Dysregulated microRNAs in progression of hepatocellular carcinoma: a systematic review

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ABSTRACT

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer and the third cause of cancer-related mortality worldwide. The primary risk factor for HCC is liver cirrhosis secondary to persistent infection with hepatitis B virus (HBV) or hepatitis C virus (HCV).

Although a number of cellular phenomena and molecular events have been reported to facilitate tumor initiation, progression, and metastasis, the exact etiology of HCC has not yet been fully uncovered. MicroRNAs (miRNAs), a class of noncoding RNAs, negatively regulate post-transcriptional processes that participate in crucial biological processes, including development, differentiation, apoptosis, and proliferation. In the liver, specific miRNAs can be negative regulators of gene expression. Recent studies have uncovered the contribution of miRNAs to cancer pathogenesis as they can function as oncogenes or tumor suppressor genes. In addition, other studies have demonstrated their potential value in the clinical management of patients with HCC as some miRNAs may be used as prognostic or diagnostic markers. In this review, we summarize the current knowledge about the roles of miRNAs in the carcinogenesis and progression of HCC.
INTRODUCTION

Hepatocellular carcinoma (HCC), the most common primary liver cancer, is the fifth most frequent cancer and the third cause of cancer-related mortality worldwide \(^1\).

A report from the World Health Organization estimated that 746,000 people died because of HCC and that 782,000 people were affected by HCC in 2012. HCC normally develops because of an underlying liver disease and is often associated with cirrhosis \(^2\).

Approximately 80%–90% of all HCC cases arise from liver cirrhosis secondary to persistent infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) \(^3\). Other risk factors for HCC include alcohol abuse, obesity, iron overload, environmental pollutants, and foodstuffs contaminated with aflatoxins \(^4, 5\).

A number of cellular phenomena, including inflammation and oxidative stress, and molecular events, such as inactivation of the tumor suppressor gene p53 \(^6, 7\), beta-catenin mutations \(^8\), overexpression of various ErbB receptor family members \(^9\), and overexpression of the MET receptor \(^10\), have been reported to facilitate tumor initiation, progression, and metastasis \(^11, 12\).

In addition, various cancer-relevant genes appear to be inactivated by methylation at the epigenetic level in human HCC \(^13\).
Moreover, genomic instability and alterations are believed to contribute to human HCC\textsuperscript{14, 15}. However, the exact etiology of HCC has not yet been fully elucidated. microRNAs (miRNAs) represent a small class of noncoding RNA molecules (21–13-nucleotide fragments) that negatively regulate post-transcriptional expression by inhibiting target mRNA translation or inducing its degradation by pairing with complementary sequences within 3′-untranslated regions (UTRs) of targeted transcripts. To date, more than 2,500 homosapiens miRNAs have been described (1,881 precursors, 2,588 mature; data obtained from miRbase 21; http://www.mirbase.org/), each of which can influence hundreds of gene transcripts (4). Studies have shown that miRNAs participate in crucial biological processes, including cell development, differentiation, apoptosis, and proliferation\textsuperscript{16, 17}. Circulating miRNAs in blood have potential as noninvasive biomarkers for cancer diagnosis and prognosis\textsuperscript{18, 19}. Additionally, previous reports indicate that miRNAs exist in other body fluids, including bile, and miRNA might be a more specific and consistent biomarker of liver pathologies\textsuperscript{20}. In the liver, miRNAs are crucial in normal liver development and biological processes, including liver differentiation, hepatocyte development, and several metabolic functions\textsuperscript{21}. Specific miRNAs were demonstrated to be negative regulators of gene expression, and these may function as tumor suppressors or oncogenes\textsuperscript{22, 23}. Sequence analysis has shown that
among the dysregulated miRNAs in HBV-related HCC compared with those in adjacent nonmalignant liver tissue, some are upregulated or downregulated \(^{24}\). A recent study demonstrated that miRNA profiling enables the classification of various tumors \(^{25}\), involving identification of signatures associated with diagnosis, prognosis, and response to treatment \(^{26}\). Several miRNAs have been identified to act as oncogenes or tumor suppressors \(^{27}\), playing an important role in tumorigenesis. It is expected that a large number of miRNAs are involved in the critical aspects of liver physiology because 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. Dysregulation of tissue and serum miRNA expression has also been observed in the context of specific liver pathologies, including hepatitis, cirrhosis, and liver cancers, as well as in chronic cholestasis \(^{24}\). Here we review recent advances in the understanding of underlying miRNA mechanisms involved in HCC development.

**Short description of miRNA biogenesis**

The maturation of miRNAs requires a multistep process that begins with transcription from nuclear genes by the RNA polymerase II into initial, approximately 1–4-kb long,
polyadenylated, and capped transcripts of either monocistronic or polycistronic primary miRNAs (pri-miRNAs) (Figure 1)\textsuperscript{28}. These pri-miRNAs are further cleaved by either the canonical (by the microprocessor complex of Drosha and DiGeorge syndrome critical region gene 8)\textsuperscript{29} or the mirtron pathway (by the spliceosome)\textsuperscript{30} into hairpin-shaped 60–100-nucleotide precursor miRNAs (pre-miRNAs). These pre-miRNAs are subsequently transported by Exportin-5/RanGTP\textsuperscript{31} from the nucleus to the cytoplasm and further cleaved by the RNase III enzyme known as Dicer as well as by TBRP\textsuperscript{32} leading to the generation of an imperfect miRNA duplex. This duplex is then separated by helicases, thereby generating a single-stranded mature miRNA that is incorporated into the RNA-induced silencing complex\textsuperscript{32}, which is then guided to the target mRNA through interactions with members of the Argonaute family. Due to complementarity with its target gene sequence, the interaction between mature miRNA and mRNAs typically induces either translational repression or mRNA degradation\textsuperscript{33}. However, several reports indicate that miRNAs and their associated protein complexes (micrornucleoproteins) can also post-transcriptionally stimulate gene expression by direct and indirect mechanisms\textsuperscript{34,35}. miRNAs primarily regulate mRNAs by interacting with their 5′-end and 3′-UTR, although it has recently been suggested that miRNA target sites may be located in the 5′-UTR or even at simultaneous 5′-UTR and
3’-UTR interaction sites \(^{36}\). Moreover, miRNAs are present in human serum and plasma within exosomes in a remarkably stable form that is protected from endogenous RNase activity and can be transferred from cell to cell with the potential to regulate gene expression in receiving cells or by acting as the paracrine agonists of receptors, such as toll-like receptors, suggesting that they can mediate both short- and long-range cell–cell communication \(^{37,38}\).

**Dysregulated miRNAs in HCC**

Accumulating evidence based on miRNA profiling demonstrates the dysregulation of miRNA expression in HCC at various stages of tumor progression \(^{24,39-41}\). In addition, recent advances in the study of liver miRNAs using gene-modified mice and *in vivo* nucleic acid delivery for the overexpression of specific miRNAs or the inhibition of miRNA function have revealed crucial biological roles for individual miRNAs in physiologically essential liver functions *in vivo*. In HCC, a set of miRNAs, including miR-221 and miR-21, is upregulated and some are downregulated, including miR-122, miR-199, and miR-200 families (Tables 1 and 2). The effect of increased miRNAs on tumor progression could be mediated by the suppression of antioncogenes.
miR-21

miR-21 is the most commonly upregulated miRNA in several human cancers, including liver cancer, and is one of the oncogenes “OncoMIRs” \(^{42}\). Recent reports revealed that the overexpression of miR-21, particularly in cancerous tissues, was effectively predictive of a worse prognosis in patients with various carcinomas \(^{43}\) and that miR-21 may play an important role in the regulation of anticancer drug sensitivity and resistance \(^{44}\). Subsequently, miR-21 could be an attractive molecular target for cancer therapy. The mechanisms underlying upregulated miR-21 expression levels include amplification of the encoding genetic locus 17q23 \(^{45,46}\) and stimulation by a variety of cancer-associated pathways, such as hypoxia, inflammation, activator protein (AP)-1, and steroid hormones \(^{46,47}\). The targets of miR-21 could include tumor suppressive factors followed by the promotion and development of cancer. With regard to the liver, several reports have been focusing on miR-21 and its potentially critical role in hepatocarcinogenesis, where miR-21 is associated with the capacity of cancer cell migration and invasion in HCC \(^{48,49}\). In addition, miR-21 could be a predictor of the clinical course in patients with HCC \(^{50,51}\). The key miRNA-21 targets tumor suppressors, including phosphatase and tensin homolog (PTEN) \(^{49,52}\) and programmed cell death-4
(PDCD4)\textsuperscript{53}. Meng \textit{et al.} demonstrated that the overexpression of miR-21 could contribute to HCC growth and spread by modulating PTEN expression and its downstream mediator focal adhesion kinase phosphorylation and expression of matrix metalloproteases 2 and 9 that are involved in cancer growth, migration, and invasion\textsuperscript{49}. Bao \textit{et al.} also noted that miR-21-mediated suppression of both hSulf-1 and PTEN leads to the activation of AKT/ERK pathways and epithelial–mesenchymal transition (EMT) in HCC cells, enhances the activity of HCC cell proliferation and movement, and promotes HCC xenograft tumor growth in mouse models\textsuperscript{52}. Zhu \textit{et al.} reported that miR-21 expression is inversely correlated with the protein expression of its targeted gene PDCD4, and miR-21 promotes migration and invasion in HCC through the miR-21–PDCD4–AP-1 feedback loop. Xu \textit{et al.} demonstrated that MAP2K3, a putative tumor repressor gene, is a direct target of miR-21 in HCC and inhibits its expression during the carcinogenesis of HCC both at transcriptional and post-translational levels\textsuperscript{54}.

\textbf{miR-221/222}

MiRNA-221 and -222, two highly homologous miRNAs, are overexpressed in several human cancers. The possibility that miR-221/222 could be involved in tumor progression could
result from the suppressive effect of miR-221 on some cellular antioncogenes. In HCC, recent studies demonstrated that miR-221/222 are the most upregulated miRNAs and have been shown to target the cyclin-dependent kinase (CDK) inhibitor p27 and DDIT and to enhance cell growth, proliferation, migration, and invasion \(^{22,55}\). Other mechanistic analyses demonstrated that PTEN and TIMP3 were targeted by miR-221/222 followed by the activation of AKT is common in HCC and could potentially confer metastatic properties to HCC cells \(^{56,57}\). In addition, there is evidence that miR-221 overexpression accelerates the growth of tumorigenic murine hepatic progenitor cells in murine liver cancer, which significantly shortens the mean time to death \(^{22}\). He et al. demonstrated that lentivirus-mediated miR-221 silencing could significantly suppress the growth of hepatoma xenografts in nude mice \(^{58}\). In addition, Park revealed the preclinical proof of efficacy of chol-antimiR-221 for reducing tumor cell proliferation and increasing the markers of apoptosis and cell cycle arrest, elevating the tumor doubling time and increasing mouse survival \(^{59}\).

**miR-155**

miR-155 plays an important role in a wide variety of cancers, such as leukemia, lymphoma, lung cancer, breast cancer, and HCC \(^{60}\). Studies suggest a role for miR-155 in cancer
development either as an onco-miRNA or as an oncosuppressor-miRNA depending on the tissue type. Wang et al. demonstrated in their CDAA-fed induced hepatocarcinogenesis mouse model that the upregulation of oncogenic targets was transactivated by nuclear factor kappa B (NF-kappaB), which suppressed its target CCAAT/enhancer binding protein (C/EBP) beta. They also demonstrated that the ectopic expression of miR-155 promoted the growth of HCC cells, whereas its depletion inhibited cell growth. Xie et al. demonstrated that miR-155 is a tumorigenic factor, with a gradual increase of miR-155 expression in cirrhotic liver tissue and in HCC tumor tissue compared with low expression levels in normal liver tissue. Further, they revealed that miR-155 enhances liver cell tumorigenesis, at least in part, through the miR-155/SOX6/p21waf1/cip1 axis. Zhang et al. performed gain-/loss-of-function studies demonstrating that miR-155 expression is regulated by p300/NF-kappaB, promoting hepatocyte proliferation and tumorigenesis by increasing Wnt signaling in vitro and in vivo. Although this pathway was not elucidated in HCC, miR-155 may play an important role in TGF-beta/Smad4-induced EMT and cell migration and invasion by targeting RhoA.
Downregulated miRNAs in HCC (Table 2)

miR-122

MiR-122, the most abundant miRNA in the liver\textsuperscript{65, 66}, has been reported to have multiple roles in the liver, including development, differentiation, and metabolism\textsuperscript{67, 68}. MiR-122 is known to be under transcriptional control by liver-enriched transcription factors, such as hepatocyte nuclear factor (HNF)-1alpha, HNF3beta, HNF4alpha, HNF6, and C/EBP alpha\textsuperscript{69}. An epigenetic modulation of miR-122 expression is involved in the suppression of miR-122 in HCC\textsuperscript{71}. Dysregulation of miR-122 appears to be involved in a variety of liver diseases\textsuperscript{72}. For instance, miR-122-deficient mice develop steatohepatitis and fibrosis\textsuperscript{73}. Recent advances in the understanding of the association between miRNA-122 and hepatitis virus gives an insight into the underlying mechanisms involved in the progression of liver diseases. The direct interaction between HCV RNA and miR-122 resulting in a heterotrimeric stable structure enhances HCV translation and protects HCV RNA from degradation\textsuperscript{74}. Chen \textit{et al.} demonstrated that miR-122 can downregulate HBV gene expression and replication by interacting with the target sequence coding for nt 2738–2760 by targeting sequences located at the coding region of the mRNA for viral polymerase and the 3′-UTR region of the mRNA for the core protein of the HBV genome via base-pairing interactions\textsuperscript{75}. Several comparative
studies have been conducted reporting that miR-122 is downregulated in HCC tissue compared with that in adjacent normal tissue, where loss of miR-122 expression in HCC is associated with metastasis and poor prognosis. Therefore, miR122 is believed to have potential as a tumor suppressor. Moreover, accumulating evidence indicates that miR-122 regulates cancer cell proliferation, apoptosis, metastasis, and drug resistance by targeting a set of cancer-related genes. Wang et al. showed that miR-122 can modulate cancer cell proliferation through cyclin G1 expression in HCC-derived cell lines and that an inverse correlation between miR-122a and cyclin G1 expression exists in primary liver carcinomas. Recent work from Wang et al. showed that HNF4a/miR-122 controls hepatocyte epithelial phenotype through modulating the RhoA/Rock inactivation pathway, indicating that the loss of miR-122 in HCC facilitates cancer invasion and metastasis with EMT. Tsai et al. suggested that miR-122 might affect HCC intrahepatic metastasis by the suppression of angiogenesis, exerting some of its action via regulating disintegrin and metalloprotease 17 (ADAM17). Recent work from Xie et al. demonstrated that the upregulation of distal-less 4, a member of the DLX family of homeobox genes that is abnormally expressed in several types of human tumors, results from a miR-122 downregulation in HCC. Koberle et al. reported that circulating miR-122 levels were...
positively correlated with liver transaminases and negatively correlated with the Model for End-Stage Liver Disease score, suggesting that serum miR-122 is a novel biomarker for liver injury but not specifically for HCC, whereas there was no significant difference in circulating miR-122 levels between patients with and without HCC.

**miR-101**

MiR-101 is an antionco-miRNA displaying a suppressive effect on cellular proliferation, migration, and invasion (51). It is significantly downregulated in multiple types of cancers, including HCC. MiR-101 exerts its function through regulating the expression of downstream target genes, including, ZEB1, Rab5a, DNMT3A, SOX9, v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS), EZH2, NLK, STMN1, and ATG4D. Li et al. demonstrated that that miR-101 directly repressed the expression of FOS, a key component of the AP-1 transcription factor. They also demonstrated that enhanced miR-101 expression inhibited invasion and migration of cultured HCC cells, suggesting that miR-101 might play an important role in HCC progression. Su et al. found that miR-101 could exert its proapoptotic function via targeting the antiapoptotic BCL-2 family member myeloid cell leukemia sequence 1 (Mcl-1), where miR-101 significantly repressed the translation of Mcl-1.
and reduced the endogenous protein levels of Mcl-1, whereas the miR-101 inhibitor upregulated Mcl-1 expression and inhibited cell apoptosis \(^8^{3}\). Moreover, the silencing of Mcl-1 phenocopied the effect of miR-101, and forced expression of Mcl-1 could reverse the proapoptotic effect of miR-101 \(^8^{3}\). Zhang et al. showed that miR-101 directly targeted SOX9 in HCC \(^8^{6}\). Ectopic expression of miR-101 significantly inhibited HCC cell proliferation and tumorigenicity by targeting sex-determining region Y (SRY)-box 9 (SOX9). Additionally, they showed that the downregulation of miR-101 in clinical HCC tissues correlates with tumor aggressiveness and poor prognosis \(^8^{6}\). Xu et al. found that autophagy was suppressed by miR-101 in the HCC cell line HepG2, in which miR-101 directly targets RAB5A, STMN1, and ATG4D \(^8^{7}\). Moreover, miR-101 enhanced cisplatin-induced apoptosis in the HepG2 cell line, probably through the inhibition of autophagy \(^8^{7}\). Shen et al. revealed that miR-101 could suppress NLK, a MAP kinase-related kinase, in HCC cells. Notably, ectopic miR-101 expression repressed cancer cell growth and proliferation by targeting NLK on HCC cells \(^8^{8}\). Sheng et al. showed that HBV can downregulate miR-101-3p expression by inhibiting its promoter activity and that downregulation of miR-101-3p promotes HCC cell proliferation and migration by targeting Rab5a, a member of the Rab subfamily of small GTPases that acts as an oncogene \(^8^{4}\). A recent report by Zhao et al. demonstrates that miR-101 inhibited HCC
EMT and migration by downregulating the transcription repressor ZEB1\(^8^9\).

**miR-200 family**

The miR-200 family, which contains the two coding clusters of miR-200b/a/429 and miR-200c/141, is an important regulator of EMT and has been implicated in human carcinogenesis\(^9^0\). ZEB1 and ZEB2 transcriptionally control the expression of the primary miR-200 for both coding clusters\(^9^0\). Conversely, ZEB1/2 regulates EMT by way of miR-200 family members, where miR-200 family members suppress ZEB1/2 production by binding to its 3′-UTR. The ZEB1/2-miR200 feedback loop regulates an EMT signaling axis that might be critical to tumor progression\(^9^1\). Although their expression in human cancers remains controversial, miR-200 family members were seen to be frequently downregulated in HCC\(^9^2\)–\(^9^5\). Using a stable lentivirus-miR-200-transduced HCC cell line, Hung et al. demonstrated that miR-200c and -200b play important roles in HCC migration by regulating E-cadherin expression by regulating its target ZEB1/2\(^9^3\).

Using miRNA expression profiles, Dhayat et al. reported that miR-200a and miR-200b are able to distinguish between cirrhotic and HCC tissue and could serve as an early marker for cirrhosis-associated HCC\(^9^4\). Although all five members of the miR-200 family inhibit
ZEB1/2 expression in HCC cell lines, there are differences between the miR-200a/141 and miR-200b/200c/429 subfamilies in the role of cancer progression due to the downregulation of different targets. Wong et al. showed that the miR-200b/200c/429 subfamily, but not the miR-200a/141 subfamily, inhibited HCC cell migration through modulating Rho/ROCK-mediated cell cytoskeletal reorganization and cell-substratum adhesion. Lin et al. confirmed that miR-141 directly regulates the expression of HNF-3beta, which has a critical role in hepatocyte differentiation and controlling liver-specific gene expression during the development of HCC, in HepG2 cells and demonstrated that the repression of HNF-3beta by miR-141 suppressed the proliferation and invasion and promoted the apoptosis of HepG2 cells.

**Let-7 miRNA family**

The let-7 miRNA family, which has 13 members, was first discovered in *Caenorhabditis elegans* and is evolutionally conserved among many species (71). It is widely accepted that let-7 miRNA family plays pivotal roles in tumorigenesis by functioning as a tumor suppressor miRNA, where several human cancers exhibit dysregulated let-7 expression when compared to normal tissues (96-98). Recently, several studies have identified the role of let-7 in...
HCC development. Using microarray analysis, Shimizu et al. found that miRNAs let-7c and let-7g negatively regulate by targeting B-cell lymphoma 2-like protein 1 (Bcl-xL), an antiapoptotic member of the Bcl-2 family, and can induce apoptosis when used with an anticancer drug targeting Mcl-1 in human HCC, indicating that the restoration of let-7 expression may be a useful therapeutic option for HCC where let-7 expression is absent. A study from Tian et al. showed that Lin28, which was identified as a risk factor for relapse and drug resistance of cancer, downregulated let-7 family levels and upregulated Bcl-xL. In addition, they found that the expression of Lin28 was closely associated with resistance to paclitaxel in an HCC cell line. Let-7g and let-7i also appeared to have a concurrent effect of regulating cell proliferation and apoptosis of hepatoma cells through Bcl-xL targeting. Wang et al. demonstrated that let-7 family was transcriptionally downregulated by HBx followed by the upregulation of its target STAT3, which positively regulates cellular proliferation. Lan et al. demonstrated in their loss-/gain-of-function study that hsa-let-7g may act as a tumor suppressor gene that inhibits HCC cell proliferation by downregulating the oncogene c-Myc and upregulating the tumor suppressor gene p16(INK4A).

Other up and downregulated miRNAs in HCC and their involvement in cellular processes are
listed in Table 1 and 2.

**MiRNAs in HCC progression**

Key signaling involved in HCC progression, including cell cycle, apoptosis, angiogenesis, EMT, and metastasis, is modulated by several miRNAs (Figure 2).

**Cell cycle**

Several miRNAs, including miR-122, miR-138, miR-15a/16-1, miR-26a, miR-195, miR-34a, miR-221, miR-222, miR-193b, and miR-519d, have been reported to be associated with cell cycle dysregulation in HCC. Of these, miR-26a, miR-34a, miR-122, miR-138, and miR-195 are significantly downregulated, whereas miR-15a/16-1, miR-221, miR-222, and miR-519d are upregulated in HCC. Liver-abundant miR-122 was found to directly target cyclin G1 in HCC and to modulate p53 activity. MiR-26a, miR-34a, and miR-195 were demonstrated to induce G1 arrest and control G1/S transition by inhibiting cyclin D1 as a direct target in HCC. MiR-26a and miR-195 target CDK6 and E2F3, whereas miR-34a targets CDK2 and CDK4. Recent date indicate that miR-138 inhibit tumor growth and cell cycle arrest of HCC by targeting cyclin D3. MiR-221 and miR-222 have been shown to target...
CDKN1B/p27/Kip1 and CDKN1C/p57/Kip2 59, 107, 108. Fornari et al. demonstrated that miR-519d promotes cell proliferation and invasion and impairs apoptosis directly through targeting CDKN1A/p21, PTEN, AKT3 and TIMP2 in HCC cells 109.

**Apoptosis**

Several miRNAs, including miR-15a/16-1, mir-106b-25, miR-221, miR-224, miR-29, miR-122, miR-125b, and let-7 family, are involved in the regulation of apoptosis in HCC. Of these, miR-15a/16-1 and miR-224 can target the antiapoptotic member B-cell lymphoma-2 (Bcl-2), and miR-224 also targets the Bcl-2-like protein 2 (Bcl-w) 110, 111. HBV transcripts can act as a sponge to bind and sequester endogenous miR-15a/16, resulting in the upregulation of its target Bcl-2, which might contribute to facilitating chronic HBV infection and hepatoma development 111. The miR-106b-25 cluster was shown to be upregulated and inversely correlated with the proapoptotic gene Bim expression in HCC and to inhibit expression of Bim as a direct target 112. Similary, Gramantieri et al. demonstrated that the downregulation of miR-221 inhibits apoptosis in HCC through controlling its target, the proapoptotic gene Bmf 107. Downregulated miRNAs, such as miR-122 and miR-29, target the antiapoptotic genes of Bcl-2 and Bcl-w along with Mcl-1 113, 114.
EMT

Several miRNAs are involved in metastasis and invasion through EMT modulation (Fig. 2). Of these, the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) and miR-205 have a central role in the modulation of the cell phenotype, such as EMT and MET, through targeting the transcriptional repressor ZEB1/ZEB2. Wong et al. identified RhoA and ROCK2, a small GTPase protein known to regulate the actin cytoskeleton in the formation of stress fibers, as specific downstream targets of the miR-200b/200c/429 subfamily, which mediated cell cytoskeletal reorganization and cell-substratum adhesion. They also demonstrated that the re-expression of miR-200b suppressed lung metastasis of HCC cells in an orthotopic liver implantation model \textit{in vivo}. Therefore, transcriptional regulation by the miR-200 family has been subject to scrutiny. Both the promoter regions of pri-miR-200 contain E-box and Z-box elements with conserved sequences for ZEB1 binding upstream of the transcription starting site. Therefore, high ZEB1 levels suppress miR-200 transcription. Low miR-200 expression levels, in turn, result in an increase of ZEB1 through less binding to its 3′-UTR. Consequently, high ZEB1 expression suppresses E-cadherin expression, maintaining a mesenchymal phenotype. In breast cancer, the loss of miR-200 increases SUZ12 expression, which results in polycomb-mediated repression of the CDH1
gene and upregulation of ZEB1/ZEB2. In a way similar to the miR-200 family, miR-101 has been shown to target the ZEB1 3′-UTR and to inhibit TGF-β1-induced EMT in hepatocytes, whereas inhibition of miR-101 promotes the EMT process as indicated by changes in morphology, cell migration, and expression profiles of EMT markers. Zheng et al. demonstrated that miR-124 plays a critical role in regulating cytoskeletal events and EMT and ultimately inhibits the invasive and/or metastatic potential of HCC, probably by directly targeting ROCK2 and EZH2 genes. Wong et al. revealed that miR-139 targeted ROCK2 and reduced its expression in HCC cells and that miR-139 expression levels are reduced in human metastatic HCC samples and correlates with prognosis.

**Angiogenesis**

Angiogenesis plays crucial roles in the progression of HCC, where miRNAs, including miR-26a, 29b, miR-126, miR-214, miR-221/222, miR-122, and miR182, might regulate various aspects of vascular development and angiogenesis. MiR-221 and miR-222 are originally known to modulate the angiogenic properties of human umbilical vein endothelial cells by directly regulating c-kit, p27Kip1, p57Kip2, and cyclin G1. In HCC, miR-221 is involved in the staphylococcal nuclease domain-containing 1.
(SND1)-induced angiogenesis pathway, where SND1 induces the production of NF-κB and subsequent miR-221 followed by the activation of angiogenin and CXCL16, which results in angiogenesis.Du et al. demonstrated that miR-182 was induced in HCC cells under hypoxia followed by the inhibition of its target RASA1. Although the direct association between RASA1 inhibition and angiogenesis requires further study, miR-182 induction displayed a better ability to promote the formation of capillary-like structures than the control group by in vitro capillary tube formation assay. The authors demonstrated that the downregulation of miR-126-3p, an endothelial cell-specific miRNA, promotes metastasis and angiogenesis by targeting LRP6 and PIK3R2 in HCC. Wang et al. revealed that miR-195 downregulation results in enhanced VEGF levels in the tumor microenvironment, which subsequently activates VEGF receptor-2 signaling in endothelial cells and thereby promotes angiogenesis. Fang et al. demonstrated that miR-29b in part exerted its antiangiogenesis function by directly suppressing MMP-2 expression in HCC cells and, in turn, impairing vascular endothelial growth factor receptor-2 signaling in endothelial cells. Downregulation of miR-214 was reported to contribute to the unusual hypervascularity of HCC via activation of the HDGF paracrine pathway in tumor angiogenesis. MiR-26a exerts its antiangiogenesis function, at least in part, by inhibiting the HGF-hepatocyte...
growth factor receptor (cMet) and PIK3C2α plus its downstream signaling pathway, in turn, suppressing VEGFA production in HCC cells and impairing VEGFR2 signaling in endothelial cells. MiR-122, a liver-specific tumor suppressor miRNA affecting HCC intrahepatic metastasis by angiogenesis suppression, exerts some of its action via regulation of ADAM17.

Conclusion and future directions

Here we summarized the involvement of miRNAs in the carcinogenesis and progression of HCC. Investigation of the association between miRNAs and expression of target genes has uncovered our understanding of many biological processes. Accumulating evidence gives us a better understanding of cancer-associated miRNA functions and their roles as tumor suppressors and oncogenes in the development, invasiveness, and metastasis of HCC. MiRNAs might have different functions related to individual cell types or tissue environments, enabling modulation of gene expression in multiple targets, thereby suggesting that miRNAs could be attractive therapeutic targets.

Despite progress in drug discovery and development, as evidenced by the emergence of novel protease inhibitors and polymerase inhibitors, the clinical use of direct-acting antivirals is
limited due to poor compliance and rapid-onset drug resistance. Use of imatinib mesylate, a platelet-derived growth factor receptor, and other tyrosine kinase inhibitors, as molecular targets against HCC progression, has been proposed \textsuperscript{128, 129}; however, clinical trials have indicated a lack of efficacy using such approaches \textsuperscript{130}. The development of more effective, cost-efficient, and better-tolerated novel treatments is crucial to controlling the development of hepatitis, cirrhosis, and HCC. Exploring different facets of the interactions among miRNA, HBV, and HCV infections and the carcinogenesis and progression of HCC could facilitate the development of novel and effective treatment approaches for liver disease.

It is interesting that circulating miRNA levels are also affected by HCC progression. MiRNAs could potentially be more accurate cancer biomarkers essential for early detection and diagnosis of HCC as well as for developing preventive screening. Recent evidence demonstrated that commonly dysregulated miRNAs in HCC, including miR-21 and miR-122, are present in the serum of patients with HCC and might serve as diagnostic markers \textsuperscript{131}. Future studies with emerging techniques, including single nucleotide polymorphisms, next generation sequencing, using large numbers of samples can identify key molecular targets including miRNA that contribute to the development of novel treatments and personalized medicine. Although relatively poorly understood in the context of human cancers, evidence continues to
accumulate indicating that long ncRNAs play a crucial role in regulating numerous developmental and biological genomic pathways (50).

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Figure 1. Illustration of miRNA maturation and biogenesis
Figure 2. A summary of aberrant expression of microRNAs in HCC progression. A set of increased and decreased miRNAs expressed in the pathway of proliferation, apoptosis, and EMT as well as invasion/metastasis is depicted.
Table 1. Upregulated miRNAs and their target genes in the progression of HCC

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<td>MiR-148a</td>
<td>PTEN, smad2, met, wnt1</td>
<td>metastasis</td>
<td>139-142</td>
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<tr>
<td>MiR-155</td>
<td>RhoA, TLR, APC, AT1R, AHIP1, C/EBPbeta, SOX6</td>
<td>metastasis</td>
<td>61-64</td>
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<tr>
<td>MiR-181b</td>
<td>TIMP3</td>
<td>proliferation</td>
<td>143, 144</td>
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<tr>
<td>MiR-182</td>
<td>IGF-1R, MTSS1, TP53INP1, CEBPA, RASA1</td>
<td>proliferation, metastasis</td>
<td>145, 146, 122, 147</td>
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<tr>
<td>MiR-210</td>
<td>VMP1</td>
<td>migration, invasion</td>
<td>148</td>
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<td>MiR-216a</td>
<td>PTEN, SMAD7</td>
<td>metastasis</td>
<td>149, 150</td>
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<td>MiR-221</td>
<td>Bmf; HDAC6,CDKN1B/p27/Kip1; CDKN1C/p57/Kip2, PTEN, DDIT4, Arnt, Erα</td>
<td>apoptosis; proliferation, angiogenesis</td>
<td>55, 107, 131, 151-153</td>
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<td>MiR-222</td>
<td>AKT, PTEN, p27, p57, PPP2R2A</td>
<td>metastasis, angiogenesis</td>
<td>56, 57, 108</td>
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<tr>
<td>MiR-224</td>
<td>Bcl-2, Bcl-w, RKIP, CDC42, CDH1, PAK2, MAPK1, API-5, Smad4</td>
<td>apoptosis</td>
<td>110, 156</td>
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<td>MiR-373</td>
<td>PPP6C</td>
<td>proliferation</td>
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<tr>
<td>MiR-519d</td>
<td>CDKN1A/p21; PTEN; AKT3; TIMP2, MKi67</td>
<td>proliferation, metastasis</td>
<td>109, 157</td>
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<tr>
<td>MiR-550a</td>
<td>CREB4</td>
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Table 2. Downregulated miRNAs and their target genes in the progression of HCC

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<td>miR-1</td>
<td>ET-1, ets1</td>
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<td>PIK3CD, mTOR, p70S6K, CCNE1</td>
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<td>miR-7</td>
<td>CDK6, cyclin D1, PIK3C2α, c-MET</td>
<td>cell cycle, metastasis</td>
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<td>miR-29</td>
<td>Bcl-2, Bcl-w, Ras, matrix metallopro-teinase-2 (MMP-2)</td>
<td>apoptosis, angiogenesis, metastasis, invasion</td>
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<tr>
<td>miR-34a</td>
<td>cyclin D1, CDK4, and CDK2, c-Met, CCL22</td>
<td>cell cycle, proliferation, apoptosis, metastasis</td>
<td>104, 167, 168</td>
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<td>miR-101</td>
<td>ZEB1, Rab5a, DNMT3A, SOX9, FOS, EZH2, NLK</td>
<td>EMT, proliferation</td>
<td>83, 85-89, 169, 170</td>
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<td>cyclin G1, Bcl-w, DLX4, Rho, N-Myc, ADAM17</td>
<td>apoptosis, angiogenesis, metastasis</td>
<td>70, 79, 81 69, 80, 114</td>
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<td>ROCK2, EZH2, PIK3CA, STAT3,</td>
<td>EMT</td>
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<td>MiR-125b</td>
<td>Bcl-2, Bcl-w, LIN28B2; PIGF, Mcl-1, IL6R, SUV39H1, eIF5A2</td>
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<td>MiR-139</td>
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<td>IRS1</td>
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<td>MiR-146a</td>
<td>HAb18G</td>
<td>metastasis</td>
<td>182</td>
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<td>MiR-195</td>
<td>CDK6, cyclin D1, CBX4, Wnt3a, VEGF, VAV2, CDC42</td>
<td>cell cycle, apoptosis, metastasis</td>
<td>105, 123, 183, 184</td>
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<td>MiR-200 family</td>
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<td>MiR-203</td>
<td>ZEB1, ZEB2, HNF-3β, Rho/ROCK, ASB4</td>
<td>EMT, metastasis</td>
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<td>MiR-214</td>
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<td>MiR-218</td>
<td>epatoma-derived growth</td>
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<td>MiR-449</td>
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<td>MiR-520b</td>
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<td>MiR-520e</td>
<td>MEKK2, cyclinD1</td>
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<td>Let-7 family</td>
<td>Bcl-xL, Stat3, c-myc, COL1A2, Mcl-1</td>
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