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Review

MicroRNAs in hepatocellular carcinoma：pathogenesis and therapy

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MicroRNAs (miRNAs) are an astonishing new class of gene regulators, and it had been documented that these molecules play a crucial role in various biological processes, including development, differentiation, metabolism, hematopoiesis, cell cycle and apoptosis. Most importantly, deregulated miRNAs have been implicated in cancer pathogenesis, behaving either as oncogenes or tumor suppressors. Hepatocellular carcinoma (HCC) is one of the most common and aggressive human malignancies worldwide. Recent work indicates selective overexpression of oncogenic miRNAs and down-regulation of tumor suppressive miRNAs in HCC. The aim of this review is to summarize results from key studies characterizing the miRNA expression profiles of HCC and give a brief introduction on the functional analyses of certain key miRNAs. In addition, this review outlines novel therapeutic strategies based on miRNA modulation.

Keywords: MicroRNA, hepatocellular carcinoma, pathogenesis, therapy.

INTRODUCTION

MicroRNAs (miRNAs) are a novel class of endogenous 20 to 22 nucleotide (nt) non-coding RNA molecules that regulate gene expression at the transcriptional or posttranscriptional level. Constituting only 1% to 3% of the human genome, miRNAs are estimated to control approximately 30% of all coding genes in the human genome (Filipowicz et al., 2008). It has been documented that miRNAs regulate critical biological processes such as development, differentiation, metabolism, hematopoiesis, cell cycle and apoptosis (He and Hannon, 2004). Further studies revealed that miRNAs are de-regulated in diverse diseases such as cancer, immune disorders, and viral infections and play a role in their pathogenesis (Calin and Croce, 2006).

Biogenesis of miRNA is a complex process initiated by the nuclear processing of a long primary transcript (pri-miRNA) into precursor miRNAs (pre-miRNAs) of 60-110 nucleotides in length by the ribonuclease Drosha/DGCR8. The pre-miRNA has a characteristic hairpin structure and is then translocated to the cytoplasm, where it is further cleaved by the ribonuclease Dicer into a mature miRNA duplex. Subsequent incorporation of the mature miRNA duplex into the RNA-induced silencing complex (RISC) leads to the degradation of the duplex into a single-stranded, mature miRNA. Within the RISC, the mature miRNA can bind to its target mRNAs, the binding affinity depends on the mature miRNA sequence, in particular the seed match (from nucleotides 2 to 8) that recognize complementarity sequences in the 3'UTR of the target mRNAs, resulting in gene silencing by translational repression (when there is imperfect complementarity between the miRNA sequence and its target mRNA) or/and mRNA degradation, when there is a perfect complementarity with the 3'UTR of the target mRNAs (Garzon et al., 2006; Lennon et al., 2009; Bartel, 2004).

Increasing evidence suggests miRNAs have been implicated in various human cancers. Genome-wide studies have shown that over half of all known human miRNA genes are located at fragile sites and genomic regions involved in cancers. In addition, abnormal miRNA

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expression have recently been reported in many human cancers, including prostate cancer, pancreatic cancer, thyroid cancer, melanoma, ovarian cancer, breast cancer and colon cancer (Calin et al., 2004; Lee et al., 2007; Shi et al., 2007; Mitomo et al., 2008, Felicetti et al., 2008; Yang et al., 2008; Schetter et al., 2008; Iorio et al., 2005).

MiRNAs involved in cancer development can behave either as oncogenes or tumor suppressors. Oncogenic miRNAs are generally overexpressed in tumors, whereas tumor suppressor miRNAs are generally down-regulated (Kent and Mendell, 2006). For example, the miR-15a and miR-16-1 cluster, which is down-regulated in approximately 70% of chronic lymphocytic leukemia cases, exert the tumor suppressor effect by targeting the oncogene Bcl-2 (Cimmino et al., 2005). Other miRNAs with known tumor suppressor functions are miR-29 and lethal-7 (*let-7*) in lung cancer, miR-10b, miR-125b,and miR-145 in breast cancer, and miR-34 in ovarian and colon cancers (Iorio et al., 2005; Fabbri et al., 2007; Johnson et al., 2007; Corney et al., 2007; Tazawa et al., 2007). Conversely, miRNAs with tumor-promoting activities include miR-21 in breast cancer and glioblastoma, miR-155 in many types of cancers, and miR-373 and miR-520c as metastasis-promoting miRNAs in breast cancer (Lee et al., 2007; Schetter et al., 2004; Iorio et al., 2005; Chan et al., 2005; Costinean et al., 2006).

Hepatocellular carcinoma (HCC) is a primary cancer of the liver. It is the fifth most common malignant cancer and the third leading cause of cancer-related death. Worldwide, there were about 626,000 new HCC cases and nearly 600,000 HCC-related deaths each year (Jemal et al., 2011). Surgical resection and liver transplantation are currently the best curative options to treat HCC. However, only 5–15% of HCC patients are eligible for surgical intervention (El-Serag et al., 2008). Clearly, there is a pressing need to elucidate the mechanisms underlying HCC pathogenesis and to develop novel and effective diagnostic and therapeutic techniques to improve clinical outcomes in patients with HCC. Most recently, the discovery of aberrantly expressed miRNAs in HCC further improved our understanding of this disease (Mott, 2009). Moreover, compelling evidence demonstrates that miRNAs have important effects in HCC progression and contribute to the cell proliferation, avoidance of apoptosis, and metastasis of HCC (Negrini et al., 2011). In this review, we discuss current advances in miRNA research, focusing on the roles of miRNAs in HCC and the potential therapeutic implications.

Important miRNAs Alterations in HCC

Several recently conducted miRNA profiling studies revealed that the expression of miRNAs is deregulated in human HCC in comparison with matched non-neoplastic tissue. Among the aberrantly expressed miRNAs, some specific miRNAs were identified in more than one study and some others were were found to be associated with the clinicopathological features of HCC, such as metastasis, recurrence, and prognosis, suggesting a list of miRNAs most likely involved in liver tumorigenesis and merit further investigation. The aberrantly expressed miRNAs in HCC identified in more than one study is reported in Table 1, and those associated with HCC clinical significance in Table 2.

Key miRNAs in HCC

miR-21

miR-21 is consistently up-regulated in many human cancers, including HCC (Krichevsky and Gabriely, 2009). Over-expression of miR-21 in cultured human cells led to protection from apoptosis (Chan et al., 2005), increased tumor cell proliferation and migration (Si et al., 2007), promoted soft agar colony formation and an in vitro metastatic phenotype (Connolly et al., 2010). In a transgenic mouse model, overexpression of miR-21 led to a pre-B malignant lymphoma, that completely regressed, when miR-21 was inactivated, partly as a result of apoptosis (Medina et al., 2010). These studies demonstrate that miR-21 is a definite oncogene. The mechanisms underlying its oncogenic activities may be referred to the many tumor suppressor genes that miR-21 can control (Krichevsky and Gabriely, 2009). PTEN is one of the proven targets repressed by over-expression of miR-21, leading to cell survival through activation of the PI3K-AKT pathway (Meng et al., 2007). miR-21 can also downregulate the tumor suppressor Programmed Cell Death 4 (Pdcd4) [57], a protein believed to have a role in invasion and metastasis. In addition to PTEN and PDCD4, miR-21 could also increase invasion and metastasis by down-regulating tropomyosin 1 (TPM1) (Zhu et al., 2007), Maspin (Zhu et al., 2008) as well as matrix metalloproteinase inhibitors, such as Timp3 (Selaru et al., 2009) and Reck (Gabriely et al., 2008). So, the upregulation of miR-21 in HCC affects multiple cancer-associated pathways and it represents a potentially attractive therapeutic target.

miR-221

In HCC, miR-221 is up-regulated in a large fraction of the cases (Fornari et al., 2008). In addition, miR-221 emerged as a significantly up-regulated miRNA in glioblastoma, pancreatic, kidney, bladder, prostate and thyroid cancer, thus suggesting an oncogenic role in several human neo-

Table 1. miRNAs aberrantly expressed in HCC reported by more than one study

miRNAs	Expression in HCC	Other cancers	References
miR-18	Up	No	(28,29)
miR-21	Up	Ovarian,glioblastoma, lung, breast	(14,29-35)
miR-221	Up	Colon,pancreas,stomach, bladder,glioblastoma,thyroid	(29,31-33,36-39)
miR-222	Up	Stomach,pancreas	(30,32,33,36)
miR-224	Up	Prostate,Thyroid	(28,30,40-42)
miR-122	Down	No	(30,32,37)
miR-125a	Down	Breast,Ovarian,Lung	(14,28,32 ,35,43)
miR-130a	Down	Breast, Lung	(29,33 ,37)
miR-150	Down	No	(29,37)
miR-199a-1-5p	Down	Ovarian	(28,29,32,34,35,37,43)
miR-200a	Down	No	(28, 29, 37,43)

Table 2. miRNAs aberrantly expressed in HCC associated with HCC clinical significance

miRNAs	Expression in HCC	Clinical Significance	References
20 miRNAs	Signature	Venous metastasis, overall survival	(44)
19 miRNAs	Signature	Poor survival	(29)
miR-125a	Up	Better survival	(45)
miR-221	Up	Multinodularity; reduced time to recurrence	(46)
miR-92,miR-20, miR-18	Up	Poor differentiation	(28)
miR-26a	Down	Poor survival	(47)
miR-122	Down	Gain of matastic properties, Early recurrence	(48-50)
Let-7 members	Down	Early recurrence	(51)
miR-199a-3p	Down	Reduced time to recurrence	(52)

plasms (Lee et al., 2007; Ciafre et al., 2005; He et al., 2005; Gottardo et al., 2007; Pallante et al., 2006; Galardi et al., 2007). miR-221 has been shown to stimulate tumor growth by inhibiting expression of p27Kip1 (Sage et al ., 2007), a cell cycle inhibitor and tumor suppressor. Other proven targets of miR-221 include the cyclindependent

kinase inhibitor (CDKN1C/p57) (Pineau et al., 2010) and DNA damageinducible transcript 4 (DDIT4) (Gramantieri et al., 2007), a modulator of the mammalian target of rapamycin (mTOR) pathway.

miR-17-92 cluster

MiR-17-92, a polycistronic miRNA cluster also designated as oncomir-1, contains 7 miRNAs, many of which have been found to be commonly overexpressed in HCC (Murakami et al., 2006). Several key molecular targets of this cluster, which have been experimentally identified, include Bim and PTEN, regulators of apoptosis; p21, regulator of proliferation; thrombospondin 1 (Tsp1) and connective tissue growth factor (CTGF), regulators of angiogenesis; and E2F1/2/3, transcription activators that stimulate cell cycle progression (Olive et al., 2010). In summary, these data suggest that miR-17-92 cluster promotes oncogenesis in HCC through the modulation of several key pathways related to growth and survival.

miR-122

More than 70% of HCC cases exhibit a down-regulation of miR-122, suggesting that its loss has a role in the tumor genesis of HCC (Wu et al., 2009). Enforced expression of miR-122 can induce apoptosis and cell cycle arrest of cancer cells; inhibit in vivo tumorigenicity of liver cancer cell lines; and sensitize cells to sorafenib or doxorubicin (Bai et al., 2009; Fornari et al., 2009). These phenotypic effects further support miR-122 may act as a tumor suppressor gene of HCC. Identified molecular targets of miR-122 include cyclin G1 (Gramantieri et al., 2007), a negative regulator on p53 tumor suppressor gene, and the antiapoptotic gene BCL-W (Lin et al., 2008).

miR-199

Located on three different chromosomes, all members of the miR-199 family emerged as frequently down-regulated in HCC, suggesting a role as tumor suppressors in HCC (Murakami et al., 2006). Various lines of evidence confirm the involvement of miR-199 as suppressor of oncogenic phenotype. Phenotipically, enforced expression of miR-199a in HCC cells leads to cell cycle arrest, reduced invasive capability and enhanced susceptibility to hypoxia (Viswanathan et al., 2009). These effects could be explained by the modulation of some target genes, such as MET, mTOR and HIF-1 (Fornari et al., 2010; Kim et al., 2008; Rane et al., 2009).

let-7 family

The *let-7* family consists of 11 very closely related genes. Members of the *let-7*
miRNA family were found to be among the commonly downregulated miRNAs in HCC (Wong et al., 2010). This miRNA family is known to directly regulates and suppresses the RAS and HMGA2 oncogenes through their 3'-UTR (Lee et al., 2007; Mayr et al., 2007). Other proposed targets of *let-7* include collagen type I alpha2 and Bcl-xL (Ji et al., 2010), indcating that *let-7* may act as a tumor suppressor by suppressing multiple oncogenic signaling pathways and metastatic factors.

MiRNA-based therapy in HCC

New therapeutic modalities are desperately needed for HCC because the prognosis of the disease is still very poor. Targeting genes associated with multiple molecular pathways involved in human tumourigenesis has become the most rational approach. The discovery that miRNAs play an important role in hepatocarcinogenesis has laid the foundation for their exploitation for molecular therapy. The therapeutic application of miRNAs involves two strategies (Havelange et al., 2008). One strategy is directed toward a gain of function and aims to inhibit oncogenic miRNAs by using miRNA antagonists, such as anti-miRs, locked-nucleic acids (LNA), or antagomiRs. These miRNA antagonists are oligonucleotides with sequences complementary to the endogenous miRNA. They carry chemical modifications that enhance the affinity for the target miRNA and trap the endogenous miRNA in a configuration that is unable to be processed by RISC, or alternatively, leads to degradation of the endogenous miRNA. The second strategy is miRNA replacement therapy, which means tumor-suppressor miRNA mimics are used to restore the expression and function of the original down-regulated miRNAs (Havelange et al., 2008).

Anti-HCC effects were observed in several recent studies aiming to decrease the expression of oncogenic miRNAs. For example, the introduction of 2 antagomiRs to reduce levels of miR-221 and miR-222 in liver cancer cell lines led to decreased cell growth (Pineau et al., 2010). In another study, depletion of miR-181b could sensitize SK-Hep1 cells to doxorubicin, implicating that antagomirs targeting miR-181b might be useful in increasing drug efficacy (Wang et al., 2010).

In a study attempting to increase levels of tumor suppressor, Kota et al (Kota et al., 2009) found that systemic administration of miR-26a-expressing adenovirus increased miR-26a expression and results in inhibition of HCC cell proliferation, induction of tumour-specific apoptosis, and dramatic protection from

disease progression without toxicity in a mouse liver cancer model. These data provide effective and promising strategy for future miRNA-targeted therapies for the treatment of HCC. Note that these deregulated miRNAs are aberrantly expressed and exert their functions only in a portion of HCC cases, it is very likely that just subtype HCC populations will benefit from the therapeutics targeting certain miRNA(s). So, categorising HCC cases into several subgroups based on their miRNA signatures will not only deepen our understanding of the molecular mechanisms of hepatic carcinogenesis but also facilitate the development of personalised miRNA-based therapeutics against HCC.

A main challenge for successful translation miRNA-based therapy into the clinic is to develop effective and safe *in vivo* delivery strategies. Although there is still a long way to go, our prospective outlook for miRNAs as potential therapeutic tool for HCC is cautiously optimistic for two reasons. One reason is that liver appeared to be the organ most efficiently and consistently targeted by intravenous injection of anti-miRNA oligonucleotides (AMOs) (Elmén et al., 2009). In a recent study performed in African green monkeys, efficient silencing of miR-122 was achieved by three doses of 10 mg/kg LNA-anti-miR without any evidence for associated toxicities or histopathological changes in the liver of the animals (Krützfeldt et al., 2005). Thus, by proving feasibility, safety and efficacy for the use of AMOs in a pre-clinical setting, these studies established the basis for their use as therapeutic molecules in clinical trials. The other reason is that, as far as treatment of liver and HCC is concerned, the limitations encountered for other organs related to an effective in vivo delivery of the drugs is partly overcome by the current clinical application of techniques able to deliver the drugs directly into the hepatic artery branches. In addition, several new systemic delivery systems for miRNA antagomirs or miRNAs are currently under intensive investigation. These delivery methods include lipid encapsulation, complex formation via a variety of liposomes or cationic polymers, liposomal nanoparticles, and chemical conjugation of miRNAs to peptides, aptamers, or antibodies (Aigner, 2007; Purow, 2011).

CONCLUSIONS AND PERSPECTIVES

MiRNAs are an astonishing new class of gene regulators, and it had been demonstrated that these molecules play a crucial role in cancer development and progression in a variety of malignancies, including HCC. Aberrantly expressed miRNAs associated with specific bio-pathological and clinical features can establish the basis for the development of a more rational system of HCC classification and therapeutic approaches. Several key oncogenic and tumor suppressor miRNAs have been identified in HCC, and a few of these have confirmed mRNA targets. More important, there is increasing evidence that reintroduction of downregulated tumor suppressor miRNAs and the silencing of overexpressed oncogenic miRNAs have great therapeutic potential in HCC, both *in vitro* and *in vivo*. Of course, it has to be acknowledged at this stage that translation of these preliminary data into“hard clinical facts” is not feasible. But these findings provide a very promising basis for further intensive investigations in this field. Although therapeutic delivery of miRNAs is still a developing field, and there is much more work to be done before these molecules can be securely applied in clinical settings, miRNA modulation may one day have a therapeutic application in HCC patients.

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