Function and clinical potential of microRNAs in hepatocellular carcinoma (Review)

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Abstract. MicroRNAs (miRNAs) are small non-coding RNAs involved in the initiation and progression of several types of human cancer, including hepatocellular carcinoma (HCC), which is one of the most common types of cancer and the third leading cause of cancer-related mortality worldwide. Mounting evidence has demonstrated that miRNAs play a vital role in HCC, hepatitis, alcoholic liver disease, liver cell development and the metabolic functions of the liver. The aim of the present review was to summarize the most recent findings on the functions of miRNAs in the liver and discuss their potential roles in the diagnosis, prognosis and treatment of HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer and third-leading cause of cancer-related mortality worldwide (1). Despite the advances in the management of HCC over the last decade, persistent remissions are usually not achieved and the high invasiveness and metastatic potential represent a major challenge in HCC treatment (2). Therefore, novel treatment options are required to improve the clinical outcome of HCC patients.

With the development of computational engines for microRNA (miRNA) target prediction, biochemical tools and techniques to modulate miRNA activity *in vitro* and *in vivo*, our knowledge of the miRNA field is rapidly expanding (3). miRNAs are a class of small non-coding RNAs that regulate a wide range of biological processes through altering the expression and translation of their target mRNA genes (4). Previous studies have demonstrated that miRNAs may be a potential target for cancer diagnosis and treatment. miRNAs, regulating 60% of human genes, are a powerful regulator of human physiological and pathological processes, including embryonic development, cell differentiation, tumorigenesis, cancer metastasis and tumor response to therapy (5-8). Thus, miRNAs may be used as diagnostic, prognostic and predictive biomarkers in cancer. Increasing evidence has demonstrated that abnormal expression of miRNAs in tumors results in the deregulation of the expression levels of oncogenes and tumor suppressors, which eventually promotes the proliferation of tumor cells (9-11). Therefore, miRNA-based anticancer therapies have emerged as an effective treatment option and may offer a curative potential in cancer therapy, either alone or in combination with other treatments (12). The aim of present study was to summarize the possible role of miRNA dysregulation in liver cancer and discuss the potential of miRNAs as diagnostic, prognostic and therapeutic biomarkers.

2. Introduction of miRNAs

The first small non-coding but functional RNAs to be identified over 20 years ago were lin-4 and let-7 (13,14), which were first identified as regulators controlling developmental timing in the nematode *Caenorhabditis elegans*. Since then, extensive studies have been conducted to investigate the role of miRNAs in multiple biological processes, ranging from embryonic development to the pathogenesis of various diseases, including cancer (7,15-17).

The biogenesis of miRNAs involves multiple steps, including transcription, nuclear processing, export and cytoplasmic processing (4,18). In the nucleus, miRNAs are transcribed as primary miRNA transcripts (pri-miRNAs)

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with 5'-end caps and 3'-end poly-A tails, mainly by RNA polymerase II (19-21). Pri-miRNAs consist of ≥1 hairpin structures, which finally become one or more functional miRNA(s) (22). The pri-miRNAs are located in intergenic and intragenic regions. The intergenic regions are processed by protein complexes, including nuclease, DiGeorge syndrome critical region gene 8 (DGCR8) and Drosha, while intragenic regions are processed by spliceosomes (23-25). Precursor miRNAs derived from the pri-miRNAs are exported from the nucleus to the cytoplasm by exportin-5 in a RanGTP-dependent manner (26), then further processed to the 22-nucleotide duplex by Dicer, a second RNase III endonuclease, and the double-stranded RNA-binding domain proteins TAR RNA-binding protein/protein activator of the interferon (IFN)-induced protein kinase, to form a duplex of mature miRNA. Constitutive disruption of either DGCR8 or Dicer, two key factors in the miRNA signaling pathway, results in a global loss of miRNAs (27). Ultimately, the miRNA duplex unwinds, and one of the strands associates with an argonaute protein within the RNA-induced silencing complex, where they direct gene expression by mRNA degradation or translational repression; the other miRNA strand is rapidly degraded (28).

3. miRNAs in liver development and homeostasis

As the largest gland in mammals, the liver serves as an endocrine and exocrine organ with numerous functions, including carbohydrate, lipid and amino acid metabolism, urea synthesis, detoxification of drugs and toxic endogenous compounds, bile production and plasma protein secretion (29). miRNAs play vital roles in several organ developmental and differentiation processes. Increasing evidence has demonstrated that miRNAs play an important role in regulating liver development and homeostasis (30). Hinton *et al* (27), Fu *et al* (31) and Kim *et al* (32) evaluated dynamic miRNA changes through analyzing definitive endoderm (DE) formation, murine and human embryonic stem cells (ESCs) and ESC-derived hepatocytes. The results of these previous studies indicated that different cell lines require definitive regulation by different miRNAs. For example, it is necessary that activin A-mediated DE formation is enhanced by the forced combined expression of RNAs, including miRNA (miR) 181, miR222, miR196a, miR196b, miR333-5p and let-7e. It was previously reported that a positive feedback loop between miR122 and hepatocyte nuclear factor 6 (HNF6) regulates proper hepatocyte-specific gene expression (33). During the process of liver differentiation and maturation, hepatoblasts exhibit morphological changes, such as epithelial-to-mesenchymal transition (EMT). Certain miRNAs are involved in the maintainance of the homeostasis of transition.

miR122, the most abundant miRNA in the liver, regulates the expression levels of 24 hepatocyte-specific genes, including HNF6, forkhead box protein A1 (FOXA1) and HNF4α. Furthermore, miR122 forms a positive feedback loop with FOXA1 and HNF4α to regulate hepatocyte maturation (34). Several other miRNAs are involved in the regulation of liver development and differentiation, including miR148a (35) and miR33 (36).

As the central organ of metabolism in mammals, the liver synthesizes plasma proteins and bile acids, maintains the energy equilibrium and detoxifies metabolic wastes and xenobiotics. The metabolism of glucose, the main energy source of the body, is primarily regulated by insulin and glucagon. The deletion of *DICER1* in mouse liver led to severe hypoglycemia in the fasting state as a result of glycogen depletion (37). Several miRNAs are associated with glucose metabolism, including miR122, miR34a, miR103/107, let-7 and miR143 (38). It has also been reported that miR122 and miR27b are associated with lipid metabolism, miR122, miR485-3p and let-7 are involved in iron metabolism, while miR132/142-3p/21, miR142-3p/21 and miR130b/185/34a are involved in the metabolism of drugs and xenobiotic substances (39). In conclusion, miRNAs play pivotal roles in regulating multiple aspects of liver physiology.

4. miRNAs and HCC development

As the miRNAs are vital regulators of liver function, their dysregulation is associated with liver dysfunction. HCC is the main type of liver cancer and the third most common cause of cancer-related mortality worldwide. miRNAs are often deregulated in HCC, and certain specific miRNAs are associated with the clinicopathological characteristics of HCC. It was recently demonstrated that miRNAs play critical roles in HCC progression and directly contribute to tumor cell proliferation, avoidance of apoptotic cell death and metastasis by targeting a large number of specific mRNAs. miRNAs may undergo aberrant regulation during carcinogenesis and act as oncogenes or tumor suppressor genes (30,40-43).

Chronic hepatitis caused by hepatotropic viruses, namely hepatitis B virus (HBV) and hepatitis C virus (HCV), are a major risk factor for HCC (44) and may affect the course of liver tumor development (45). Numerous genetic and epigenetic alterations are involved in hepatocellular carcinogenesis. Park *et al* (46) demonstrated that the inflammatory responses induced by obesity or administration of diethylnitrosamine contribute to HCC development in mice.

It was previously indicated that transient inhibition of HNF4α, which is essential for liver development and hepatocyte function, initiates hepatocellular transformation through an miRNA-inflammatory feedback loop circuit consisting of miR124, interleukin 6 receptor (IL6R), signal transducer and activator of transcription 3 (STAT3), miR24 and miR629 (47). In this feedback loop circuit, miR24 and miR629 inhibit HNF4α expression, directly resulting in hepatocellular transformation. STAT3 is a direct regulator of miR24 and miR629 expression, the increase of which exerts vital effects on HCC initiation. As a direct downstream effector of HNF4α activity, miR124 targets IL6R and consequently modulates the IL6R/STAT3 pathway during hepatocellular transformation. The activation of this circuit suppresses HNF4α expression to sustain oncogenesis. Administration of miR124, a modulator of inflammatory signaling, induces tumor-specific apoptosis, thus suppressing hepatocellular carcinogenesis. Accordingly, manipulation of this miRNA feedback inflammatory loop may be of clinical value in the treatment of liver cancer. In order to elucidate the mechanisms underlying the effect of miRNAs on HCC development, some of the associated miRNAs are summarized below.

miRNAs and hepatic cancer stem cells (CSCs). Mounting evidence in cancer biology indicates that a small population of cells in tumor tissues, referred to as CSCs, have the ability to

maintain tumorigenesis (44). CSCs in the liver sustain tumor formation and development and endow tumor cells with stem cell properties (49,50).

As a result of the high renewal capacity, currently available chemotherapeutic and radiotherapeutic regimens fail to eliminate the bulk of cancer cells (51,52). Several signaling pathways, including MET, MYC, transforming growth factor-β, Hedgehog, p53, WNT/β-catenin and epidermal growth factor, have been demonstrated in hepatocarcinogenesis, a number of which may overlap with other pathways associated with hepatic progenitor cells (53). According to Oishi and Wang (54), hepatic progenitor cells are considered to be the origin of a proportion of HCCs, whereas miR181 may be involved in HCC progression by targeting HCC CSCs. Ji *et al* (55) demonstrated that conserved miR181 family members were highly expressed in embryonic livers and isolated hepatic stem cells using a global microarray-based miRNA profiling approach, followed by validation with reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Furthermore, deletion of miR181 resulted in a reduction in the number of HCC cells and tumor-initiating ability; however, exogenous miR181 expression in HCC cells restored their growth. miR181 has been found to directly target hepatic transcriptional regulators of differentiation and is an inhibitor of WNT/β-catenin signaling. It may be concluded that miR181 is involved in the regulation of human liver CSCs (55). Therefore, miRNAs are vital regulators of the maintainance of stemness characteristics of hepatic CSCs through modulation of tumor-suppressive and oncogenic signaling pathways associated with tumorigenesis and tumor development.

miRNAs and cell cycle regulation. As defects in cell cycle control are among the hallmarks of HCC, multiple tumor activators and suppressors involved in cell cycle regulation are often aberrantly targeted by deregulated miRNAs in HCC. For example, regulated miRNAs may target essential cell cycle regulators, including cell cycle inhibitors of the cyclin-dependent kinase (CDK) interacting protein/kinase inhibitory protein family, cyclin-CDK complexes, the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin signaling cascade, and other cell growth regulatory genes (43). The direct targets of miR26a involved in the cell cycle are cyclins D2 and E2, from a family of proteins that control cell cycle progression by activating CDK cyclins, which exhibit reduced expression in HCC (56). The expression of miR26a induces HCC cell cycle arrest through direct targeting of these two cyclins.

miR122 suppresses HCC cell growth by directly targeting cyclin G1 expression (57). miR122 shortens the G2-M phase, leading to a reduction in the invasive ability of HCC-derived cells through modulating cyclin G1, p53 protein stability and transcriptional activity (58). In addition, miR124, which is silenced through CpG methylation in HCC, targets CDK6 to induce cell cycle arrest at the G1-S checkpoint (59).

miRNAs and apoptosis. The ability to escape apoptosis enables tumor cells to survive in the tumor environment, even after invading distal tissue. Cytochrome *c*, a potent catalyst of apoptosis, is released from mitochondria when proapoptotic signals emerge. An increasing number of studies have demonstrated that miRNAs play an important role in regulating the B-cell lymphoma-2 (Bcl-2) family of proteins, which are associated with mitochondrial apoptosis signaling. The Bcl-2 protein family includes Bcl-2-interacting mediator of cell death (Bim), Bcl-2-modifying factor (Bmf), Bcl-2, Bcl-2-like protein 2 (Bcl-W), Bcl-extra large (Bcl-XL) and myeloid leukemia cell differentiation protein (Mcl-1). Shimizu *et al* (60) demonstrated that the let-7 family of miRNAs enhanced sorafenib-induced apoptosis by repressing Bcl-XL expression in HCC. miR29 may promote apoptosis by targeting Mcl-1 and Bcl-2 in the mitochondrial pathway (61). Therefore, upregulated let-7 and miR29 expression may improve the sensitivity of HCC cells to certain apoptotic signals, thus exerting antitumor effects. Bcl-2, Bcl-W, Bcl-XL and Mcl-1 exert antiapoptotic effects; however, Bim and Bmf exert a proapoptotic effect. miR221 and miR25 exert their antiapoptotic effect through targeting and inhibiting Bmf and Bim, respectively (62,63). Other apoptosis-related genes are also targeted by miRNAs. Yang *et al* (64) reported that miR602 repressed HCC cell apoptosis by inhibiting Ras association domain family 1, isoform A. In addition to cell cycle regulation, miR221 and miR222 render tumor cells more resistant to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis through inhibiting the expression of phosphatase and tensin homolog (PTEN) and metalloproteinase inhibitor 3 (65).

miRNAs and cancer metastasis. The aberrant expression of miRNAs has been found to be closely associated with HCC cell metastasis (66). Among these miRNAs, the reduced expression of miR122 in HCC may suppress the hepatic phenotype and enhance the metastatic properties of HCC (67). miR34a downregulates c-Met in HCC, resulting in the reduction of cell migration and invasion (68). By contrast, miR21 promotes cell growth, invasion and metastasis by inhibiting PTEN gene activity in HCC (69). miR224 was found to be highly expressed during extensive metastasis of HCC (70). Li *et al* (71) confirmed that miR224 promotes the expression of the tumor invasion-associated proteins phosphorylated-p21-activated kinase 4 and matrix metalloproteinase-9 by directly targeting homeobox D10. A series of miRNAs, including 3 upregulated miRNAs (miR10a, miR100 and miR122) and 2 downregulated miRNAs (miR145 and miR198), were found to be expressed in HCV-HCC tissues, but not in normal liver parenchyma (72). In addition, decreased levels of miR126 have been specifically observed in the HCC subgroups associated with alcohol consumption (73).

Other miRNAs are also involved in the regulation of HCC invasion and metastasis, including miR17-5p (74), miR30d (75) and miR151 (76). In conclusion, miRNAs may be considered as a neoteric modulator of tumor cell migration and invasion in HCC, and provide a novel approach to the treatment of HCC. More miRNAs involved in HCC development are listed in Table I.

5. miRNAs and clinical management of HCC

miRNAs for HCC diagnosis. Due to the lack of reliable markers for early diagnosis, the overall 5-year survival rate of HCC remains extremely low (77). Liver damage induced by multiple agents, ranging from chemicals to viruses, is clinically

evaluated by measurement of serum aminotransferase levels (alanine and aspartate aminotransferase). However, these markers are associated with several limitations, including the requirement of fresh blood samples, lack of tissue specificity, and the inability to distinguish between hepatocyte damage and inflammation. Similarly, the marker α-fetoprotein, traditionally used to monitor patients at high risk of HCC, may only be detected in a proportion of HCC patients, with an associated risk of false-negative and -positive results (78). Therefore, there is an urgent need for novel molecular biomarkers to assist in the early diagnosis and prognosis of HCC. Increasing evidence suggests that unique miRNA signatures may serve as valuable diagnostic and prognostic biomarkers in HCC (79).

miRNAs are present in various body fluids, including serum and plasma (80,81). Furthermore, miRNAs exhibit significant stability under extreme conditions, such as low pH (acidic environment) and resistance to RNAase, and are considered as alternative non-invasive biomarkers (80,82). Several studies have demonstrated that specific circulating miRNAs are present in various diseases (81,82). The above-mentioned traits of miRNAs render them optimal biomarkers for liver diseases.

Since miRNAs have the advantages of being released from cancer cells into body fluids, accessible non-invasiveness and stability, a number of unique circulating miRNAs have the potential to serve as diagnostic markers for HCC. In 2010, Li *et al* (83) employed a 'proof-of-principle' approach, which included Solexa sequencing of pooled serum samples, followed by multiple RT-qPCR validation sets at an individual level, and identified unique expression profiles for HBV- and HCC-related serum miRNAs. Ultimately, the results suggested that let-7f, miR25 and miR375 may distinguish HCC patients from healthy subjects [area under the curve (AUC) = 99.67±0.15%; sensitivity, 97.9%; and specificity, 99.1%] (83). Additionally, miR23a, miR23b, miR342-3p, miR375 and miR423 may differentiate between HBV-positive HCC patients and control subjects (AUC=99.9±0.1%; sensitivity, 96.9%; and specificity, 99.4%) (83). Zhou *et al* (84) made these findings more comprehensive in another study; their results suggested that a 7-miRNA signature, including 3 upregulated miRNAs (miR21, miR192 and miR801) and 4 downregulated miRNAs (miR26a, miR27a, miR122 and miR223), may be used to distinguish HCC patients from healthy subjects, chronic hepatitis B and cirrhosis patients. Together with other biochemical tests, quantitative analysis of circulating miRNA may significantly improve the early detection rate and screening of potential HCC patients.

miRNAs for prediction of prognosis in HCC. In recent years, an increasing number of studies have indicated that miRNAs may be applied not only as diagnostic biomarkers, but also as prognostic factors for cancer. Identifying relevant biomarkers may help classify patients at a higher risk for tumor recurrence following radical resection for HCC. Furthermore, relevant biomarkers play a pivotal role in improving the therapeutic strategies for patients with early-stage disease, without evident vascular invasion, regional lymph node or distant metastasis (85). In order to simplify the evaluation of prognostic miRNA signatures in cancer, Aguirre-Gamboa *et al* (86) developed SurvMicro, a freely accessible and easy to use web tool that assesses miRNA signatures from publicly available miRNA profiles using multivariate survival analysis. SurvMicro consists of a wide and updated database of >40 cohorts in different tissues, and is a bioinformatics tool that aids the evaluation of multivariate prognostic miRNA signatures in several types of cancer, including HCC. This bioinformatics tool provides strong evidence regarding the potential of miRNAs as biomarkers for the prognosis of HCC.

Of note, Zhu *et al* (87) reported that the expression of miR29a-5p in formalin-fixed paraffin-embedded HCC tissues may provide useful information for predicting early recurrence following HCC resection through studying two independent large cohorts of patients with long-term follow-up; they found that the sensitivity and specificity of miR29a-5p as a predictor of early recurrence of Barcelona Clinic Liver Cancer stage 0/A HCC were 74.2 and 68.2%, respectively, by multivariate analysis. However, the mechanism underlying the involvement of miR29a-5p in early recurrence of HCC has not yet been elucidated, although it may include HCC invasion and metastasis, which are the main causes of early recurrence following HCC resection (88,89). In addition, other miRNAs are considered as potential biomarkers for the prognosis of HCC. For example, downregulation of the expression of miR26 indicates that HCC patients are sensitive to IFN-α therapy (90). A characteristic feature of tumors with low miR26 expression is unique activation of IFN-α signaling via the nuclear factor-κB-IL-6 signaling pathway. As a consequence, HCC cells exhibiting low expression of miR26 become more sensitive to growth repression by IFN-α through IL-6-STAT3 signaling. In conclusion, miRNA profiling may be able to predict clinical response to therapy for HCC and provide novel prognostic tools, paving the way for the personalized therapy of HCC patients.

miRNAs in HCC treatment. The mechanisms underlying miRNAs acting as regulators of multiple aspects of liver development is an appealing research focus in HCC treatment. Preclinical models constructed to elucidate the biological role of any specific miRNA lay the foundation for the emergence of the first indications of the feasibility and efficacy of miRNA-based therapy in cancer. The strategies targeting miRNA expression in HCC mainly encompass direct and indirect methods of preventing the expression of an oncogenic miRNAs or reintroducing a tumor suppressor miRNA that is lost in cancer, and using drugs to modulate miRNA expression by separately targeting their transcription and processing. For example, silencing oncogenic miR221 generates a proapoptotic and antiproliferative response *in vitro* in different cellular models of HCC (91), while reinduction of miR26a suppresses cancer cell proliferation and activates tumor-specific apoptosis *in vivo*, resulting in significant suppression of tumor progression without toxicity (57). By contrast, miRNAs may be used as adjuvant tools, largely due to their involvement in specific networks, including apoptosis, proliferation, or receptor-driven pathways. Therefore, miRNAs may affect the response of HCC to targeted therapy or chemotherapy. Xu *et al* (92) demonstrated that miR122 renders HCC cells sensitive to Adriamycin® and vincristine by reducing the expression of multidrug resistance-related genes, including the antiapoptotic genes Bcl-W and cyclin B1. DNA methylation of miR193a-3p enhances the resistance of HCC cells to 5-fluorouracil through

Table I. Dysregulated microRNAs in hepatocellular carcinoma.

	Upregulated		Downregulated	
Function	miRs	References	miRs	References
Proliferation	miR155, miR18a, miR210, miR221, miR224, miR519d, miR590-5p	(101-108)	let-7a, let-7b, let-7c, let-7d, let-7f-1, miR1, miR124, miR200a,miR203, miR219-5p, miR223, miR376a, miR449, miR450a, miR520b	(109-122)
Apoptosis	miR210, miR221, miR224, miR519d	(58,62,91, 105-107, 123,124)	let-7a, let-7b, let-7c, let-7d, let-7f-1, miR101, miR122, miR125b, miR195, miR376a, miR449	(60,109,111, 115-117,122, 125-137)
Cell cycle	miR373	(140)	miR138, miR195, miR26a/b	(134,138,139)
Metastasis	miR10a, miR135a, miR143, miR181b, miR182, miR21, miR200a, miR210, miR224, miR301a, miR550a, miR590-5p	(68,69,105, 107,120, 144-157)	let-7g, miR122, miR125a, miR125b, miR139, miR3a, miR7	(109,114,126, 133,141-143)

miR, microRNA.

downregulation of the serine/arginine-rich splicing factor 2, resulting in upregulation of the proapoptotic splicing form of caspase 2 (93). IFN is widely employed in the treatment of HCC. However, miR146a induces resistance of HCC cells to IFN-α by downregulating mothers against decapentaplegic homolog 4 (94).

miRNAs may alter the sensitivity of tumor cells to chemotherapy and/or radiotherapy. Weidhaas *et al* (95) reported that specific inhibition of miR210 may increase the sensitivity of HCC cells to radiotherapy. A direct target of miR210 in human HCC cells is apoptosis-inducing factor M3 (AIFM3), also referred to as AIF-like, as it is a gene homolog of AIF. AIFM3 is mainly present in mitochondria, resulting in cytochrome *c* release and apoptosis in a caspase-dependent manner (96,97). AIFM3 downregulation by small interfering RNA-impaired radiation-induced apoptosis in human HCC cells was associated with reduced miR210 expression. miR210 downregulation enhances radiation-induced apoptosis in human HCC cells by targeting the AIFM3 gene (95). Therefore, specific regulation of miRNAs in combination with radiotherapy may be expected to exert strong antitumor effects on HCC cells.

Therapies based on directly targeting miRNAs are faced with several challenges. First, steadily and effectively delivering a therapeutic RNA to target tissues remains a major obstacle. A direct method is difficult, since it would involve exiting the circulatory system, transiting the cell membrane, escaping from endosomal vesicles into the cytoplasm and avoiding being filtered and excreted by the kidney. In addition, escaping removal by phagocytic immune cells in the bloodstream, such as macrophages and monocytes, is another challenge.

A number of studies have investigated methods to overcome these difficulties. Among the established approaches to *in vivo* delivery of miRNAs, adeno-associated viral vectors have been considered to be a promising therapeutic strategy for cancer, due to the lower risk of vector-related toxicities and the higher gene transfer efficacy (57,98). In 2009, Kota *et al* (56) utilized an adeno-associated virus carrying the miR26a gene to infect a mouse model of HCC, resulting in the inhibition of cancer cell proliferation and induction of tumor-specific apoptosis. However, the utilization of a viral system to reintroduce an miRNA is inevitably associated with certain shortcomings. The delivered material may be integrated into the host DNA or remain episomal, depending upon the nature of the system. For example, retroviral and lentiviral vectors integrate their DNA into the host genome, resulting in the risk of insertional mutagenesis and activation of protooncogenes due to the unpredictable site of integration. In addition to miRNA delivery using viral vectors, artificially synthesized miRNA or anti-miRNA oligonucleotides (AMOs) are other noteworthy therapeutic approaches (99). Since synthesized miRNA or AMOs consist of single-stranded 2'-*O*-methyl-modified antisense oligonucleotides fully complementary to the predicted miRNA binding sites in the 3'-untranslated region of a specific target mRNA, this strategy may markedly reduce unwanted or off-target effects. Hatakeyama *et al* (100) encapsulated AMOs including 2-*O*-methyl and phosphorothioate modifications against miR122 (AMO122) into the YSK05-MEND, which is a pH-sensitive multifunctional envelope-type nanodevice (MEND) containing a pH-sensitive lipid YSK05. YSK05-MEND was then utilized to regulate liver-specific miR122. Compared with Lipofectamine® 2000 (LFN2k), YSK05-MEND displayed a higher activity in liver cancer cells due to efficient endosomal escape, despite the lower uptake. Furthermore, YSK05-MEND exhibited minimal cytotoxicity at 100 nM of AMO122 in treated cells, whereas LFN2k exhibited cytotoxicity at 50 nM. Compared with free AMO122, the YSK05-MEND delivered higher amounts of AMO122 to the liver. In addition, free AMO122 is more

easily eliminated via the kidney due to its molecular weight. The dose at which systemic administration of YSK05-MEND results in the knockdown of miR122 and an increase in target gene expression in the liver, with a subsequent reduction of plasma cholesterol, is significantly lower compared with that of free AMO122. The duration of the effect of AMO122 delivered by YSK05-MEND is also longer compared with that of free AMO122. In conclusion, these results suggest that YSK05-MEND is a promising system for *in vivo* delivery of AMOs to the liver (100).

6. Conclusions and perspectives

Small non-coding RNAs as regulators of gene expression have been demonstrated to be involved in all biological systems. Several miRNAs are deregulated to promote hepatocellular carcinogenesis through inducing translational inhibition and degradation of target mRNAs critical for HCC development. miRNAs may be used as biomarkers for diagnosis and prognosis and may be a potential therapeutic tool for HCC. Although significant progress has been achieved in the miRNA field, a number of questions remain to be further elucidated. Undoubtedly, the identification of novel miRNAs and novel miRNA functions in liver development and abnormity pave the way to designing effective and safe strategies for the diagnosis and treatment of this life-threatening disease. With the identification of HCC-associated miRNA signatures and the overcoming of certain obstacles, including unwanted off-target effects and inefficient miRNA delivery, the use of miRNAs as a diagnostic and therapeutic tool in HCC appears to be a promising research focus in the immediate future.

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