines silenced their expression [21]. The next step of miRNA processing, when Dicer cleaves out the mature miRNA, can be affected in HCC, e.g. Dicer expression is altered in many cancers [22]. This results in miRNA dysregulation and as a consequence, in an abnormal gene expression that may lead to cancer phenotype. Finally, the stability of the mature miRNA molecule can be affected by differential polyadenylation modifications. miR-122, a liver-specific miRNA, is selectively 3′ adenylated, which will result in a higher stability in the liver while it will be destabilized in fibroblasts due to poly(A) polymerase GLD-2 depletion [23,24].

Key Points 2

- miRNA polymorphisms have been associated with increased risk of developing hepatocellular carcinoma (HCC)
- miRNA processing is altered in HCC
- Hepatic miRNAs are dysregulated in HCC and this results in changes in the expression profile of target genes involved in HCC onset and progression
- Cellular miRNAs can be released into the circulation, and circulating miRNA levels are also affected in HCC

miRNA polymorphisms

Besides the possible alterations in the miRNA processing, miRNA polymorphisms can also be associated with an increased risk of HCC. A miRNA polymorphism consists of a single nucleotide polymorphism (SNP) in the miRNA gene. Although rare, a SNP in a miRNA can affect its transcription, processing, or target recognition. Since binding of a miRNA to its mRNA target is limited only to the seed sequence, even one nucleotide change would result in a different group of genes that would be regulated. Recently, two groups of investigators have described that a variant of miR-196a-2 is positively associated with HCC susceptibility, in two populations of distinctive ethnical background [25,26]. Yet, the field of HCC-associated miRNA polymorphisms and their relevance to disease progression as a result of regulation of different pools of genes is only starting to develop.

Detection of miRNAs

Since miRNAs are involved in fine-tuning of physiological responses, they have become of interest for diagnosis and therapy of a number of diseases; nevertheless, reliable miRNA detection is a key requirement. Currently, the three most commonly used detection methods are microarray, RT-qPCR and next-generation sequencing (NGS). Much less common is the use of Northern blot, *in situ* hybridization and bead-based flow cytometry. Microarray is based on annealing of DNA oligonucleotides to the homologous sequences, on a microchip. Main advantages are the relatively low price and the high throughput, but the method has a low sensitivity and specificity, i.e. miRNAs with similar sequences

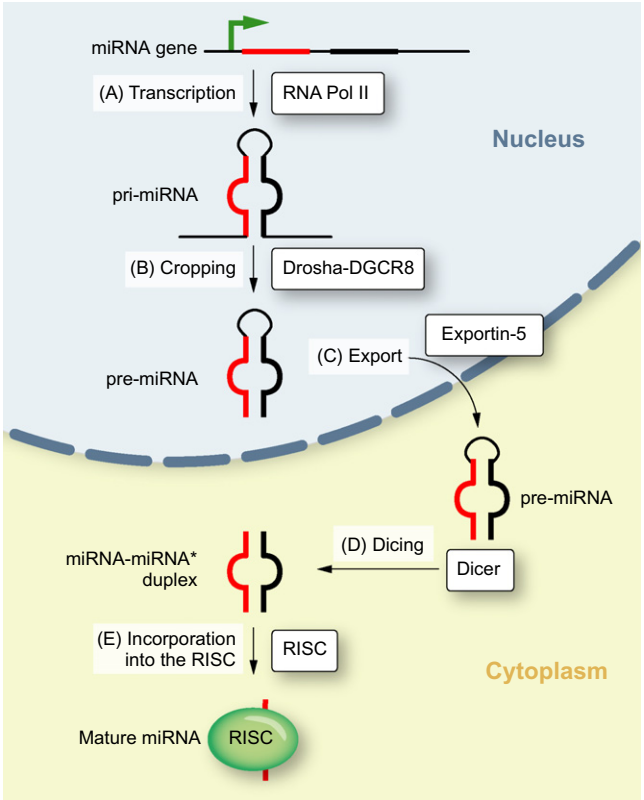

Fig. 1. miRNA biogenesis. miRNA biogenesis involves multiple steps requiring (A) RNA Pol II for transcription of the 1–4 kb primary transcript called pri-miRNA, (B) nuclease Drosha-DGCR8 for cropping of the single-stranded sequences flanking double-stranded stem–loop structure of the pre-miRNA precursor of ~70-nt long, (C) export of the pre-miRNA via Exportin-5 from the nucleus to the cytoplasm and (D) nuclease Dicer cleavage of the loop to generate the mature ~22-nt long miRNA that will be incorporated into the RISC (E). miRNA guide strand is represented in red, and passenger strand is represented in black.

(miRNA families) can hybridize with the same probe. The use of DNA locked nucleic acid (LNA) oligonucleotides in microarrays ensures a greater specificity by increasing the melting temperature. In addition, the sensitivity has also been increased [27]. miRNA RT-qPCR is based on a stem–loop primer binding to the mature miRNA during the reverse transcription, making it a highly specific technique that can distinguish 1-nt differences between related miRNAs [28]. Although preamplification step sometimes required before the RT-qPCR can induce some bias and underestimate the concentration of lowly expressed miRNAs, this method is more sensitive than microarray. Despite its higher cost, it is currently the method of choice for validation of miRNA signatures. NGS is a high-throughput technology that provides global information on all miRNAs in a certain sample. Costs are much higher and data analysis is more laborious, but NGS provides quantitative data, allows miRNA discovery and provides data on miRNA polymorphisms and differential processing. Finally, the nCounter developed by Nanostring Technologies (Seattle, WA) is based on annealing of a fluorescent barcode probe followed by single molecule imaging, without preamplification step, offering high sensitivity and specificity. This technology offers high-throughput when using up to 800 multiplexed targets.

Key miRNAs dysregulated in HCC

Dysregulation of miRNAs in cancer has been repeatedly described, e.g. deregulated miRNAs in prostate, bladder, and kidney cancer [29], breast cancer [30], colon cancer [31]. Importantly, miRNAs are predominantly downregulated in tumor tissue [32]. Hepatocellular carcinoma is no exception and various HCC-specific miRNA signatures have been described (Table 1). Screens of clinical samples are qualitatively heterogenic, firstly because of the variability in the technical procedure, from method of sampling (surgery or biopsy), time to and procedure of freezing, RNA isolation, to method of detection. Most miRNA screens are done using miRNA RT-qPCR, but some publications report microarray and NGS as described in Table 1. Secondly, the disease etiology is a significant factor of variation. This should be taken into account when pooling data, as the patient group can have a single etiology (alcohol or viral) or mixed etiologies (alcohol plus viral). Thirdly, the stage of the disease should also be considered, although miRNA dysregulations occur from an early stage [33], it is not clear how miRNA expression changes during disease progression. Finally, the control tissue used for normalization is also of importance, as it can be the healthy liver from patients with a different pathology or no known pathology, or non-tumoral liver tissue from the same patient, i.e. with the same underlying liver disease (e.g. cirrhosis, viral infection), the latter allowing to look only at intra-individual changes [34]. Nevertheless, dysregulation of several key miRNAs appears to be common to different screens, as described in Table 1.

Use of miRNAs in molecular classification of HCC and in prognosis

Key miRNAs are affected in HCC, and different dysregulation patterns can be used to discriminate tumors based on molecular characteristics. For instance, downregulation of miR-375 has been associated with β-catenin mutation, and downregulation of miR-107 with hepatocyte nuclear factor 1α (HNF1α) [35]. Toffanin *et al.* recently proposed a miRNA-based classification of HCC in three subclasses: the wingless-type MMTV integration site, interferon-related, and proliferation subclasses [36]. Such miRNA-based determination of molecular subclasses of HCC could allow subtype-specific treatment. miRNA signatures can also be used to determine disease prognosis, e.g. Budhu *et al.* identified a 20-miRNA signature as a predictor of survival and recurrence [37]. In addition, low tumoral miR-26 expression has been associated with high interleukin-6 (IL6) expression, and shorter survival [38]; Ji *et al.* showed a better response of these tumors to interferon therapy compared to tumors with high miR-26 levels [38]. It hence appears that miRNA profiling may play a crucial role in the clinic, not only for HCC classification and subtype-specific treatment allocation, but also for prognosis.

Gene targets of miRNAs and their association with HCC

Oncogenic and tumor-suppressive miRNAs

Prior to inhibition of gene expression, mature miRNAs are loaded into RISC which will mediate recognition of the target mRNAs and lead to either mRNA degradation or translational repression. This negative regulation of gene expression by miRNAs leads to a

balance between miRNA and gene expression level. In the context of HCC, miRNAs can act either as oncogenes, by inducing progression of a cell to cancer, or as tumor-suppressors, by preventing cell progression to cancer miRNAs (Fig. 2, in red) repressing the expression of oncogenic targets; when downregulated in HCC, a higher expression of their targets is allowed, hence promoting the malignant phenotype. Alternatively, upregulation of oncogenic miRNAs (Fig. 2, in green) in HCC will cause downregulation of their gene targets, again promoting the malignant phenotype.

Targets of tumor-suppressing miRNAs downregulated in HCC

Being downregulated in HCC, tumor-suppressor miRNAs cause upregulation of oncogenic target genes, stimulate and/or increase cellular mechanisms such as cell proliferation, cell cycle regulation, cell migration and invasion, apoptosis, and hence participate in the establishment and maintenance of the cancer phenotype, as described in Fig. 2. Cyclin G1 (CCNG1) is one of the most well-characterized targets of miR-122 [39]. However, miR-122 also targets the anti-apoptotic BCL-W [40] and ADAM17 (a disintegrin and metalloprotease family 17) involved in metastasis [41]. Additional validated miRNA targets are described in Fig. 2.

Targets of oncogenic miRNAs upregulated in HCC

Oncogenic miRNAs are upregulated in HCC, thus causing downregulation of target genes and decrease of cell mechanisms such as apoptosis, which eventually leads to onset and progression of the disease, as described in Fig. 2. Overall, less miRNAs are upregulated than downregulated in cancer [32]. miR-221 targets the cyclin-dependent kinase inhibitors CDKN1B/p27 [42] and CDKN1C/p57 [43], which results in an increase in the G1 to S phase shift and induces cell growth. Another target of miR-221 is the pro-apoptotic BMF of the BCL-2 family [44], therefore in HCC, BMF downregulation inhibits apoptosis. Enforced miR-221 expression also induces downregulation of DNA damage-inducible transcript 4 (DDIT4), leading to modulation of the mTOR pathway [45] and of the tumor suppressors thymidylate synthase (TIMP3) and PTEN [46], which results in enhanced cell migration.

Circulating miRNAs

Origin and clinical relevance of circulating miRNAs

Recently it has been described that miRNAs are found in many body fluids including plasma [47]. Vesicles (microvesicles or exosomes) released from cells to the circulation do contain miRNAs [48]. A positive correlation between cellular and exosomal miRNAs levels was reported for a subset of 8 miRNAs, both presenting a profile significantly different in ovarian cancer compared to benign ovarian disease [49]. Circulating miRNAs can therefore be considered representative of some pathological conditions. Moreover, their accessibility and high stability in the circulation system [50] make them perfect biomarkers, especially for surveillance of early stage, pre-symptomatic diseases in at-risk patients. For example, a serum diagnostic test based on a 34-miRNAs signature, could diagnose early stage lung cancer with 80% accuracy [51]. This study underscores the remarkable potential of circulating miRNAs in early, pre-symptomatic disease diagnosis.

Key Points 3

- Circulating plasma miRNA signatures may provide a novel diagnosis method for early, pre-symptomatic HCC patients, and may prove useful as prognosis biomarkers
- The field of miRNA-based gene therapy holds promise for HCC therapy by potentially affecting several targets with only one miRNA; yet more research is required in particular concerning possible off-targeting

Circulating miRNAs associated with HCC

As described above, many miRNAs are dysregulated in HCC. Therefore, it is anticipated that circulating miRNAs are also affected during HCC progression. A few studies reported altered levels of circulating miRNAs in association with HCC (Table 2). For instance, the serum level of miR-221 was shown to be 4.8-fold elevated in HCC patients [52]. Additionally, high level of miR-221 positively correlated with cirrhosis, tumor size and tumor stage, and negatively correlated with overall survival. These promising results should be validated in a larger patient cohort; nevertheless, miR-221 serum level monitoring could be of clinical relevance as a potential diagnosis tool and biomarker of treatment efficacy. Indeed, no optimal blood tumor marker has been developed so far, the performance of α-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive AFP (AFP-L3) and des-γ-carboxyprothrombin (DCP) [53] is limited in a surveillance mode and for early HCC detection. In addition, the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines (July 2010) discarded AFP for surveillance and diagnosis. Therefore, there is a need for novel markers that would combine the less invasiveness of a blood test and serve as a reliable early detection method. miRNAs definitely have this potential because not only they can be detected in plasma, but their sensitivity and stability are suitable for a clinical setting. Depending on the method, as little as one copy can be detected (see paragraph on detection). Nevertheless, appropriate controls should be used, since HCC is most often accompanied by viral infection, cirrhosis, or other underlying liver conditions. Therefore, in order to assess the HCC-specificity of a miRNA, it is critical to ensure not only an age- and gender-matched control group but they should also be matched for etiology and severity of underlying liver disease. For instance, the miRNA profile of three patient groups was compared: 105 patients with HCC (19.1% HBV, 62.9% HCV, 17.1% other etiology), 107 with chronic liver disease (CLD; 7.5% HBV, 55.1% HCV, 37.4% other etiology) and 71 normal controls [54]. In another study, miR-16 and miR-199a levels were decreased in serum and significantly associated with HCC [54]. miR-16 was more sensitive as HCC detection marker than AFP, DCP, and AFP-L3. Combination of miR-16 with AFP, DCP, and AFP-L3 allowed detection of 92.4% HCC cases with a high specificity (78.5%), and interestingly, it could detect tumors $\leqslant$3 cm with the same sensitivity (92.4%). This research demonstrates the feasibility of plasma markers for diagnosis of HCC. Circulating miRNAs could therefore be used as a first-line testing in HCC patients if they would outperform the currently used tumor markers. The discovery of circulating miRNAs offers interesting clinical perspectives but this field of research is quite recent and more work has to be done. It remains to be established which miRNA can

Table 1. Key cellular miRNAs dysregulated in HCC (studies based on patient material) compared to the healthy liver (HL).

miRNA	Dysregulation in HCC	Experimental settings	HCC etiology	miRNA detection method	[Ref.]
let-7a	Down	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
let-7a	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
let-7a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
let-7b	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
let-7c	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
let-7d	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
let-7e	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
let-7f	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
let-7g	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-9-3p	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-9	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-10a	Up	3 HL, 43 HCC	HCV	RT-qPCR	Varnholt *et al.*, 2008 [84]
miR-10b	Up	4 HL, 28 HCC	Mixed etiologies	RT-qPCR	Ladeiro *et al.*, 2008 [35]
miR-15a	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-15a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-16	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-17	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-17	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-18a	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-18a	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-18b	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-18	Down	22 HL, 24 HCC including 22 pairs	Mixed etiologies	Microarray	Murakami *et al.*, 2006 [82]
miR-18	Up	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-19b	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-20a	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-21	Up	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-21	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-21	Up	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-21	Up	4 HL, 28 HCC	Mixed etiologies	RT-qPCR	Ladeiro *et al.*, 2008 [35]
miR-21	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-22	Down	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-24	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-25	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-25	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-25	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-27a	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-27a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-29c	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-33	Up	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-34a	Up	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-92	Down	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-92	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-93	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-93	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-96	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-99a	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-99a	Down	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-100	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-100	Up	3 HL, 43 HCC	HCV	RT-qPCR	Varnholt *et al.*, 2008 [84]

Table 1. continued.

miRNA	Dysregulation in HCC	Experimental settings	HCC etiology	miRNA detection method	[Ref.]
miR-101	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-101	Down	17 paired HL and HCC	Mainly HBV	Northern blot	Su *et al.*, 2009 [83]
miR-101	Down	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-106b	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-107	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-122a	Down	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-122a	Up	3 HL, 43 HCC	HCV	RT-qPCR	Varnholt *et al.*, 2008 [84]
miR-122a	Down	4 HL, 28 HCC	Mixed etiologies	RT-qPCR	Ladeiro *et al.*, 2008 [35]
miR-122a	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-122a	Down	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-122a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-122	Down	9 paired HL and T1-HCC (stage 1 TNM) and 11 paired HL and T3-HCC		Northern blot	Tsai *et al.*, 2009 [41]
miR-124a	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-124a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-124	Down	In 11 out of 19 paired HCC and HL	NI	RT-qPCR	Furuta *et al.*, 2009 [21]
miR-125a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-125a	Down	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-125a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-125a	Down	22 HL, 24 HCC including 22 pairs	Mixed etiologies	Microarray	Murakami *et al.*, 2006 [82]
miR-125b	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-125b	Down	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-125b	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-126-3p	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-126	Down	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-126	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-127-3p	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-128b	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-129	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-130a	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-130a	Down	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-130a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-130b	Up	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-132	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-135a	Up	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-136	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-137	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-139	Down	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-139	Down	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-141	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-142	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-143	Up	25 paired HL and HCC	12 HBV-, 13 HBV+	RT-qPCR	Zhang *et al.*, 2009 [87]
miR-143	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-143	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-145	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-145	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-145	Down	3 HL, 43 HCC	HCV	RT-qPCR	Varnholt *et al.*, 2008 [84]
miR-145	Down	19 paired HL and HCC	Mixed etiologies, mainly alcohol	RT-qPCR	Borel *et al.*, 2011 [34]

Table 1. continued.

miRNA	Dysregulation in HCC	Experimental settings	HCC etiology	miRNA detection method	[Ref.]
miR-145	Down	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-146	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-148a	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-150	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-150	Down	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-151	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-152	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-155	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-155	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-181a	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-181c	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-182-3p	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-182	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-183	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-185	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-186	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-194	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-195	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-195	Down	22 HL, 24 HCC including 22 pairs	Mixed etiologies	Microarray	Murakami *et al.*, 2006 [82]
miR-195	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-195	Up	10 paired HL and HCC	HBV-, HCV-	Microarray	Huang *et al.*, 2008 [79]
miR-195	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-198	Down	3 HL, 43 HCC	HCV	RT-qPCR	Varnholt *et al.*, 2008 [84]
miR-199a-3p	Down	19 paired HL and HCC	Mixed etiologies, mainly alcohol	RT-qPCR	Borel *et al.*, 2011 [34]
miR-199a-3p	Down	22 HL, 24 HCC including 22 pairs	Mixed etiologies	Microarray	Murakami *et al.*, 2006 [82]
miR-199a-3p	Down	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-199a	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-199a	Down	22 HL, 24 HCC including 22 pairs	Mixed etiologies	Microarray	Murakami *et al.*, 2006 [82]
miR-199a	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-199a	Down	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-199a	Down	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-199a	Down	19 paired HL and HCC	Mixed etiologies, mainly alcohol	RT-qPCR	Borel *et al.*, 2011 [34]
miR-199a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-199b-3p	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-199b	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-199b	Down	19 paired HL and HCC	Mixed etiologies, mainly alcohol	RT-qPCR	Borel *et al.*, 2011 [34]
miR-199b	Down	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-200a	Down	22 HL, 24 HCC including 22 pairs	Mixed etiologies	Microarray	Murakami *et al.*, 2006 [82]
miR-200a	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-200b	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-200b	Down	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-203	Down	4 HL, 28 HCC	Mixed etiologies	RT-qPCR	Ladeiro *et al.*, 2008 [35]
miR-205	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-207	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-210	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-210	Up	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]

Table 1. continued.

miRNA	Dysregulation in HCC	Experimental settings	HCC etiology	miRNA detection method	[Ref.]
miR-213	Up	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-214	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-214	Down	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-214	Down	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-215	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-216a	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-216	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-221	Up	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-221	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-221	Up	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-221	Up	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-221	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-222	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-222	Up	42 paired HL and HCC	Mixed etiologies, mainly HBV	RT-qPCR	Wong *et al.*, 2008 [86]
miR-222	Up	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-222	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-222	Up	4 HL, 28 HCC	Mixed etiologies	RT-qPCR	Ladeiro *et al.*, 2008 [35]
miR-222	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-223	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-223	Down	42 paired HL and HCC	Mixed etiologies, mainly HBV	RT-qPCR	Wong *et al.*, 2008 [86]
miR-223	Down	21 HL, 17 HCC including 13 pairs	Mixed etiologies, mainly HCV	Microarray	Gramantieri *et al.*, 2007 [39]
miR-223	Down	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-224	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-224	Up	22 HL, 24 HCC including 22 pairs	Mixed etiologies	Microarray	Murakami *et al.*, 2006 [82]
miR-224	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-224	Up	4 HL, 28 HCC	Mixed etiologies	RT-qPCR	Ladeiro *et al.*, 2008 [35]
miR-224	Up	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-292-3p	Down	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-294	Up	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-301	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-301	Up	28 paired HL and HCC	Mixed etiologies	RT-qPCR	Jiang *et al.*, 2008 [80]
miR-324	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-324	Up	19 paired HL and HCC	HBV	NGS, Northern blot	Connolly *et al.*, 2008 [77]
miR-338	Down	20 paired HL and HCC	Mixed etiologies	Microarray	Huang *et al.*, 2009 [78]
miR-362	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-365	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-373	Up	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-374	Up	19 paired HL and HCC	NI	RT-qPCR	Wang *et al.*, 2008 [85]
miR-376a	Up	3 paired HL and HCC	NI	Microarray	Meng *et al.*, 2007 [81]
miR-378	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-382	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-422a	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-422b	Down	4 HL, 28 HCC	Mixed etiologies	RT-qPCR	Ladeiro *et al.*, 2008 [35]
miR-424	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-491	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-500	Up	40 paired HL and HCC	Mixed etiologies, mainly viral	RT-qPCR	Yamamoto *et al.*, 2009 [91]
miR-519	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-520c-3p	Down	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]
miR-527	Up	3 HL, 5 HCC	Mainly HBV	Microarray	Su *et al.*, 2009 [83]

NI, no information; RT-qPCR, reverse-template quantitative PCR; NGS, next-generation sequencing, HCV, hepatitis C virus; HBV, hepatitis B virus.

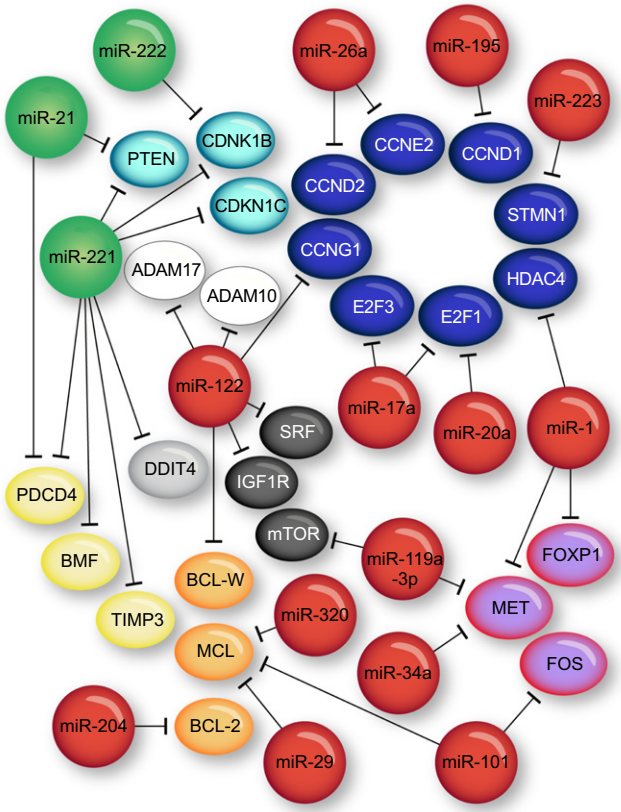

Fig. 2. miRNAs and their oncogenic and tumor-suppressing targets associated with HCC. Tumor suppressing miRNAs which are downregulated in HCC are indicated in red and oncogenic miRNAs which are upregulated in HCC are indicated in green. miRNAs post-transcriptionally repress the expression of genes involved in cell cycle regulation, (blue); cell proliferation, (grey); apoptosis, (yellow); cell migration and invasion, (white); and of proto-oncogenes, (purple). Genes having a positive effect on the cell process are marked in the dark shade, while genes having a negative effect on the cell process are marked in the light shade. For example, *CCND1* is marked in dark blue, because it causes cell cycle progression, and has been linked to the development and progression of cancer. Vice-versa, PTEN is marked in light blue as it causes cell cycle arrest. Additionally, one gene can be targeted by several miRNAs, for example tumor-suppressor PTEN was shown to be simultaneously repressed by oncogenic miR-21 and miR-221. Bar-headed lines indicate post-transcriptional repression of gene expression. Data presented in this figure is non-exhaustive and based on literature.

sensitively and reliably be correlated with the presence of HCC at early stages of disease development and prognosis.

Cellular miRNAs as therapeutic targets in HCC

Potential of miRNA-based gene therapy

RNAi was identified in *Caenorhabditis elegans* in 1998 by Fire and Mello [55] and in mammalian cells in 2001 by Tuschl [56]. Since then, RNAi has generated increasing interest and publications in diverse research areas. The combination of RNAi with the latest developments in the field of gene therapy which rendered it safer, and the delivery more efficient, opens the door to novel therapeutic perspectives. Among the many possibilities currently investigated, the use of cellular miRNAs as therapeutic agents is one of the most promising from a clinical point of view. Many miRNAs are downregulating genes that are highly relevant to HCC and therefore contribute to disease progression. Because a single miRNA could potentially affect several clinically relevant targets, artificially increasing or decreasing the expression level of a given miRNA offers interesting therapeutic perspectives. Such therapy could even be combined with local chemotherapy via the transarterial route (transarterial chemoembolization, TACE) to increase the treatment effectiveness. Nevertheless, because a miRNA can affect the expression of several downstream targets, modulating the expression of a miRNA of interest could also lead to undesirable off-target effects.

miRNA-based gene therapy for HCC

The main question raised by RNAi-based gene therapy is the delivery of the effector molecule, which should preferably be controllable, sustained and tissue-specific. Several groups have chosen for non-viral delivery of synthetic miRNA molecules. miRNA mimics or miRNA antagomirs can be repeatedly delivered locally or systemically and that would cause transient suppression of target gene expression. To prevent rapid degradation of naked molecules, miRNAs are modified or conjugated to improve stability or target delivery to a specific tissue. They can be incorporated into stable nucleic acid lipid particles (SNALPs), a lipid bilayer coated by polyethylene glycol (PEG) which will protect them from degradation, prevent immunostimulation and facilitate their uptake in endosomes [57]. Similarly, 2′O-methyl modifications increase the stability of synthetic molecules, additionally preventing off-targeting [58]. Mimicking the external viral protein structure, virus-like particles (VLP) can also be used for synthetic miRNA delivery, yet they are not suitable for all applications since they stimulate the immune response [59]. However, VLP vehicles take advantage of the natural virus tropism, e.g. HBV in the liver, and can efficiently mediate hepatic gene transfer [60]. Finally, miRNA conjugation to HDL, LDL or cholesterol will also lead to hepatic uptake [61]. However, even with the described improvements, miRNAs would need to be delivered monthly or bimonthly. Landford *et al.* inhibited miR-122 expression in 4 chimpanzees using SPC3649 LNA-modified oligonucleotides. Because miR-122 stimulates HCV RNA accumulation, miR-122 inhibition leads to an efficient suppression of HCV replication and stable reduction of viremia in chimpanzees [62]. A phase I trial for SPC3649 (Miravirsen from Santaris Pharma) showed that SPC3649 was well-tolerated and the drug is now in phase II trial. This approach holds promise for HCV patients and has the advantage that it should not allow the development of viral escape variants. Nevertheless Li *et al.* reported that mutations in miR-122 binding site in HCV 5′ UTR reduced SPC3649 treatment efficacy [63]. This indicates that viral escape could still be possible.

Alternatively, gene therapy using virally-delivered miRNAs is desirable when chronic and genetic diseases need to be treated. Viral delivery indeed can offer sustained expression after single dosing, however, in clinical perspective, it raises several questions concerning safety. Pri-miRNA can be delivered as an expression cassette using different types of viral vectors. The advantages and disadvantages of the viral vectors used in gene therapy clinical trials are summarized in Table 3. Briefly, the main disadvantage of lentivirus and retrovirus is their integration into the host genome, which raises safety issues. On the contrary,

Table 2. Circulating miRNAs candidate biomarkers for HCC.

miRNA	Dysregulation in plasma	Experimental settings	Result	[Ref.]
miR-16	Down	71 HL, 105 HCC, 107 CLD	Significant association with HCC, combination with traditional markers improves diagnostic	Qu *et al.*, 2011 [54]
miR-21	Up	89 HL, 101 HCC, 48 CH	Unspecific marker of liver injury	Xu *et al.*, 2010 [90]
miR-21	Up	20 HL, 46 HCC	Elevated in HCC	Li *et al.*, 2011 [52]
miR-92a	Down	10 HL, 10 HCC	Decreases post-resection	Shigoka *et al.*, 2010 [89]
miR-122	Up	89 HL, 101 HCC, 48 CH	Unspecific marker of liver injury	Xu *et al.*, 2010 [90]
miR-195	Down	71 HL, 105 HCC, 107 CLD	Significant association with HCC	Qu *et al.*, 2011 [54]
miR-199a	Down	71 HL, 105 HCC, 107 CLD	Significant association with HCC	Qu *et al.*, 2011 [54]
miR-221	Up	20 HL, 46 HCC	Elevated in 35/46 HCC, correlates with HCC stage and prognosis	Li *et al.*, 2011 [52]
miR-222	Up	20 HL, 46 HCC	Elevated in HCC	Li *et al.*, 2011 [52]
miR-223	Up	89 HL, 101 HCC, 48 CH	Unspecific marker of liver injury	Xu *et al.*, 2010 [90]
miR-224	Up	20 HL, 46 HCC	Elevated in HCC	Li *et al.*, 2011 [52]
miR-500	Up	10 HCC	Levels increased in 3/10 HCC, reduced 6 months post-operation	Yamamoto *et al.*, 2009 [91]
miR-885	Up	10 HL, 15 HCC, 10 LC	Marker of liver cirrhosis	Gui *et al.*, 2011 [88]

HL, healthy liver; HCC, hepatocellular carcinoma; CH, chronic hepatitis; LC, liver cirrhosis, CLD, chronic liver diseases.

Table 3. Gene therapy vectors used in clinical trials.

Viral vector	% Gene therapy clinical trials[1]	Maximal transgene size (kb)	Transgene expression	Tropism	Host genome interaction	Immunogenicity
Adenovirus	24.1	7.9	Transient	Broad	Non-integrating	High
Retrovirus	20.8	8	Long lasting	Dividing cells only	Integrating	Low
Vaccinia and poxvirus	13.5	250	Transient	Broad. Natural tumor tropism	Non-integrating	High
Adeno-associated virus	4.8	4.7	Potential long lasting	Broad	Non-integrating	Low
Herpes simplex	3.3	40	Potential long lasting	Broad	Non-integrating	High
Lentivirus	2.2	8	Long lasting	Both dividing and non-dividing cells	Integrating	Low

Kb, kilobases. [(1)]Source: gene therapy clinical trials worldwide database, *The Journal of Gene Medicine*, 2011.

adeno-associated virus (AAV) genome remains episomal, which gives it an advantageous safety profile. However, the episomal presence of AAV questions its relevance for cancer therapy. Up to now, adenovirus has been widely used in HCC gene therapy clinical trials [64–70], as well as *Vaccinia* virus [71]. Despite the fact that many virus-delivered "classical" gene therapy products have been developed for HCC and are currently progressing through clinical trial phases, no virus-delivered miRNA-based gene therapy has been tested in clinical trials yet. Indeed, more research still needs to be done to carefully evaluate potential risks of this approach. Kota *et al.* showed that self-complementary AAV serotype 8 (scAAV8)-delivery of miR-26a in tumor-bearing tet-o-myc; LAP-tTA mice restored miR-26a expression [72]. Re-expression of miR-26a specifically reduced cancer cell proliferation, induced tumor-specific apoptosis, and suppressed tumorogenesis. At 3 weeks post-transduction, most liver tissue in the control group was replaced with tumor while in 8 out of 10 mice of the treated group no or small tumors only were found [72]. This research demonstrated for the first time the therapeutic potential of restoring the expression of a dysregulated miRNA in the liver. Additional advantages of this approach are that the miRNA is well-tolerated, given that it is only downregulated in tumor cells, and therefore only tumor cells are affected. However, its significance in patients still needs to be determined.

With the first successes of RNA agents in clinical trials, it becomes clear that miRNAs and their inhibitors hold a great potential as therapeutics for different cancers including HCC.

Conclusions

It is now well-established that miRNAs are key players in many various biological processes, including development, cellular proliferation, apoptosis, and oncogenesis [3]. In HCC, miRNAs have aberrant processing and expression profiles, in addition, the profile of circulating miRNAs is also affected, which renders them potential biomarkers, with possible applications in diagnosis, especially for early, pre-symptomatic disease, and prognosis of HCC. One miRNA may target several genes that are involved in the development and maintenance of the HCC phenotype. Therefore, miRNA-based gene therapy offers promising perspectives compared to classical gene therapy for HCC. An additional advantage of miRNAs is that since they encode no protein, they are generally not immunogenic. However, activation of Toll-like

receptors (TLRs), involved in initiation of inflammatory responses to pathogens, can occur, as reviewed by O'Neill *et al.* [73], indicating that unanticipated off-target effects can occur in a clinical setting. For instance, the let-7 family regulates the expression of TLR4 and this can create off-target effects [74]. A positive aspect of gene therapy for HCC is that the delivery route seems not to be questioned, up to now direct imaging-guided intratumoral injection has been the most used strategy in clinical trials, but tumor-selective intra-arterial administration could be a good alternative [70]. In HCC, combination of classical and miRNA-based therapies appears a desirable goal. First, chemo- or radiation therapy can improve gene transfer efficiency and transgene expression [75,76]. Second, in the case of combined chemo- and gene- therapies, a direct co-injection of both via the intravenous route used for the TACE procedure, could be suitable, offering a gene therapy delivery route already clinically approved and in practice. The discovery of miRNA-mediated gene regulation as a fundamental post-transcriptional mechanism increases the complexity of cancer genetics. However, understanding the molecular mechanisms by which miRNAs regulate development and tumorogenesis may lead to novel concepts in the diagnosis and treatment of cancer. Besides the fact that miRNAs have shown promising results in pre-clinical studies, miRNA-based gene therapy is not yet suitable for routine clinical practice.

Conflict of interest

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