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REVIEW

MicroRNAs in Hepatocellular Carcinoma: Insights into Regulatory Mechanisms, Clinical Significance, and Therapeutic Potential

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Abstract: Hepatocellular carcinoma (HCC) is one of the most common malignant tumors. Tumor immune microenvironment (TIME), angiogenesis, epithelial-mesenchymal transformation (EMT), invasion, metastasis, metabolism, and drug resistance are the main factors affecting the development and treatment of tumors. MiRNAs play crucial roles in almost all major cellular biological processes. Studies have been carried out on miRNAs as biomarkers and therapeutic targets. Their dysregulation contributes to the progression and prognosis of HCC. This review aims to explore the molecular cascades and corresponding phenotypic changes caused by aberrant miRNA expression and their regulatory mechanisms, summarize and analyze novel biomarkers from somatic fluids (plasma/serum/urine), and highlight the latent capacity of miRNAs as therapeutic targets.

Keywords: hepatocellular carcinoma, microRNAs, regulatory mechanisms, biomarker, targeted therapy

Introduction

Primary liver cancer is one of the most common malignant tumors and the third leading cause of cancer-related death globally.[1,2] HCC accounts for 75% to 85% of primary liver cancer and has become the fourth most common malignant tumor and the second cause of cancer-related death in China.[3] Main treatments of HCC include surgical resection,[4] liver transplantation, radiofrequency ablation (RFA), transarterial chemoembolization (TACE),[5] transarterial embolization (TAE), radiation therapy, molecular targeted therapy.[6] The radiomic immunoscore (RIS), a new radiomic model, not only showed high accuracy in predicting TIME status in the testing cohort (area under the curve = 0.753) but also the capability of predicting therapeutic response to anti-programmed cell death 1 (PD-1) immunotherapy.[7] Jiedu Recipe (JR), a Chinese herbal remedy, can prolong overall survival time and decrease recurrence and metastasis rates in patients with HCC via abating the Wnt/β-catenin pathway under hypoxic conditions.[8] Great breakthroughs have been made in treating HCC with the emergence of new or multi-modal treatment approaches. However, the prognosis of HCC remains poor, especially in advanced patients, due to the lack of biomarkers for prognosis and treatment response.[9] Therefore, deepening the molecular mechanisms of HCC pathogenesis and searching for biomarkers for early diagnosis is of great significance for the prevention and treatment of HCC.

There has been a paradigm shift in treating malignancies with the further study of immuno-oncology.[10] Since immune checkpoint inhibitors (ICIs) were approved, immunotherapy is considered a new-generation therapy.[11] TIME, angiogenesis, EMT, and drug resistance are the key factors affecting the prognosis of HCC. The TIME includes tumor cells, immune cells, cytokines, etc. The interactions and function of these components determine antitumor immune efficacy.[12] Consequently, it is indispensable to elucidate the role of TIME in tumor progression.[13] Angiogenesis is a complex process of new and abnormal blood vessel network formation.[14] It not only delivers oxygen and nutrients but also transports tumor cells, which plays an important role in tumor growth and metastasis.[15] EMT is a complex biological process in which epithelial cells are transformed into cells with mesenchymal phenotypes, which plays a vital role in the

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malignant features of cancers, such as migration, invasion, metastasis, stem-like properties, and drug resistance.[16] Therefore, exploring mechanisms of tumor invasion-metastasis cascade responses is significant for treating tumors.[17] Tumor metabolism is characterized by active "aerobic glycolysis", which can rapidly provide ATP and biosynthetic materials for tumors.[18] Even in the presence of sufficient oxygen, tumors prefer 'aerobic glycolysis'. Studies have shown that metabolic reprogramming of tumors is a survival strategy to adapt to harsh environments with limited glucose and oxygen supply, which is considered a hallmark of tumors and is associated with poor prognosis.[19] What is unexpected is that mortality due to drug resistance accounts for more than 90% of cancer-specific mortality.[20]

MiRNAs, small non-coding RNAs (ncRNAs) about 22 nucleotides in size, play a role in gene expression regulation and almost all major cellular biological processes by targeting the 3′UTR of mRNA. Abnormal expression and dysregulation of miRNAs contributes to tumor development and progression and influences drug resistance in HCC. Accordingly, miRNAs have been extensively investigated as both biomarkers and therapeutic targets as oncogenes or tumor suppressors.[21] To date, approximately 2000 human miRNA precursor genes have been annotated in miRBase. Almost 30% of human genes are regulated by miRNAs. Hereafter, we will focus on the latest findings on miRNAs involved in cellular biological processes, including TIME, angiogenesis, EMT, invasion, metastasis, metabolism, and drug resistance, and review the potential value of miRNAs as novel biomarkers and therapeutic candidates for HCC. Then, we will review the main therapeutic strategies of HCC-targeted miRNAs.

Regulatory Mechanisms of MiRNAs in HCC

MiRNAs and TIME

The TIME in HCC involves interactions among tumor cells like HCC cells, the blood arteries around them, stromal cells, immune cells, cytokines, etc. These interactions are essential for tumorigenesis, angiogenesis, and metastasis.[22] Evidence suggests that miRNAs play an important role in the TIME of HCC by changing immune phenotypes, hypoxic conditions, and acidification, as well as angiogenesis and extracellular matrix components.[23] TIME-based HCC treatment strategies have attracted more and more interest from scholars.[24,25] This section comprehensively reviews the roles and molecular mechanisms of miRNAs in regulating immune cell subsets and tumor immune responses in the TIME.

T Cells

CD8+ T Cells

CD8+ (cytotoxic) T cells, which mediate target cell apoptosis by secreting perforin and granzyme or expressing Fas ligand, are the major players performing antitumor immune functions in HCC.[26] However, they are always in a dysfunctional state characterized by impaired activity and proliferation, reduced cytokine production, and compromised cell-killing ability.[27] Emerging investigations have shown that targeting miRNAs can improve the activity of CD8+ T cells and restore the state of immunosuppression in the TIME. MiR-206 promotes the recruitment of CD8+ T cells and prevents HCC by the M0-to-M1 transition of Kupffer cells driven by the activation of the KLF4/CCL2/CCR2 axis.[28]

CD4+ T Cells

CD4+ T cells are not only able to kill tumor cells directly but also exert an indirect role in the TIME as T helper cells. Certain CD4+ T cells directly lyse tumor cells.[29] They not only cooperate with CD8+ T cells and macrophages to enhance antitumor effects but also differentiate into different subpopulations with different anti-tumor responses.[30] MiR-26b-5p can promote the secretion of TNF-α, IFN-γ, IL-6, and IL-2 in CD4+ and CD8+ T cells by targeting PIM-2 in HCC, leading to enhanced T-cell responses.[31] T helper cells (Th17) participate in many organ-specific autoimmune diseases. One report showed that the expression of miR-132 is higher in CD4+ IL-17+ cells than in CD4+ IL-17- cells. MiR-132 mediates the differentiation of Th17 cells and the secretion of IL-17 and IL-22, which increase the activation of hepatic stellate cells and strongly promote HCC migration and EMT.[32] Regulatory T cells (Tregs) play an important role in maintaining self-tolerance and immune homeostasis and closely correlate with tumor progression, invasion, and metastasis.[33] They can induce an immunosuppressive microenvironment by compromising immune surveillance against cancers and impairing antitumor immune responses. Accumulating evidence implies that miRNAs are considered key

regulators in Tregs. Multiple reports have shown that miR-34a, miR-23a, miR-15a, and miR-16-1 play an important role in the regulation of Tregs.[34–36]

B Cells

Research on the immunobiology of HCC is of great significance for promoting the development of immunotherapy. There has been an exponential increase in research on the biological properties of T cells, but less on the role of B lymphocytes. Interestingly, B lymphocytes play a dual role in regulating tumor immunity.[37] They can not only enhance humoral and cellular immunity but also promote tumor progression. One view put forward by Han is that the immune-related miRNAs harbored in exosomes may promote the differentiation of immunosuppressive B-cell subtypes in HCC.[38] Hutter found that the miR-15a/16-1 and miR-15b/16-2 clusters limit progenitor B-cell proliferation by suppressing several prominent cell cycle regulators, such as Ccne1, Ccnd3, and Cdc25a, in vivo. In addition, loss of the miR-15 family (miR-15a/16-1 and miR-15b/16-2) in B-cell progenitors enhances IL7R expression and signaling which acts as a key regulator of early B-cells survival, proliferation, and differentiation.[39] Therefore, it is difficult to determine their specific role,[40] the regulatory effects and molecular mechanisms of miRNAs on B cells need to be further studied.

Natural Killer Cells

Natural killer (NK) cells are the first line of defense against cancers and virus infection by directly killing malignant cells without antibody involvement or MHC restriction. NK cells not only induce target cell apoptosis by releasing perforins and granzymes, expressing FasL, and mediating antibody-dependent cellular cytotoxicity (ADCC), but also directly act on target cells by secreting a large number of cytokines, such as ifn-v, tnf-x, gm-csf, il-3, and m-csf, or attack target cells.[41] NK cells are beginning to become an attractive complement to T-cell-based immunotherapies based on the properties of independent antigen expression and rapid activation. Changes in the phenotype and function of NK cells have been described in patients with HCC, who also show perturbations of NK activating receptor/ligand axes.[42]

MiRNAs can enhance the cytotoxicity of NK cells. MiR-92b is a highly expressed microribonucleic acid in serum exosomes that improves the migration of liver cancer cell lines. Hepatoma-derived exosomal miR-92b may be transferred to NK cells, resulting in the downregulation of CD69 and weakening NK cell cytotoxicity.[43] The target gene of miR-449c-5p is T-cell immunoglobulin and mucin domain 3 (TIM-3), which inhibits antitumoral immunity. HCC-derived exosomal circUHRF1 inhibits the cytotoxicity of NK cells by upregulating the expression of TIM-3 through degrading miR-449c-5p, leading to the promotion of immune evasion of HCC and resistance to anti-PD1 immunotherapy.[44] The overexpression of miR-182 enhances the cytotoxicity of NK cells against tumorigenic Huh-7 cells by increasing the expression of activating receptor NKG2D and suppressing the receptor NKG2A.[45] MiR-561-5p attenuates the antitumor response by downregulating CX3CL1 (a potential target of miR-561-5p) to reduce the infiltration and function of CX(3)CR1(+) NK cells.[46]

Tumor-Associated Macrophages in the TIME

Tumor-associated macrophages (TAMs), as the main type of inflammatory cells in the tumor microenvironment, exert either anti-tumorigenic (M1) or pro-tumorigenic (M2) functions.[47] Monocytes differentiate into M1-like macrophages with antitumor properties under certain stimuli and signaling. However, M2-like macrophages can promote tumors, which promote tumor growth directly or indirectly, via the suppression of cytotoxic cell populations, including CD8+ T cells and NK cells.[48] In the TIME, TAMs mainly show the properties of M2-type macrophages, leading to the promotion of tumor cell invasion, angiogenic switching, and immune escape of malignant cells.

Studies have reported the role of miRNAs in macrophage differentiation and polarization, which mediates the occurrence and progression of HCC. MiR-200b-3p exosomes are internalized by M0 macrophages and induce M2 polarization by down-regulating ZEB1 and up-regulating interleukin-4. Meanwhile, high levels of PIM1 and VEGFα expression are detected in M2 macrophages by activating the JAK/STAT signaling pathway, resulting in increased proliferation and metastasis of HCC.[49] A study reported by Zhao elucidated that the expression of miR-144/miR-451a is regulated via chromatin remodeling dependent on DNA methylation. CpG island deletion of the miR-144/miR-451a promoter increases the expression of miR-144/miR-451a and reduces the expression of hepatocyte growth factor and macrophage migration inhibitory factor, leading to the promotion of M1-like polarization and the antitumor effect.[50]

Other Immune Cells

In addition to the immune cells mentioned above, miRNAs also play a role in regulating the biological function of other immune cells, such as tumor-associated neutrophils (TANs) and dendritic cells (DCs). TANs are neutrophils recruited into tumor tissues under the action of chemokines and involved in the genesis, development, and metastasis of tumors. They exhibit both antitumor (phenotype-N1) and protumor (phenotype-N2) activities in response to different stimuli.[51] Ye pointed out that deregulated miR-223 participates in the pathogenesis of various liver diseases by influencing neutrophil infiltration, macrophage polarization, and inflammasome activation.[52] The expression levels of CCL2 and CCL17 in TANs are significantly higher than neutrophils in peripheral blood. TANs contribute to HCC progression not only by recruiting macrophages and Treg cells but also by promoting the infiltration of macrophages and Treg cells through CCL2-CCR2 and CCL17-CCR4.[53] Although a large number of studies have been conducted on miRNAs, none has adequately covered the mechanism by which miRNAs regulate immune cells in the TIME.

Regulation of the Angiogenesis

Under normal physiological conditions, angiogenesis is a highly ordered process regulated by complex and balanced interactions between pro-angiogenic and anti-angiogenic factors. With the rapid growth of tumors, the balance between proangiogenic and antiangiogenic factors is broken, and angiogenesis is subsequently activated. HCC is a solid tumor with a high degree of angiogenesis, these changes are advantageous to the growth, progression, invasion, and metastasis of HCC.[54] Studies have revealed that miRNAs regulate tumor angiogenesis through different pathways (Figure 1). As early as 2013, Wang et al pointed out that miR-195 can directly inhibit VEGF levels and VEGF receptor 2 signaling in endothelial cells, leading to the suppression of angiogenesis and metastasis in HCC.[55] MiR-375 affects antiangiogenesis by inhibiting platelet-derived growth factor C.[56] MiR-200b-3p promotes angiogenesis by enhancing endothelial ERG (erythroblast transformation-specific (ETS)-related gene) expression.[57]

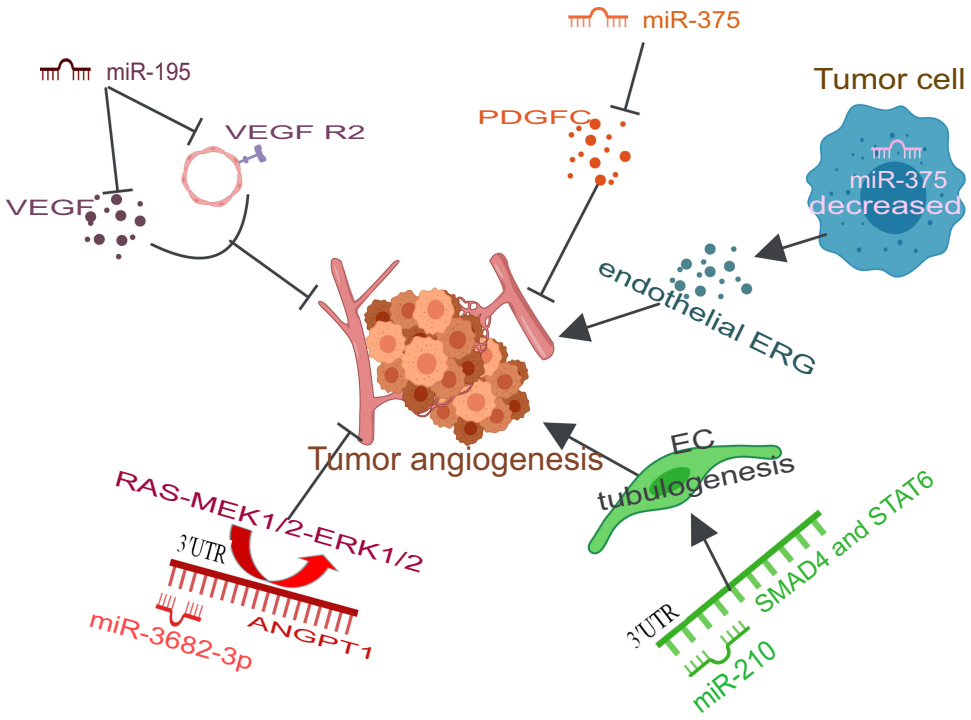

Figure 1 The role of miRNAs in the regulation of tumor angiogenesis.

Abbreviations: VEGF, vascular endothelial growth factor; VEGF R2, vascular endothelial growth factor receptor2; EC, epithelial cells; PDGFC, platelet-derived growth factor C; ERG, ETS-related gene; ANGPT1, angiopoietin-1; 3′UTR, 3′-untranslated region; SMAD4, mothers against decapentaplegic homolog 4; STAT6, signal transducer and activator of transcription 6; RAS, rat sarcoma; MEK1/2, Mitogen-activated protein kinase kinases 1/2; ERK1/2, signaling via extracellular regulated kinase 1/2.

Cancer-associated endothelial cells (ECs) are essential for angiogenesis by affecting tube formation. MiRNAs play an important role in this process. MiR-210 directly targets SMAD4 and STAT6 and inhibits their expression to stimulate EC tubulogenesis, leading to the promotion of angiogenesis.[58] Exosomal miR-638 from HuH-7Mb decreases the expression of VE-cadherin and ZO-1, resulting in attenuating endothelial tight junctions and increasing the permeability of FITC-dextran.[59] Nevertheless, the role of antiangiogenic agents in cancers is disappointing, partly because the precise molecular mechanisms of angiogenesis are unknown and partly because antiangiogenic agents may restrict drug delivery to the tumor, resulting in reduced clinical efficacy.[60]

Regulation of EMT

Epithelial-mesenchymal transition (EMT) is a complex phenotypic event characterized by the morphologic transformation of cells from an epithelioid to a mesenchymal appearance, which plays an important role in embryogenesis, stem cell biology, and cancer progression. Studies have shown that miRNAs regulate the EMT by affecting different transcription factors and signaling pathways (Figure 2).

MiR-509-3p inhibits EMT by targeting Twist which is a critical inducible transcription factor for EMT.[61] There is a growing body of reports suggesting that miRNAs affect EMT by regulating signaling pathways. miR-92a-3p is involved in the regulation of EMT by inhibiting the PTEN/Akt/Snail pathways.[62] MiR-300 inhibits the EMT by targeting FAK and the downstream PI3K/AKT pathway, leading to reduced migration and invasion of HCC.[63]

In addition to these, studies have shown that miRNAs participate in the regulation of EMT by interacting with other ncRNAs, such as lncRNAs, and circRNAs. CircTOLLIP serves as a sponge for miR-516a-5p to alleviate its inhibition of PBX3, leading to the promotion of EMT and the progression in HCC.[64] Similar results were found that lncRNA SNHG12 can upregulate HEG1 by targeting and inhibiting miR-516a-5p, resulting in the promotion of EMT in HCC.[65]

Furthermore, miRNAs are involved in the EMT by regulating metabolic pathways, which has received increasing attention. MiR-612 inhibits the EMT by directly targeting HADHA which promotes β-oxidation of fatty acids in HCC.[66]

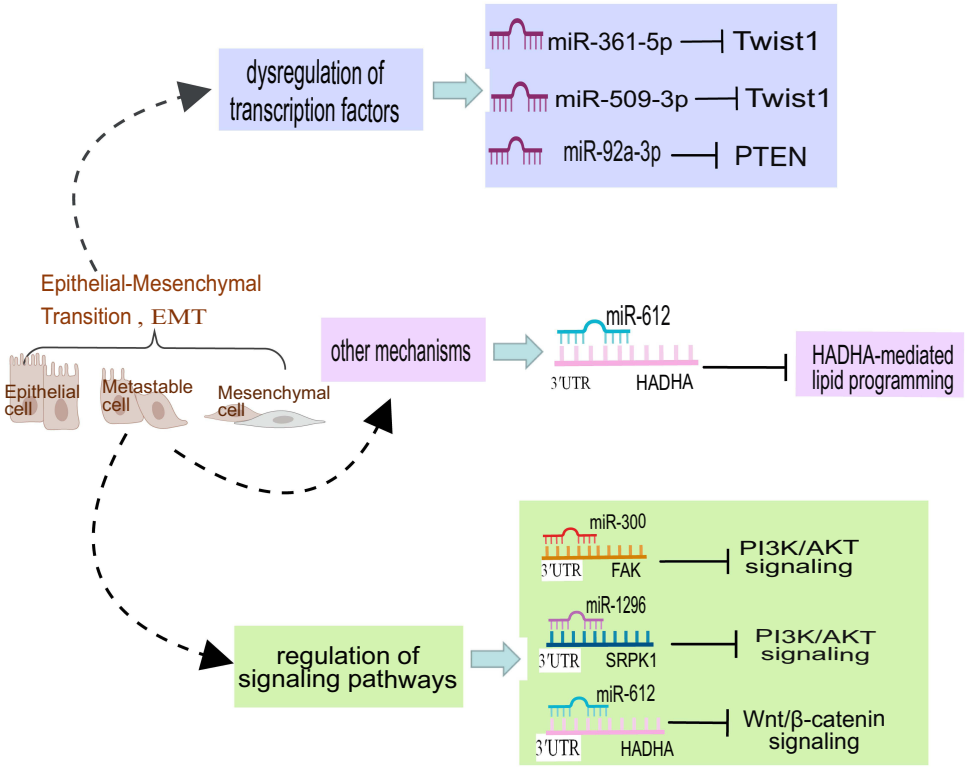

Figure 2 The role of miRNAs in the regulation of epithelial-mesenchymal transformation.
Abbreviations: Twist1, Twist-related protein 1; EMT, epithelial-mesenchymal transition; PTEN, phosphatase and tensin homolog; 3′UTR, 3′-untranslated region; HADHA, hydroxyl CoA dehydrogenase alpha subunit; FAK, focal adhesion kinase; SRPK1, serine-arginine protein kinase 1; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B.

MiR-186 inhibits the EMT, migration, and invasion in HepG2 and HUH7 cells by directly targeting cyclin-dependent kinase 6 (one of the cell cycle-related genes).[67]

However, it is difficult to obtain the same results, due to the molecular heterogeneity of HCC as well as different experimental approaches and control groups selected by laboratories, even though contrary results may be obtained in some cases. The regulatory functions and underlying mechanisms of miRNAs in EMT need further investigation.

Regulation of Tumor Invasion and Metastasis

HCC is always accompanied by intrahepatic vascular microinvasion and micrometastasis at an early stage, resulting in high aggression and poor prognosis, with a recurrence rate of over 70% in 5 years. Therefore, the molecular mechanism of invasion and metastasis in HCC urgently needs further studies. Research about miRNAs on the complex invasive-metastatic cascade response provides new insights into the HCC treatment.

On the one hand, miRNAs play a role in promoting tumor invasion and metastasis in HCC. A-kinase anchor protein 12 (AKAP12), as a scaffolding protein in signal transduction, exerts an antitumor role in various cancers including HCC. MiR-1251-5p promotes the migration and invasion of HCC in vitro by directly targeting and negatively regulating the expression of AKAP12.[68] On the other hand, miRNAs exert an inhibitory effect on the invasion and metastasis of HCC. MiR-7 can inhibit the invasion and metastasis of hepatoma cells by downregulating EMT-related proteins.[69] MiR-145 suppresses HCC metastasis and invasion by directly targeting ARF6 and inhibiting its expression.[70]

In addition, scientists have explored how miRNAs regulate the invasion and metastasis in HCC from new perspectives. Chronic inflammatory stimuli are indispensable in tumorigenesis and progression such as IL-8, IL-6, and IL-10. Peng found that IL-8 negatively modulates the expression of miR370-3p by recruiting histone deacetylase 1 (HDAC1) to the miR-370-3p promoter. MiR-370-3p attenuates IL-8 protumoral effects on liver cancer cells by directly targeting Snail and Twist1. The novel axis IL-8/STAT3/miR-370-3p/Twist1 and Snail relying on HDAC1 recruitment provides new insights into the diagnosis and treatment of HCC.[71] Studies have also shown that abnormal lipid metabolism is involved in the regulation of miRNAs on the invasion and metastasis of tumors. The miR-377-3p/CPT-1C axis regulates the proliferation, migration, and invasion of HCC mainly through continuous fatty acid oxidation (FAO), which is essential for cancers to produce extra energy to maintain the rapid proliferation of cells.[72]

The invasion and metastasis in HCC are complex processes involving multiple factors, the regulatory functions and underlying mechanisms of miRNAs in these processes have yet to be fully elucidated. More in-depth research is needed in the future.

Regulation of Tumor Metabolism

To accommodate the insufficient supply of nutrients and oxygen, metabolic reprogramming becomes an essential survival strategy for tumors. The most classic event of metabolic reprogramming in cancer is the Warburg effect, also known as aerobic glycolysis, which is considered a hallmark of cancers and an indicator of poor prognosis. Even so, malignant cells prefer aerobic glycolysis in case of sufficient oxygen.[18]

Studies have revealed that miRNAs are involved in regulating the aerobic glycolysis. Forkhead box K1 (FOXK1) is a transcription factor that promotes the progression of multiple cancers. Xing et al reported that miR-144-3p inhibits glycolysis by targeting FOXK1, thereby inhibiting the malignant progression of HCC.[73] MiR-183-5p could increase aerobic glycolysis by targeting PTEN and then activating Akt/mTOR signaling, leading to the promotion of migration and invasion in HCC.[74]

The Warburg effect results in a large production and accumulation of lactate, which enhances the immunosuppressive properties of the TIME. Komoll et al found that miR-342-3p plays a suppressor role in HCC by targeting and modulating the lactate transport function of member 1/ monocarboxylate transporter 1 (SLC16A1/MCT1). Mechanistically, the downregulation of MCT1 expression by miR-342-3p impairs lactate transport, resulting in an increase in extracellular lactate level and a decrease in intracellular lactate level. This change attenuated immunosuppression, resulting in a significant suppression of tumor progression and prolonged survival in HCC patients.[75] In addition to participating in glycolytic and lactate metabolism, miR-377-3p and miR-21 play important roles in hepatic lipid metabolism.[72,76]

Amino acid metabolic remodeling is another important factor affecting the malignant activities of cancers. MiR-122 is involved in Gln metabolism and transport in hepatic cells by inhibiting the expression of GLS (Gln metabolism) and SLC1A5 (Gln transport). HCC patients with higher levels of GLS and SLC1A5 have a significantly lower survival rate than patients with lower levels.[77] TDO2 (tryptophan 2,3-dioxygenase), is highly expressed in HCC and involved in immune tolerance. MiR-126-5p affects cell proliferation and metastasis by directly targeting and promoting the expression of TDO2.[78] Targeting tumor metabolic pathways may become an effective therapeutic strategy for HCC treatment. The underlying mechanisms of miRNAs in regulating tumor metabolism (Figure 3) provide new insights into HCC treatment.

Regulation of Drug Resistance

With the advent of molecularly targeted drugs and immune checkpoint inhibitors (ICIs), a breakthrough has been made in HCC treatment. Nonetheless, treatment resistance has become one of the major barriers to the failure of HCC treatment, and more than 90% of cancer patients' deaths are associated with chemotherapy resistance.[79] Studies have shown that miRNAs play important roles in drug resistance by fine-tuning key physiological and pathophysiological processes.

Sorafenib, a kinase inhibitor, is commonly used in the treatment of patients with advanced HCC. MiR-124-3p.1 enhances sorafenib-induced apoptosis by affecting the acetylation and nuclear localization of FOXO3a by targeting

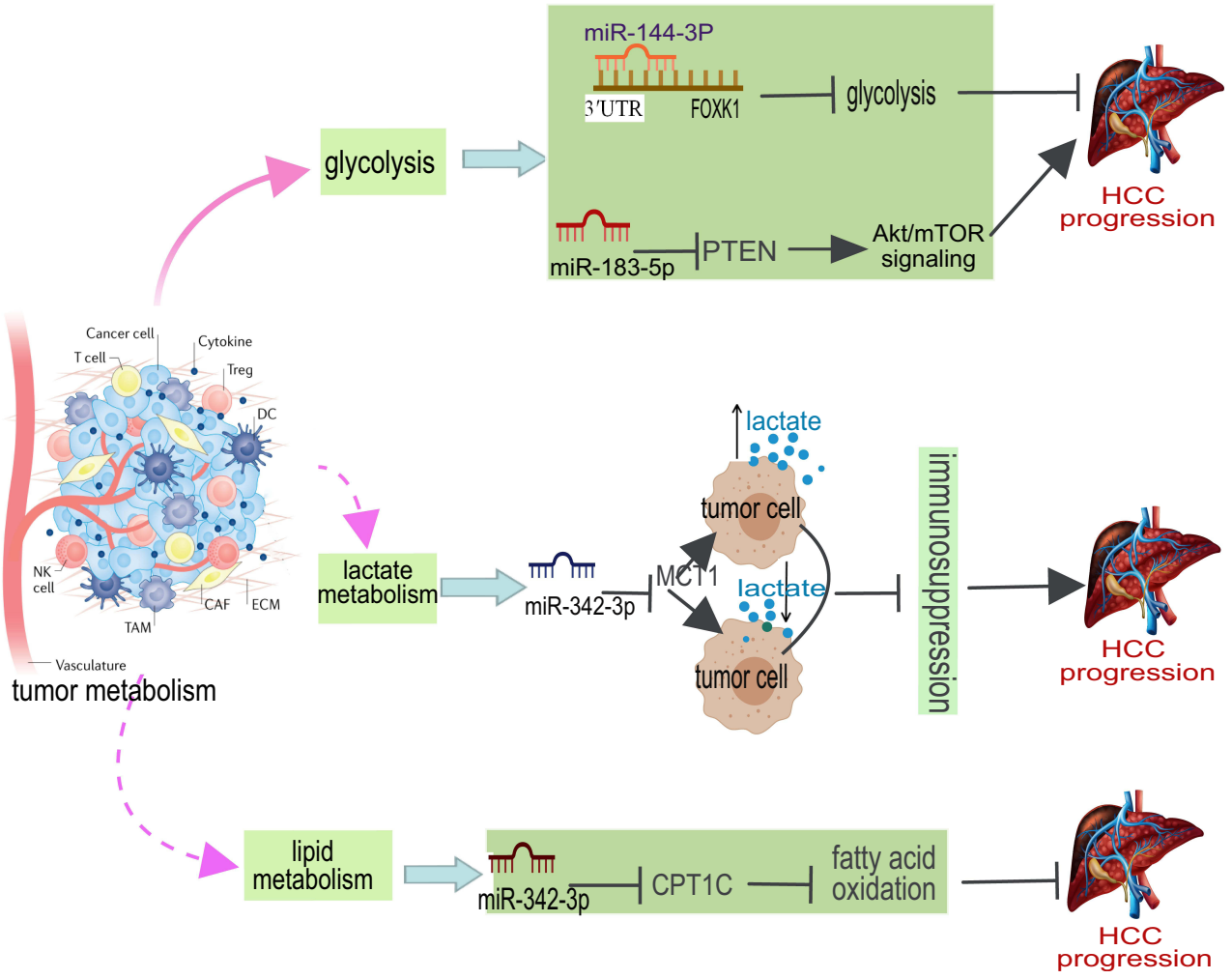

Figure 3 The role of miRNAs in the regulation of tumor metabolism.
Abbreviations: DC, dendritic Cells; NK cell, natural killer cell; TAM, tumor-associated macrophage; CAF, cancer-associated fibroblast; ECM, extracellular matrix; PTEN, phosphatase and tensin homolog; 3′UTR, 3′-untranslated region; FOXK1, forkhead box K1; Akt, protein kinase B; mTOR, mammalian/mechanistic target of rapamycin; HCC, hepatocellular carcinoma; MCT1, monocarboxylate transporter 1; CPT1C, carnitine palmitoyltransferase 1C.

SIRT1 and AKT2, respectively.[80] MiR-138-1-3p directly targets PAK5 and inhibits its expression, up-regulation of PAK5 contributes to the sorafenib resistance of HCC via β-catenin/ABCB1 signaling pathway.[81] Lenvatinib is another first-line multikinase inhibitor for patients with advanced HCC. MiR-128-3p regulates lenvatinib resistance through proliferation and apoptosis-related signaling pathways by downregulating c-Met.[82]

EMT-associated miRNAs play a crucial role in drug resistance. MiR-125b-5p is involved in sorafenib resistance by inducing EMT through up-regulating the Snail.[83] However, miR-541 increases the sensitivity of HCC cells to sorafenib treatment by directly targeting and down-regulating autophagy-related gene 2A (ATG2A).[84] Besides, miRNAs regulate drug resistance through the ferroptosis. The overexpression of miR-23a-3p attenuates sorafenib-induced ferroptosis by targeting ACSL4, resulting in sorafenib resistance.[85]

The mechanisms of miRNAs in regulating drug resistance are being investigated in depth (Figure 4). However, it is difficult to fully elucidate the mechanism of miRNAs in regulating drug resistance because it is the result of the synergistic effect of multiple genes and mechanisms.

Clinical Application of MiRNAs in HCC

Being diagnosed at an advanced stage and a high recurrence rate are leading causes of the poor prognosis of HCC. Looking for diagnostic/prognostic biomarkers, especially in the early stage, is significant for the prevention and treatment of HCC. The pathological diagnosis of HCC is the "gold standard", but its clinical application should be very cautious because it is an invasive approach and has a risk of metastasizing. However, traditional biomarkers have shown poor

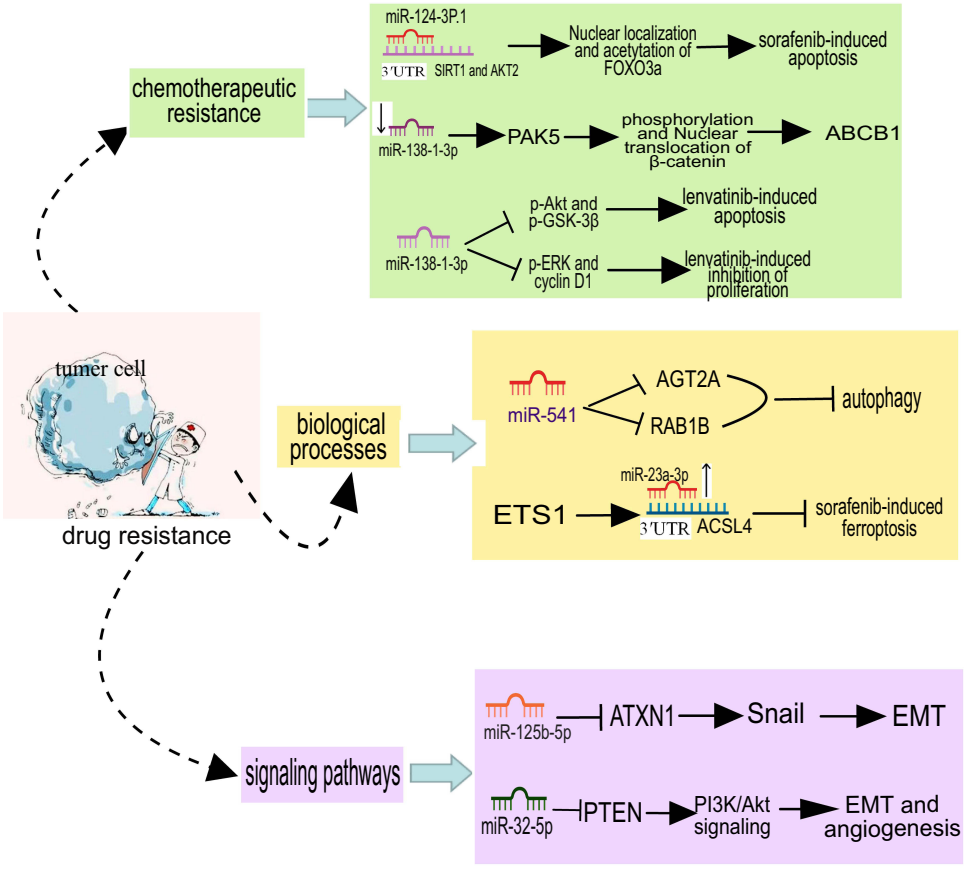

Figure 4 The role of miRNAs in the regulation of drug resistance.

Abbreviations: 3′UTR, 3′-untranslated region; SIRT1, sirtuin 1; AKT2, protein kinase B2; PAK5, P21-activated kinase 5; p-AKT, gemcitabine-induced phosphorylated AKT; p-GSK-3β, phospho-glycogen synthase kinase-3beta; p-ERK, phosphorylated extra-cellular signal-regulated kinase; FOXO3a, Forkhead box class O3a; ABCB1, P-glycoprotein; ATG2A, autophagy-related gene 2A; RAB1B, Ras-related protein Rab-1B; ETS1, ETS proto-oncogene 1; ACSL4, acyl-CoA synthetase long-chain family member 4; ATXN1, the three prime untranslated regions (3′-UTRs) of ataxin 1; PTEN, phosphatase and tensin homolog; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; EMT, epithelial-mesenchymal transition.

performance in the monitoring, diagnosis, and prognosis of HCC. Circulating miRNAs are secreted into extracellular spaces and extremely stable in biological fluids (eg, serum, plasma, and urine), their changes indicate the status of cancers and the prognosis of patients. Accumulating studies have shown that circulating miRNAs have potential clinical application in the diagnosis and prognosis of HCC as minimally invasive biomarkers. This section summarizes the main diagnostic/prognostic indicators which are currently being studied.

MiRNAs Act as Diagnostic Biomarkers

The utility of circulating miRNAs as diagnostic markers for HCC is evaluated through a clinical trial database or clinical trials. More details are shown in Table 1. As early as 2015, it was reported that miR-21 can be used as an early diagnostic marker for HCC, the change of serum miR-21 occurs earlier and more accurately reflects the pathogenesis of HCC than the change of AFP.[86] Studies have shown that both plasma and urine miR-39-5p can be used to detect early, advanced, and overall HBV-associated HCC cases with more than 85% sensitivities and 93% specificities. Interestingly, miR-93-5p in urine could be used to predict the prognosis of patients with HBV-related HCC.[87] Emerging investigations demonstrate that developing a platform of multiple miRNAs could improve their sensitivity and specificity for HCC detection.

Table 1 MiRNAs as Diagnostic Biomarkers for HCC

MiRNAs	Sample	Expression	Patients Enrolled	Diagnostic Accuracy (HCC vs Non-HCC)	Confidence Interval (CI)	References
MiR-96	Exosome	↑	50 hCC, 50 LC, 50 hC	AUC 0.80	0.016–0.832	[89]
MiR-122	Exosome	↓	50 hCC, 50 LC, 50 hC	AUC 0.85	0.027–0.903	[89]
MiR-21	Exosome	↑	50 hCC, 50LC, 50 hC	AUC 0.92	0.024–0.241	[89]
MiR-21+miR-12 +miR-96	Exosome	↑	50 hCC, 50LC, 50 hC	AUC 0.924, sensitivity 82%, specificity 92%	/	[89]
MiR-221	Serum	↑	45 hCC, 45 hC	AUC 0.945, sensitivity 77.8%, specificity 90.90%	0.655–0.894	[90]
MiR-221+AFP	Serum	↑	45 hCC, 45 hC	Sensitivity 96.49%, specificity 88%	/	[90]
MiR-122-5p	Exosome	↑	124 hCC, 46 hC	AUC 0.84	0.743–0.929	[88]
Let-7d-5p	Exosome	↑	124 hCC, 46 hC	AUC 0.77	0.575–0.801	[88]
MiR-425-5p	Exosome	↑	124 hCC, 46 hC	AUC 0.71	0.532–0.775	[88]
MiR-93-5p	Plasma	↑	64 hCC, 65 hC	AUC 0.905, sensitivity 86.2%, specificity 95.4%	/	[87]
MiR-93-5p	Urine	↑	64 hCC, 65 hC	AUC 0.910, sensitivity 87.7%, specificity 95.4%	/	[87]
MiR-155	Serum	↑	80 hCC, 80CHB, 40 hC	AUC 0.743, sensitivity 80%, specificity 62.5%	/	[91]
MiR-10b-5p	Exosome	↑	38 hCC	AUC 0.65, sensitivity 76%, specificity 55%	0.54–0.77	[92]
MiR-221-3p	Exosome	↑	38 hCC	AUC 0.69, sensitivity 87%, specificity 52%	0.58–0.80	[92]
MiR-21-5p	Exosome	↑	38 hCC	AUC 0.78, sensitivity 74%, specificity 77%	0.69–0.87	[92]

(*Continued*)

Table 1 (Continued).

MiRNAs	Sample	Expression	Patients Enrolled	Diagnostic Accuracy (HCC vs Non-HCC)	Confidence Interval (CI)	References
MiR-223-3p	Exosome	↑	38 hCC	AUC 0.63, sensitivity 61%, specificity 70%	0.51–0.75	[92]
MiR-484	Plasma	↓	41 hCC, 47 hF, 40 LC, 40 hC	AUC 0.67	0.5067–0.8307	[93]
MiR-224	Serum	↑	89 hCC, 50 hC	AUC 0.910	0.84–0.98	[94]
MiR-148a	Plasma	↓	155 hCC, 95LC, 95 hC	AUC 0.949, sensitivity 90.6%, specificity 92.6%	0.916–0.981	[95]
MiR-409-3p	Serum	↓	20 hCC	AUC 0.80, sensitivity 85%, specificity 70%	0.66–0.95	[96]
MiR-125a-3p	Serum	↓	12 hCC	AUC 0.98, sensitivity 80%, specificity 100%	/	[97]
MiR-125b	Plasma	↓	64 hCC, 59 LC, 63 CHB, 56 hC	AUC 0.891, sensitivity 85.9%, specificity 78.6%	0.835–0.947	[98]
MiR-338-5p	Plasma	↑	47 hCC, 29 LC, 31 hC	AUC 0.909, sensitivity 72.3%, specificity 99.68%	/	[99]
MiR-764	Plasma	↑	47 hCC, 29 LC, 31 hC	AUC 0.791, sensitivity 74.5%, specificity 77%	/	[99]
MiR-15b-5p	Plasma	↑	47 hCC, 29 LC, 31 hC	AUC 0.765, sensitivity 68.1%, specificity 80%	/	[99]
MiR-497	Serum and tissue	↓	50 hCC, 50 hC	AUC 0.726, sensitivity 74%, specificity 66%	0.628–0.810	[100]
MiR-1246	Serum and tissue	↑	50 hCC, 50 hC	AUC 0.865, sensitivity 82%, specificity 80%	0.783–0.925	[100]
MiR-326	Serum	↑	70HCC, 25HC	AUC 0.784, sensitivity 97%, specificity 52%	0.677–0.891	[101]

Notes: ↑ represents that the expression is up-regulated in samples; ↓ represents that the expression is down-regulated in samples; / represents that the data can't be found in references.
Abbreviations: HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; LC, liver cirrhosis; HC, healthy control; AUC, Area Under Curve.

A combination of these three miRNAs (miR-122-5p, let-7d-5p, and miR-425-5p) improves the accuracy of diagnoses for HCC, with an area under the curve (AUC) of 0.97.[88] The exosomal miRNA panel containing miRNA-122, miRNA-21, and miRNA-96 has high accuracy in discriminating HCC from the cirrhosis group and healthy volunteers' group with a higher AUC value of 0.924.[89] A view that the combination of miRNAs with AFP may be a more desirable diagnostic modality. Li reported that the combined detection of serum miR-221 and AFP had a sensitivity of 96.49% and an accuracy of 93.10% for HCC.[90] Clinical trials of miR-21 and miR-221 as early diagnostic markers for HCC have been conducted, and the details can be found by querying NCT05449847 and NCT02928627, respectively.

MiRNAs Act as Prognostic Biomarkers

Searching for specific biomarkers of the prognosis is crucial to HCC treatment. Abnormal expression and regulation of miRNAs in serum/plasma, especially in exosomes, is regarded as a "beacon" of cancer prognosis.

Lee pointed out that the overall survival and progression-free survival are significantly lower in HCC patients with higher level of exosomal miRNA-21 (≥0.09) (Log rank test: $p<0.05$). MiRNA-21 is associated not only with TNM

staging but also with T staging and portal vein thrombosis.[102] Another study has shown that a low expression of miR-33b is strongly associated with tumor volume, metastasis, and higher clinical stage of HCC.[103] Studies show that abnormal expressions of miR-637, miRNA-29a, miR-21, and miR-122 could be used as promising prognostic markers for HCC.[104–106] Details are shown in Table 2.

Therapeutic Potential of miRNAs in HCC

For a long time, Proteins have been widely considered to be the targets of most drugs in human diseases, and many new drugs targeting them have been introduced.[114,115] Studies have shown that most proteins are "nondruggable", only 0.2% of the genome codes for disease-related proteins.[116] Therefore, the feasibility of targeting RNA has attracted increasing attention. MiRNAs are involved in the occurrence and development of cancers. The regulatory mechanisms of miRNAs are delicate and complex, one miRNA may have multiple target genes, and one gene may be regulated by several miRNAs. So, miRNAs are at the core of a large and fine regulatory network. In this section, we will summarize the research progress of miRNAs in the treatment of HCC, especially its precision therapy.

Table 2 MiRNAs as Prognostic Biomarkers for HCC

MiRNAs	Sample	Expression	Patients Enrolled	Associated factors and Clinicopathological characteristic	References
MiR-92a-3p	Plasma	↑	42 hCC	OS, DFS	[62]
MiR-1251-5p	Tissue	↑	50 hCC	OS, larger tumor size, vascular invasion, and TNM stage	[68]
MiRNA-21	Serum	↑	/	OS, PFS, TNM stage, T stage and portal vein thrombosis	[102, 107]
MiR-484	Serum	↑	/	OS, PFS	[102, 107]
MiR-33b	Tissue	↓	/	Tumor volume, metastasis, and higher clinical stage	[103, 108]
MiR-637	Tissue	↓	/	OS	[104, 109]
MiRNA-29a	Serum	↑	50 hCC, 50 LC, 50HC	Tumor number, size, stage, and outcome	[105]
MiRNA-122	Whole blood	↑	54 hCC, 28LC, 12 hC	PFS	[106]
MiR-93-5p	Urine	↑	64 hCC, 65 hC	OS, PFS	[87]
MiR-497	Serum and tissue	↓	50 hCC, 50 hC	Tumor size, TNM staging, differentiation, and metastasis	[100]
MiR-1246	Serum and tissue	↑	50 hCC, 50 hC	TNM staging, differentiation, and metastasis	[100]
MiR-4454	Serum	↑	86HCC(40curativetreatment, 46TACE)	OS, DFS	[110]
MiR-4530	Serum	↑	86HCC(40curativetreatment, 46TACE)	OS, DFS	[110]
MiR-122	Serum	↑	122 hCC	OS	[111]
MiR-122	Plasma	↑	120 hCC	OS, DFS, TNM stage	[111]
MiR-122	plasma	↑	112 hCC	TUMOR number, tumor size, TFS	[112]
MiR-139	Plasma	↓	31 hCC, 31 hC	OS	[110, 113]
MiR-326	Serum	↑	70HCC, 25HC	Tumor, node, (TNM) staging, tumor differentiation, metastases	[101]

Notes: ↑ represents that the expression is up-regulated in samples; ↓ represents that the expression is down-regulated in samples; / represents that the data can't be found in references.

Abbreviations: HCC, hepatocellular carcinoma; TACE, transhepatic arterial chemotherapy and embolization; LC, liver cirrhosis; HC, healthy control; OS, overall survival; PFS, progression-free survival; DFS, disease-free survival; TNM stage, tumor, node, and metastasis stage; TFS, transplantation-free survival.

There are two main therapeutic strategies for miRNAs: replacement, restoration, or overexpression therapy, such as miRNA mimics, and miRNA-targeted therapeutics, including miRNA reduction, inhibition, or downregulation, such as antagomiRs.[117] MiRNA replacement therapeutics are achieved by importing chemically synthesized miRNAs or miRNA mimics into hepatocytes to restore/enhance tumor suppressor miRNAs.[118] A report conducted by Komoll indicated that administration of adeno-associated virus vector (AAV)-mediated miR-342-3p (AAV-miR-342-3p) can significantly attenuate tumor development and prolong overall survival In different mouse models of HCC.[75] Intravenously injected liposome-based miR-34 (MRX34) is the first-in-class miRNA replacement therapy for patients with advanced HCC. MiR-34a plays a vital role in inhibiting the progression of HCC by suppressing the expression of hexokinase-1, TRAF5, E2F1, and E2F3. Its Phase I trial was initiated in May 2013. Although the preliminary results promised a tolerable safety profile for patients with advanced HCC, several patients died during MRX34 replacement therapy owing to serious immune-related adverse events.[119] Similar findings were shown in another study.[120] Now, the development of MRX34 has been halted. However, whether miR-34a has the specific gene-suppressing activity, the double-stranded RNA (dsRNA) of the MRX34 has the nonspecific inflammatory effect or the effective delivery of these RNA constructs affects the clinical effects (both toxicity and antitumor activity) remains to be further studied.

MiRNA-targeted therapeutics are achieved by regulating the expression of oncogenic miRNAs with specific miRNA inhibitors. Miravirsen, against miR-122, is the first miRNA inhibitor. The Phase II clinical trial of Miravirsen for the treatment of hepatitis C virus was successfully completed.[121] AC1MMYR2, a small-molecule inhibitor of miR-21, reverses EMT and inhibits tumor growth, invasion, and metastasis by blocking miR-21 maturation without significant tissue cytotoxicity.[122] What excited us is that a study from Hassan holds much promise for the miR-122 mimic/miR-221 inhibitor combination as an innovative therapeutic strategy for HCC in a mouse model induced by DEN. Combined treatment with miR-122 mimic /miR-221 antagonist is the most effective technique compared to treatment with miR-122 mimic or miR-221 inhibitor alone. Mechanistically, coadministration of miR-122 mimic and miR-221 inhibitor dramatically downregulates the expression of cyclin D1, TGF-β, and β-catenin genes which play significant roles in the regulation of the cell cycle, EMT, and cell proliferation, respectively.[123]

With the rapid development of sequencing technologies and significant advances in miRNA research, miRNA therapeutics remain the most promising treatments for tumors, although they are still in the experimental stage.

Conclusion

In this review, we focus on the roles of miRNAs in regulating TIME, angiogenesis, EMT, invasion, metastasis, metabolism, and drug resistance and their molecular mechanisms in HCC. MiRNAs are not only large in number but also complex in function, one miRNA may have multiple target genes, or several miRNAs may simultaneously target and regulate one gene. Whether the dysregulation of miRNAs is a cause or a consequence of cancers has not been fully elucidated, and more in-depth studies are needed in the future. In addition, we provide a comprehensive overview of the roles and promising approaches of miRNAs in the diagnosis and therapy of HCC. However, there are no miRNA therapies for HCC approved by the FDA yet. The main reason is that miRNA therapies for HCC are still in the experimental stage, and there are still many challenges to be solved. One challenge is that the roles and underlying molecular mechanisms of miRNAs in HCC have not been fully elucidated. Another challenge is the toxicity of miRNAs in patients, such as severe immune responses and off-target effects on the other genes. Facing the challenges, the following strategies are needed. First, the next-generation sequencing technology is vital to explore the vast uncharted territory of miRNAs and their emerging roles. Studying the function of miRNAs in clinical Settings is as important as at the cellular or animal level. Second, improving targeting methods and delivery systems of therapeutic miRNAs can minimize the immune responses and overcome the off-target effects. Finally, interdisciplinary cooperation in several fields such as immunology, molecular biology, pharmacology, and nanotechnology can promote the development of HCC therapeutic strategies based on miRNAs. Nevertheless, it remains a focus of attention in the pharmaceutical industry and will be a promising tool for personalized therapy alone or in combination with other therapies shortly.

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