Role of microRNAs in the development of hepatocellular carcinoma and drug resistance

Lucas Tricoli[1], Suresh Niture[1], Uche Chimeh[1], Habtom Ressom[2], Deepak Kumar[1,2,3]

[1]Julius L. Chambers Biomedical Biotechnology Research Institute, [2]Department of Pharmaceutical Sciences, North Carolina Central University, Durham NC; [3]Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC.

TABLE OF CONTENTS

1. ABSTRACT

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths worldwide and has a relatively poor survival rate. Aside from liver transplantation, the most effective and leading curative measure for HCC is the chemotherapeutic agent sorafenib, which is a multikinase inhibitor used for treating late-stage HCC. However, the effects of sorafenib are short-lived because of rapid acquisition of multi-drug resistance (MDR) by tumor cells. MicroRNAs (miRNAs) have emerged as crucial regulatory molecules for almost every biochemical pathway in humans. The progression of HCC and acquired MDR are critically influenced by miRNAs through regulation of key genes in cell regulatory pathways. This review explores the involvement of miRNAs in the development of HCC and their role in key signaling pathways leading to MDR in HCC.

2. INTRODUCTION

Hepatocellular carcinoma (HCC) is among the leading causes of cancer-related deaths worldwide (1, 2). According to the American Cancer Society, the survival rate for HCC in the United States is relatively poor, with 42,220 new cases diagnosed in 2018 and 30,200 of these individuals dying of this malignancy. Many external and environmental factors, such as a viral disease and drug usage, contribute to rapid onset of liver damage and HCC. Liver resection and subsequent transplantation are the most effective and leading curative measures for HCC (2). The chemotherapeutic agent sorafenib remains the most effective and widely used multikinase inhibitor for treating late-stage HCC. However, the effects of sorafenib are short-lived because of the tumor's rapid acquisition of drug resistance (1, 2).

MicroRNAs (miRNAs) are small (approximately 20 bp) non-coding RNAs that target specific mRNA sequences for degradation, thereby inhibiting protein translation (3). The emergence of miRNAs as regulatory molecules has proven critical to almost every biochemical pathway in humans (3). Because of their short-lived and immediate effects on multiple pathways and downstream targets, miRNAs are an attractive target for therapy. In addition, miRNAs are frequently found to change their regulatory patterns based on the presence of external stimuli or progression of disease, making them an attractive target for biomarker development (4).

A plethora of new research has been recently published regarding the role of miRNAs in HCC and, specifically, sorafenib resistance (2, 4-8). In this review,
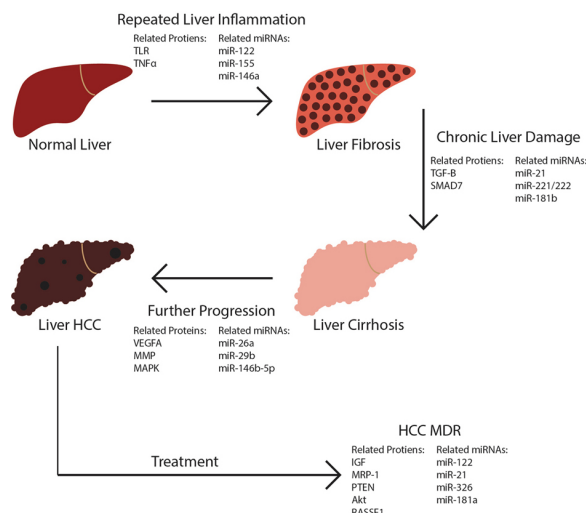
Figure 1. Schematic depiction of liver disease progression listing the major proteins involved at each step and some of the major accompanying regulatory microRNAs involved.

we compiled data from the literature to present a cohesive story of current understanding regarding the function of key liver miRNAs in HCC development, progression, and drug resistance. Discovering the mechanisms of HCC development and progression to resistance can uncover new methods for circumventing multidrug resistance (MDR) and optimizing HCC treatment.

3. HEPATOCELLULAR CARCINOMA

3.1. Critical pathways in HCC development

The general progression for HCC begins with steatosis, followed by fibrosis and subsequent cirrhosis of the liver. A multitude of factors, including viral infections, liver disease, alcohol abuse, and diabetes, cause chronic inflammation and damage to the liver and are primarily responsible for the development of HCC (9). HCC is a highly vascularized tumor, in which angiogenesis is critical for development and metastatic potential (8, 10). Dysregulation of cell cycle progression and failure of apoptosis/autophagy are classic hallmarks of progression to HCC, as with many types of neoplasia. However, unlike some tumors, such as prostate or breast cancer, that are hormonally driven, dysregulation of key cellular, proliferative and developmental pathways leads to HCC (11). There are no tissue-related, hormone-specific components to target in HCC, making study and treatment of HCC particularly difficult (11).

Several major pathways are involved in liver cell dysregulation and progression to HCC: phosphatase and tensin homolog (PTEN), phosphatidylinositol-3-kinase (P13K)/protein kinase B (AKT), mitogen-activated protein kinase (MAPK), Janus kinase (JAK)/signal transducer and activator of transcription (STAT),

and epidermal growth factor receptor (EGFR)/insulin-like growth factor (IGF) pathways. The EGFR/IGF pathway is particularly noteworthy because of its important role in vascularization and angiogenesis of the liver, through regulation of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) (1, 10). The MAPK pathway is a major therapeutic target and is strongly associated with the P13K/AKT pathway, which also interacts with VEGF and PDGF. The STAT pathway is likewise critical to liver regulation; it is involved with regulation of cell proliferation, inflammation, differentiation, survival, motility, and apoptosis (1, 12). STAT3 is upregulated in HCC tumor cell lines, inhibiting an immunogenic response (12). Figure 1 illustrates the general progression of a healthy liver to HCC, including the pathways, proteins, and associated regulatory miRNAs.

3.2. HCC therapies

Except for liver transplantation, the most effective current treatment for HCC is the first-line chemotherapeutic drug sorafenib. Sorafenib is a multikinase inhibitor developed as primary treatment for advanced stage HCC. It inhibits VEGF and PDGF and blocks the MAPK/rapidly accelerated fibrosarcoma (Raf) pathway, all of which are critical for angiogenesis and tumor growth (1). Sorafenib can also induce autophagy in HCC, reducing the size of an established tumor (7). Unfortunately, HCC is resistant to many chemotherapeutic agents because of its high genetic variability and ability to develop MDR. Sorafenib is no exception: some patients exhibit primary resistance upon initiation of treatment, and a majority of patients develop resistance within months of beginning sorafenib (1, 13). The average prolongation of life with sorafenib treatment is slightly less than 1 year, which is a relatively small improvement in survival (12). In addition, sorafenib was initially approved by the U.S. Food and Drug Administration (FDA) in 2007, making this drug a decade old.

New therapies, such as ramucirumab, have recently undergone trials for patients with HCC who previously received sorafenib. Ramucirumab is an anti-VEGFR-2 monoclonal antibody shown to increase survival of patients with baseline α-fetoprotein levels of 400 ng/mL or greater (14). Hepatic expression of α-fetoprotein after damage or the development of cirrhosis is a risk factor for HCC (15).

In addition, pre-existing chemotherapeutic agents have emerged, such as regorafenib, which was approved by the FDA in 2017 for the treatment of HCC. Regorafenib is a multikinase inhibitor with a mechanism of action similar to sorafenib, blocking VEGF. However, regorafenib is a more potent angiogenesis inhibitor than sorafenib (16). Other existing multikinase inhibitors targeting the VEGF pathway are also being

considered for clinical trials and HCC approval, including dovitinib, sunitinib, cabozantinib, and vectibix (12). The mechanism of dovitinib (which is currently undergoing clinical trials) is downregulation of the STAT3 pathway through induction of SH2 domain-containing tyrosine phosphatase-1 (SPH-1) and subsequent apoptosis (12). Sunitinib, which is also undergoing clinical trials, has demonstrated activity against HCC (17). Further study and combinatorial use of these chemotherapeutic agents have been driven by sorafenib's only moderate effectiveness against HCC and the development of resistance.

3.3. HCC drug resistance

The development of drug resistance is an important factor to consider when treating malignancies. In general, HCC is difficult to treat because the lack of liver-specific targets available for therapeutic development. Pathways that are key to HCC development (EGFR, PDGF receptor [PDGFR], fibroblast growth factor receptor [FGFR], MAPK, IFG, and transforming growth factor-beta [TGF-β]) are also vital to many other tissue types (11). MDR is a major mode of acquired HCC resistance, which occurs through a multitude of mechanisms, including hypoxic environment, DNA damage, epithelial-mesenchymal transition (EMT), autophagy, and epigenetic regulation, circumventing shutdown of any single pathway (18). Forkhead box protein M1 (FoxM1) is a transcription factor involved in cell cycle progression and proliferation, which is essential for tumorigenic metastatic potential (19). Mouse models in which FoxM1 is deleted demonstrate significant resistance to the development of HCC (11). Expression of FoxM1 in HCC is also linked to recurrence and poor survival (11). Thiazole compounds and proteasome inhibitors have been demonstrated to repress FoxM1, leading to decreased tumor size in mice (11). A DNA damage response-related protein, metadherin (MTDH), is believed to be linked to DNA repair through binding of RNA, and high levels of MTDH are expressed in HCC (11). In addition, ion channels and cellular pumps are a major source of MDR in carcinogenesis. Regulation of transient receptor potential calcium channel (TRPC6) is one example by which STAT3 regulates calcium-dependent MDR in HCC (18).

Mechanisms of sorafenib resistance involve interactions between the JAK/STAT and EMT pathways. The development of advanced HCC is characterized by vascularization and angiogenesis, which are promising targets for HCC chemotherapy. However, blocking the MAPK/Raf pathway is only a small part of the chemotherapeutic mechanistic pathway. The PI3K/Akt pathway cross talks with MAPK/Raf and can be activated in response to sorafenib, thereby stimulating resistance (1, 7). Sorafenib is also able to induce expression of growth arrest and DNA damage-inducible 45 beta (GADD45β), which is a regulator of autophagy

in HCC (20). Sorafenib inhibits STAT3, which is involved in the metastatic potential of HCC through protein phosphorylation (21). HCC cells that have become resistance to sorafenib exhibit increased STAT-3 levels, highlighting the involvement of the JAK/STAT pathway in chemoresistance (1). However, dovitinib can re-sensitize cells that have acquired sorafenib resistance by inducing expression of SHP-1, which is a negative regulator of STAT3 that inhibits HCC proliferation (12).

miRNAs are emerging as a major factor contributing to the acquisition of drug resistance through regulation of MDR related pathways (18). Developing a working knowledge of critical miRNAs involved in the development of drug resistance can aid in promoting the efficacy and longevity of current chemotherapeutic agents for HCC. A list of currently known miRNAs, their roles in liver function and HCC, and their corresponding protein targets are shown in Table 1. Figure 2 presents a graphic representation of these miRNAs and their corresponding functions.
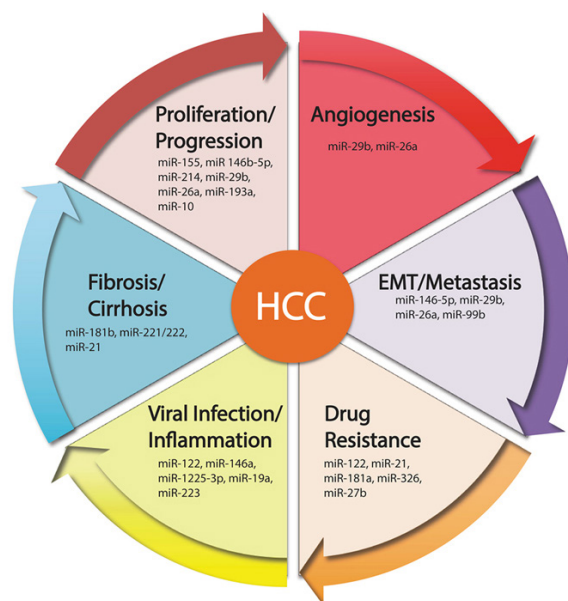
4. miRNAs

4.1. miRNAs involved in HCC development

miR-122 is the most abundant miRNA in the liver, composing 70% of the total liver miRNA; it plays a major role in basic liver function and homeostasis (22, 23). Because of its abundance and importance in liver function and HCC development, a substantial amount of research has been dedicated to miR-122 (5, 24-26). miR-122 is relatively specific to the liver, exhibiting almost no expression in other tissues; is highly conserved among vertebrate animals; and is regulated in a circadian-like cycle (24). Transcription factors in the liver—hepatocyte nuclear factor (HNF)1α, HNF3β, HNF4α, and CCAAT/enhancer-binding protein (C/EBP)α—bind to the miR-122 promoter, increasing its expression and thereby facilitating liver development (5). In addition, miR-122 plays a primary role in lipid and cholesterol metabolism (5).

Because of its vital role in liver regulation, it is common to see dysregulation of miR-122 during liver damage (24). Nearly every phase in the molecular progression to HCC involves miR-122 (24, 25). Repeated liver inflammation is a significant molecular perturbation that contributes to the development of HCC (27). During drug- and alcohol-related liver damage and subsequent inflammation, serum levels of miR-122 are elevated (22). Because of its liver specificity and upregulation with liver damage, miR-122 has been proposed as a biomarker for liver disease and the development of HCC (22). However, when HCC appears, miR-122 becomes downregulated in the tumor (22, 23). The progression from upregulation in the serum to loss of expression in the primary tumor, suggests that molecular pathways initially attempt to rescue damaged tissue through overregulation, but

Table 1. Role of different miRNAs in HCC development and drug resistance

miRNA	Function	Target	Reference
miR-122	Role in normal Liver function, HCC (including HBV-related HCC), sorafenib resistance, and doxorubicin sensitivity	IGF-1R, Cyclin G1, HNF1α, HNF3β, HNF4α, C/EBPα, MDR, MDR1, MRP, GST-p, Bcl-w, CCNG1	(25), (25), (45), (46), (5), (39)
miR-155	Involved in the tumorigenesis and clinical characteristics of HCC	SOCS1, MMP9	(47)
miR-146a	Contribution to inflammatory response	TLRs	(22)
miR-146b-5p	Inhibits tumor growth and metastasis of HCC	TRAF6	(34)
miR-214	Suppresses progression of HCC	β-catenin	(33)
miR-29b	Represses angiogenesis, invasion, and metastasis of HCC	MMP-2	(8)
miR-26a	Suppresses tumor growth and metastasis of HCC; suppresses tumor angiogenesis of HCC	VEGFA, VEGFR2, HGF-cMet	(48), (6)
miR-19a	Involved in hepatocarcinogenesis in HBV-related HCC	PTEN	(26)
miR-223	Involved in hepatocarcinogenesis in HBV-related HCC	c-myc	(26)
miR-21	Involved in fibrosis and acquired sorafenib resistance by suppressing autophagy in HCC	TGF-b, PTEN	(7), (22)
miR-99b	Promotes metastasis of HCC and is correlated with poor clinicopathological characteristics of HCC patients	CLDN11	(36)
miR-181a	Induces sorafenib resistance of HCC cells	RASSF1	(2)
miR-181b	Role in liver fibrosis	TGF-b	(31)
miR-193a	Facilitates sorafenib inhibition of HCC proliferation	uPA	(42)
miR-101	Inhibits HCC cell proliferation, migration and invasion abilities	Girdin	(37)
miR-221/222	Involved in fibrosis	TGF-b, PTEN/Akt, RTK	(22), (32)
miR-326	Role in MDR	MRP-1	(41)
miR-27b	Role in MDR	BAX, P53, FoxO1, KRAS	(38)

Figure 2. Graphic representation of microRNAs and their corresponding functions from Table 1.

when cell rescue fails, pathway dysregulation occurs, leading to tumorigenesis. Low levels of miR-122 in HCC have been associated with hepatic fibrosis (23). The role of miR-122 in liver fibrosis has been demonstrated by an increase in infiltrating inflammatory cells observed in miR-122 knockout mice (28).

Potent external factors promote accelerated deterioration of the liver and are major factors contributing to chronic inflammation. Drug-induced liver injury, alcoholic liver disease, non-alcoholic fatty liver disease, and hepatitis B and C viral infection are major contributing external factors. Because of its ubiquity in the liver, miR-122 contributes to each of these forms of externally-induced liver inflammation and subsequent damage (22). For example, miR-122 interacts directly with the hepatitis B virus (HBV) genome (22, 29). Two other miRNAs, miR-19a and miR-223, have been demonstrated to regulate PTEN and c-myc respectively, playing a role in the regulation of HBx protein in HBV and promoting progression to HCC (26). In addition to miR-122, miR-146a and miR-155 are strongly associated with liver inflammation. Both miR-155 and miR-146a are upregulated during liver inflammation and have been implicated in

hepatitis C virus (HCV) infection and progression to HCC. Each of these miRNAs plays a role in the Toll-like receptor signaling and immune response (22, 23); however, only miR-155 is a direct positive regulator of the inflammatory cytokine TNFα (30).

Chronic inflammation of the liver, caused by either disease or drug exposure, promotes fibrosis in an attempt to repair the damaged organ. A primary driver of fibrosis is the TGF-b pathway, which is responsible for the development of ECM and cell structure (22, 27). TGF-b is a cytokine that directly stimulates the development of fibrosis in the liver through stimulation of extracellular matrix (ECM) protein synthesis (27). Repeated inflammation from chronic hepatic disease or other external factors leads to the accumulation of fibrosis, hepatic cirrhosis, and ultimately HCC. miR-21, miR-221/222, and miR-181b play a role in liver fibrosis through regulation of TGF-b. Levels of miR-181b are significantly upregulated in the presence of TGF-b and have been shown to increase proliferation of immortalized hepatic stellate cells (31). miR-21 enhances TGF-b signaling through negative regulation of SMAD7 (27).

EGFR has also been associated with TGF-b and could have a crucial role in the progression to HCC. miRNAs that control TGF-b and EGFR are important to characterize and potentially modulate because of their downstream roles in liver vascularization and angiogenesis, which are critical for HCC development and metastasis. Repeated fibrosis caused by chronic damage to the liver has a clear impact on miRNAs related to liver development and repair. Chronic stress on these pathways because of liver damage leads to dysregulation of these pathways and progression to HCC. As the liver accumulates more damage and dysregulation emerges as a function of this damage, early-stage HCC appears.

4.2. Role of miRNAs after HCC onset

Both miR-221/222 and miR-214 are commonly involved in carcinogenesis and have an established role in the development of HCC. miR-221/222 is linked to cell growth and cell cycles across tissues, whereas miR-214 is an important regulator of the PTEN/AKT, b-catenin, and tyrosine kinase receptor pathways (4, 32). In HCC, miR221/222 is significantly upregulated, whereas miR-214 is downregulated and is a marker of poor outcome and HCC metastasis. Expression of miR-214 directly downregulated b-catenin in HePG2 cells and decreased cell proliferation, highlighting its potential role as a tumor suppressor (33). These miRNAs are important because of their broad carcinogenetic implications in many tumor types, aside from HCC (4). It is important to note that general carcinogenic miRNAs may have different roles in different tissues.

Progression of HCC is characterized by significant vascularization and angiogenesis, leading to growth and metastasis (6, 8). Through angiogenesis, the PDGFR and VEGF pathways are crucial for HCC growth, aggressiveness, and metastasis. Two miRNAs, miR-26a and miR-29b, are important for the angiogenesis of HCC (6, 8). Downregulation of miR-26a is more common in metastatic HCC tissue than in normal tissue and is linked to angiogenesis (6). miR-26a inhibits angiogenesis in HCC through suppression of VEGFA and inhibition of hepatocyte growth factor (HGF), both of which are critical for cell growth and motility (6). A second miRNA, miR-29b, is also significantly downregulated in HCC tissue and involved in HCC metastasis (8). miR-29b suppresses angiogenesis through inhibiting matrix metalloproteinase (MMP)-2 and capillary tube structure formation, which is critical for halting HCC metastasis (8). Downregulation of miR-29b has been associated with poor survival in patients with HCC (8). Poor prognosis in HCC is also associated with miR-146b-5p (similar to miR-146a, which has a previously mentioned role in inflammation) (34). Low miR-146b-5p expression in HCC tissue is strongly associated with HCC proliferation and, specifically, metastasis (34). miR-146b-5p has been shown to suppress TNF receptor associated factor 6 (TRAF6), which is a major regulator of MAPK signaling (35). Additionally, overexpression of miR-99b in HCC promotes growth and potential metastasis through regulation of claudin 11 (CLDN11) (36). HCC's proliferative and migratory abilities are also modulated by miR-101 (37). Girdin, a protein providing support to actin filaments, is elevated in HCC, and miR-101 has inhibitory effects on girdin, greatly reducing proliferation and migration of HCC (37).

Each miRNA discussed in the development and progression of HCC is critical to assess as potential biomarkers for early-stage HCC and as possible therapeutic targets in the path to metastasis development. Knowledge pertaining to the health of the liver and possible presence of HCC should be coupled with a specific panel of miRNAs to monitor HCC progression.

4.3. miRNAs that play a role in drug resistance

As with other aspects of HCC, differential expression of miRNAs plays a critical role in the acquisition of MDR, through regulation of key proteins in cell regulatory pathways (38). Vincristine and doxorubicin are two chemotherapeutic agents to which HCC demonstrates inherently high resistance (39). When miR-122 and vincristine/doxorubicin are used in combination, sensitivity of HCC cells to these chemotherapeutic agents is enhanced (39). In the presence of miR-122, HCC cell lines exhibited decreased protein levels of Bcl-w and cyclin G1 (CCNG1), which are involved in apoptosis and the

cell cycle (39). In addition, exposing HCC cell lines to miR-122 reduced expression of genes related to MDR: MDR1, multidrug resistance related protein (MRP), and glutathione-S-transferase p (GST-p) (39). MRP-1 is of particular interest in MDR because of its role in molecular transport and exportation of drugs and metabolites (40). In addition to miR-122, miR-326 also downregulates MRP-1 expression (41).

To further identify miRNAs that function in HCC resistance, analysis of differentially regulated miRNAs in the HCC line Huh-7 has been conducted (38). Huh-7 cell lines resistant to doxorubicin, cisplatin, carboplatin, mitomycin C, and vincristine were developed, and resistant cells exhibited significantly different expression of a multitude of miRNAs compared with the parental line (38). Five of the most differentially expressed miRNAs were miR-27b, miR-181a, miR-146b-5p, miR-181d, and miR-146a, which are believed to target key proteins, including PTEN, P53, and KRAS (38).

Treatment of HCC with one of the few FDA-approved multikinase inhibitors for the disease, sorafenib, is complicated by primary and acquired resistance. Again, miR-122 appears to play a role in sorafenib resistance (25). When sorafenib-resistant cell lines were compared to parental lines, markedly decreased miR-122 was observed (25). miR-122 is essential for the regulation of IGF, which is also an important pathway in the development of HCC (10). IGF-1R is a direct target of miR-122, and IGF-1R suppression resensitizes HCC cell lines to sorafenib through apoptosis (25). A series of miRNAs could be used preemptively in combination with therapeutic agents, such as sorafenib, to enhance their effectiveness. Urokinase-type plasminogen activator (uPA) is a factor commonly upregulated in HCC (42). miR-193a is a negative regulator of uPA, which increased inhibition of proliferation when combined with sorafenib in HCC cell lines (42).

Progression and stage of HCC can play a major role in drug resistance. miR-181a is upregulated in the more aggressive Hep3B HCC cell line and plays a role in sorafenib resistance through repression of Ras association domain-containing protein 1 (RASSF1) (2). Introduction of miR-181a inhibited less-aggressive HepG2 cells, whereas reduced miR-181a increased apoptosis in Hep3B cells. The miR-181 family's role in sorafenib resistance highlights the importance of HCC aggressiveness to acquired resistance, whereby more aggressive cell lines have differentially modulated miRNAs that attenuate HCC to resistance (2).

Another important pathway in sorafenib-resistant cell lines involves PTEN, a key player in cellular proliferation. PTEN inhibits AKT activation; thus, when PTEN is downregulated, AKT is upregulated (7). Increased miR-21 in sorafenib-resistant cell lines is a significant inhibitor of PTEN, and miR-21 has been shown to inhibit sorafenib-induced autophagy in these cell lines through the PTEN/Akt pathway (7). In addition to miR-21, a series of other miRNAs—miR-10a-5p, miR-153, miR-216a, miR-217, and miR-494—are highly expressed in sorafenib-resistant cells (43). Tang *et al* made the important comment that targeting one miRNA may have little effect on a given pathway when a number of different miRNAs are modulated by a single event (43). The redundant and overlapping functionality of miRNAs are key aspects of drug-resistant HCC, suggesting that a multifaceted approach should be adopted when evaluating personalized miRNA therapy. A combinatorial approach, modulating many miRNAs related to a signal pathway, may be critical. Important research by Tang *et al* demonstrated that artificial long non-coding RNAs targeting multiple miRNAs reduced cellular resistance to sorafenib (43).

5. CONCLUSION

As highlighted in this review, regulating pathways to circumvent MDR in HCC through miRNA manipulation will be complicated and require molecular calibration and fine-tuning. HCC development reflects the accumulation of molecular changes that occur through cellular inflammation and damage caused by liver disease and other external stimuli, ultimately leading to cellular dysregulation (44). To monitor liver health, inflammation, fibrosis, and disease progression, which are integral steps in the development of HCC, miRNAs integral to these processes should be assessed, including miR-122, miR-21, miR-221/222, miR-181b, and miR-146b-5p. These miRNAs play a critical role in the development of MDR in HCC and can be a potent target for modulating the pathways related to developing resistance. According to the National Institutes of Health website ClinicalTrials.gov, there were 7 active trials involving miRNAs across the world as of February 21, 2018 (Table 2). The trials detailed in Table 2. primarily involve the development of diagnostic and prognostic biomarkers, clinical significance of miRNAs, and characterization of circulating miRNAs. These areas reflect the early phases of miRNA clinical development; however, they are critical steps in developing effective clinical strategies and therapies using miRNAs. Careful observation of changes in miRNA expression profiles is the key to monitoring HCC and tracking its progression to drug-resistance disease. Most of the same miRNAs involved in disease development—miR-122, miR-21, miR-146b-5p, and the miR-181 family—play critical roles in MDR and should be continually assayed throughout treatment. Monitoring and targeting these miRNAs to halt disease progression and overcome drug resistance are critical strategies for effectively dealing with the ever-changing landscape of HCC disease progression.

Table 2. Clinical trials involving miRNA and HCC

ClinicalTrials.gov Identifier	Study Title	Conditions
NCT03429530	Study of miRNAs as a Diagnostic Tool for HCV-related HCC	miRNA in HCC
NCT03227510	miRNAs as Diagnostic Biomarkers in HCC Among Somali Patients	HCC
NCT02928627	Clinical Significance of Hepatic and Circulating miRNAs miR-221 and miR-222 in HCC	HCC
NCT02507882	Impact of IL-28B rs12979860 and rs4803217 Gene Polymorphisms Associated With miRNAs Deregulation on HCV-related HCC	HCV Infection (Genotype 4)
NCT02448056	miRNA as a Diagnostic and Prognostic Biomarker of HCC	Carcinoma, Hepatocellular, Marker, Biological
NCT03414554	Risk of HCC in Cirrhotic Patients Post DAAs Ttt	HCC
NCT0124750	Different Genetic Features Associated with Hepatic Carcinogenesis	HCC
NCT02412579	Genetic Profiling of Liver Cancer in Patients Undergoing Liver Transplantation	HCC
NCT03416803	A Study of Individualized Radiotherapy Based on a Prediction Model of Lymph Node Metastasis in HCC	Lymph Node Metastasis, HCC, Radiotherapy
NCT01210495	Axitinib for The Treatment of Advanced HCC	HCC

6. ACKNOWLEDGEMENTS

7. REFERENCES

1. B. Zhai and X. Y. Sun: Mechanisms of resistance to sorafenib and the corresponding strategies in hepatocellular carcinoma. *World J Hepatol*, 5 (7), 345-52 (2013)
DOI: 10.4254/wjh.v5.i7.345

2. J. Azumi, T. Tsubota, T. Sakabe and G. Shiota: miR-181a induces sorafenib resistance of hepatocellular carcinoma cells through downregulation of RASSF1 expression. *Cancer Sci*, 107 (9), 1256-62 (2016)
DOI: 10.1111/cas.13006

3. J. A. Vidigal and A. Ventura: The biological functions of miRNAs: lessons from *in vivo* studies. *Trends Cell Biol*, 25 (3), 137-47 (2015)
DOI: 10.1016/j.tcb.2014.11.004

4. E. Penna, F. Orso and D. Taverna: miR-214 as a key hub that controls cancer networks: small player, multiple functions. *J Invest Dermatol*, 135 (4), 960-9 (2015)
DOI: 10.1038/jid.2014.479

5. J. Hu, Y. Xu, J. Hao, S. Wang, C. Li and S. Meng: MiR-122 in hepatic function and liver diseases. *Protein Cell*, 3 (5), 364-71 (2012)
DOI: 10.1007/s13238-012-2036-3

6. X. Yang, X. F. Zhang, X. Lu, H. L. Jia, L. Liang, Q. Z. Dong, Q. H. Ye and L. X. Qin: MicroRNA-26a suppresses angiogenesis in human hepatocellular carcinoma by targeting hepatocyte growth factor-cMet pathway. *Hepatology*, 59 (5), 1874-85 (2014)
DOI: 10.1002/hep.26941

7. C. He, X. Dong, B. Zhai, X. Jiang, D. Dong, B. Li, H. Jiang, S. Xu and X. Sun: MiR-21 mediates sorafenib resistance of hepatocellular carcinoma cells by inhibiting autophagy via the PTEN/Akt pathway. *Oncotarget*, 6 (30), 28867-81 (2015)
DOI: 10.18632/oncotarget.4814

8. J. H. Fang, H. C. Zhou, C. Zeng, J. Yang, Y. Liu, X. Huang, J. P. Zhang, X. Y. Guan and S. M. Zhuang: MicroRNA-29b suppresses tumor angiogenesis, invasion, and metastasis by regulating matrix metalloproteinase 2 expression. *Hepatology*, 54 (5), 1729-40 (2011)
DOI: 10.1002/hep.24577

9. S. Huang and X. He: The role of microRNAs in liver cancer progression. *Br J Cancer*, 104 (2), 235-40 (2011)
DOI: 10.1038/sj.bjc.6606010

10. A. Schlachterman, W. W. Craft, Jr., E. Hilgenfeldt, A. Mitra and R. Cabrera: Current and future treatments for hepatocellular

carcinoma. *World J Gastroenterol*, 21 (28), 8478-91 (2015)
DOI: 10.3748/wjg.v21.i28.8478

11. E. J. D. Xiangbing Meng, Shujie Yang, Brandon M. Schickling and Kimberly K. Leslie: Role of MTDH, FOXM1 and microRNAs in Drug Resistance in Hepatocellular Carcinoma *Diseases*, 209-225 (2014)

12. W. T. Tai, A. L. Cheng, C. W. Shiau, C. Y. Liu, C. H. Ko, M. W. Lin, P. J. Chen and K. F. Chen: Dovitinib induces apoptosis and overcomes sorafenib resistance in hepatocellular carcinoma through SHP-1-mediated inhibition of STAT3. *Mol Cancer Ther*, 11 (2), 452-63 (2012)
DOI: 10.1158/1535-7163.MCT-11-0412

13. M. J. Blivet-Van Eggelpoel, H. Chettouh, L. Fartoux, L. Aoudjehane, V. Barbu, C. Rey, S. Priam, C. Housset, O. Rosmorduc and C. Desbois-Mouthon: Epidermal growth factor receptor and HER-3 restrict cell response to sorafenib in hepatocellular carcinoma cells. *J Hepatol*, 57 (1), 108-15 (2012)
DOI: 10.1016/j.jhep.2012.02.019

14. A. X. Zhu, A. D. Baron, P. Malfertheiner, M. Kudo, S. Kawazoe, D. Pezet, F. Weissinger, G. Brandi, C. A. Barone, T. Okusaka, Y. Wada, J. O. Park, B. Y. Ryoo, J. Y. Cho, H. C. Chung, C. P. Li, C. J. Yen, K. D. Lee, S. C. Chang, L. Yang, P. B. Abada and I. Chau: Ramucirumab as Second-Line Treatment in Patients With Advanced Hepatocellular Carcinoma: Analysis of REACH Trial Results by Child-Pugh Score. *JAMA Oncol* (2016)

15. O. Arrieta, B. Cacho, D. Morales-Espinosa, A. Ruelas-Villavicencio, D. Flores-Estrada and N. Hernandez-Pedro: The progressive elevation of alpha fetoprotein for the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis. *BMC Cancer*, 7, 28 (2007)
DOI: 10.1186/1471-2407-7-28

16. C. T. Frenette: The Role of Regorafenib in Hepatocellular Carcinoma. *Gastroenterol Hepatol (N Y)*, 13 (2), 122-124 (2017)

17. A. L. Cheng, Y. K. Kang, D. Y. Lin, J. W. Park, M. Kudo, S. Qin, H. C. Chung, X. Song, J. Xu, G. Poggi, M. Omata, S. Pitman Lowenthal, S. Lanzalone, L. Yang, M. J. Lechuga and E. Raymond: Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J Clin Oncol*, 31 (32), 4067-75 (2013)
DOI: 10.1200/JCO.2012.45.8372

18. L. Wen, C. Liang, E. Chen, W. Chen, F. Liang, X. Zhi, T. Wei, F. Xue, G. Li, Q. Yang, W. Gong, X. Feng, X. Bai and T. Liang: Regulation of Multi-drug Resistance in hepatocellular carcinoma cells is TRPC6/Calcium Dependent. *Sci Rep*, 6, 23269 (2016)
DOI: 10.1038/srep23269

19. P. Raychaudhuri and H. J. Park: FoxM1: a master regulator of tumor metastasis. *Cancer Res*, 71 (13), 4329-33 (2011)
DOI: 10.1158/0008-5472.CAN-11-0640

20. D. L. Ou, Y. C. Shen, S. L. Yu, K. F. Chen, P. Y. Yeh, H. H. Fan, W. C. Feng, C. T. Wang, L. I. Lin, C. Hsu and A. L. Cheng: Induction of DNA damage-inducible gene GADD45beta contributes to sorafenib-induced apoptosis in hepatocellular carcinoma cells. *Cancer Res*, 70 (22), 9309-18 (2010)
DOI: 10.1158/0008-5472.CAN-10-1033

21. F. M. Gu, Q. L. Li, Q. Gao, J. H. Jiang, X. Y. Huang, J. F. Pan, J. Fan and J. Zhou: Sorafenib inhibits growth and metastasis of hepatocellular carcinoma by blocking STAT3. *World J Gastroenterol*, 17 (34), 3922-32 (2011)
DOI: 10.3748/wjg.v17.i34.3922

22. C. N. Hayes and K. Chayama: MicroRNAs as Biomarkers for Liver Disease and Hepatocellular Carcinoma. *Int J Mol Sci*, 17 (3), 280 (2016)
DOI: 10.3390/ijms17030280

23. G. Szabo and S. Bala: MicroRNAs in liver disease. *Nat Rev Gastroenterol Hepatol*, 10 (9), 542-52 (2013)
DOI: 10.1038/nrgastro.2013.87

24. C. Jopling: Liver-specific microRNA-122: Biogenesis and function. *RNA Biol*, 9 (2), 137-42 (2012)
DOI: 10.4161/rna.18827

25. Y. Xu, J. Huang, L. Ma, J. Shan, J. Shen, Z. Yang, L. Liu, Y. Luo, C. Yao and C. Qian: MicroRNA-122 confers sorafenib resistance to hepatocellular carcinoma cells by targeting IGF-1R to regulate RAS/RAF/ERK signaling pathways. *Cancer Lett*, 371 (2), 171-81 (2016)
DOI: 10.1016/j.canlet.2015.11.034

26. G. Yu, X. Chen, S. Chen, W. Ye, K. Hou and M. Liang: MiR-19a, miR-122 and miR-223

are differentially regulated by hepatitis B virus X protein and involve in cell proliferation in hepatoma cells. *J Transl Med*, 14 (1), 122 (2016)
DOI: 10.1186/s12967-016-0888-7

27. R. Bataller and D. A. Brenner: Liver fibrosis. *J Clin Invest*, 115 (2), 209-18 (2005)
DOI: 10.1172/JCI24282

28. S. H. Hsu, B. Wang, J. Kota, J. Yu, S. Costinean, H. Kutay, L. Yu, S. Bai, K. La Perle, R. R. Chivukula, H. Mao, M. Wei, K. R. Clark, J. R. Mendell, M. A. Caligiuri, S. T. Jacob, J. T. Mendell and K. Ghoshal: Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J Clin Invest*, 122 (8), 2871-83 (2012)
DOI: 10.1172/JCI63539

29. Y. Chen, A. Shen, P. J. Rider, Y. Yu, K. Wu, Y. Mu, Q. Hao, Y. Liu, H. Gong, Y. Zhu, F. Liu and J. Wu: A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. *FASEB J*, 25 (12), 4511-21 (2011)
DOI: 10.1096/fj.11-187781

30. S. Bala, J. Petrasek, S. Mundkur, D. Catalano, I. Levin, J. Ward, H. Alao, K. Kodys and G. Szabo: Circulating microRNAs in exosomes indicate hepatocyte injury and inflammation in alcoholic, drug-induced, and inflammatory liver diseases. *Hepatology*, 56 (5), 1946-57 (2012)
DOI: 10.1002/hep.25873

31. B. Wang, W. Li, K. Guo, Y. Xiao, Y. Wang and J. Fan: miR-181b promotes hepatic stellate cells proliferation by targeting p27 and is elevated in the serum of cirrhosis patients. *Biochem Biophys Res Commun*, 421 (1), 4-8 (2012)
DOI: 10.1016/j.bbrc.2012.03.025

32. Y. Li, C. Di, W. Li, W. Cai, X. Tan, L. Xu, L. Yang, G. Lou and Y. Yan: Oncomirs miRNA-221/222 and Tumor Suppressors miRNA-199a/195 Are Crucial miRNAs in Liver Cancer: A Systematic Analysis. *Dig Dis Sci*, 61 (8), 2315-27 (2016)
DOI: 10.1007/s10620-016-4156-8

33. L. L. Zhang, Y. J. Guo, C. N. Zhao and J. Y. Gao: Effects and mechanism of miR-214 on hepatocellular carcinoma. *Asian Pac J Trop Med*, 8 (5), 392-8 (2015)
DOI: 10.1016/S1995-7645(14)60350-3

34. C. Li, R. Miao, S. Liu, Y. Wan, S. Zhang, Y. Deng, J. Bi, K. Qu, J. Zhang and C. Liu: Down-regulation of miR-146b-5p by long noncoding RNA MALAT1 in hepatocellular carcinoma promotes cancer growth and metastasis. *Oncotarget*, 8 (17), 28683-28695 (2017)
DOI: 10.18632/oncotarget.15640

35. J. S. Arthur and S. C. Ley: Mitogen-activated protein kinases in innate immunity. *Nat Rev Immunol*, 13 (9), 679-92 (2013)
DOI: 10.1038/nri3495

36. J. Yang, X. Liu, X. Yuan and Z. Wang: miR-99b promotes metastasis of hepatocellular carcinoma through inhibition of claudin 11 expression and may serve as a prognostic marker. *Oncol Rep*, 34 (3), 1415-23 (2015)
DOI: 10.3892/or.2015.4104

37. K. Cao, J. Li, Y. Zhao, Q. Wang, Q. Zeng, S. He, L. Yu, J. Zhou and P. Cao: miR-101 Inhibiting Cell Proliferation, Migration and Invasion in Hepatocellular Carcinoma through Downregulating Girdin. *Mol Cells*, 39 (2), 96-102 (2016)
DOI: 10.14348/molcells.2016.2161

38. L. Zhuo, J. Liu, B. Wang, M. Gao and A. Huang: Differential miRNA expression profiles in hepatocellular carcinoma cells and drug-resistant sublines. *Oncol Rep*, 29 (2), 555-62 (2013)
DOI: 10.3892/or.2012.2155

39. Y. Xu, F. Xia, L. Ma, J. Shan, J. Shen, Z. Yang, J. Liu, Y. Cui, X. Bian, P. Bie and C. Qian: MicroRNA-122 sensitizes HCC cancer cells to adriamycin and vincristine through modulating expression of MDR and inducing cell cycle arrest. *Cancer Lett*, 310 (2), 160-9 (2011)
DOI: 10.1016/j.canlet.2011.06.027

40. J. Yin and J. Zhang: Multidrug resistance-associated protein 1 (MRP1/ABCC1) polymorphism: from discovery to clinical application. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*, 36 (10), 927-38 (2011)

41. Z. Liang, H. Wu, J. Xia, Y. Li, Y. Zhang, K. Huang, N. Wagar, Y. Yoon, H. T. Cho, S. Scala and H. Shim: Involvement of miR-326 in chemotherapy resistance of breast cancer through modulating expression of multidrug resistance-associated protein 1. *Biochem Pharmacol*, 79 (6), 817-24 (2010)
DOI: 10.1016/j.bcp.2009.10.017

42. A. Salvi, I. Conde, E. Abeni, B. Arici, I. Grossi, C. Specchia, N. Portolani, S. Barlati and G. De Petro: Effects of miR-193a and sorafenib on hepatocellular carcinoma cells. *Mol Cancer*, 12, 162 (2013)
DOI: 10.1186/1476-4598-12-162

43. S. Tang, G. Tan, X. Jiang, P. Han, B. Zhai, X. Dong, H. Qiao, H. Jiang and X. Sun: An artificial lncRNA targeting multiple miRNAs overcomes sorafenib resistance in hepatocellular carcinoma cells. *Oncotarget*, 7 (45), 73257-73269 (2016)
DOI: 10.18632/oncotarget.12304

44. K. Kitisin, M. J. Pishvaian, L. B. Johnson and L. Mishra: Liver stem cells and molecular signaling pathways in hepatocellular carcinoma. *Gastrointest Cancer Res*, 1 (4 Suppl 2), S13-21 (2007)

45. L. Gramantieri, M. Ferracin, F. Fornari, A. Veronese, S. Sabbioni, C. G. Liu, G. A. Calin, C. Giovannini, E. Ferrazzi, G. L. Grazi, C. M. Croce, L. Bolondi, M. Negrini: Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res,* 67 (13), 6092-9 (2007)
DOI: 10.1158/0008-5472.CAN-06-4607

46. F. Fornari, L. Gramantieri, C. Giovannini, A. Veronese, M. Ferracin, S. Sabbioni, G. A. Calin, G. L. Grazi, C. M. Croce, S. Tavolari, P. Chieco, M. Negrini, L. Bolondi: MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res,* 69 (14), 5761-7 (2009)
DOI: 10.1158/0008-5472.CAN-08-4797

47. X. L. Yan, Y. L. Jia, L. Chen, Q. Zeng, J. N. Zhou, C. J. Fu, H. X. Chen, H. F. Yuan, Z. W. Li, L. Shi, Y. C. Xu, J. X. Wang, X. M. Zhang, L. J. He, C. Zhai, W. Yue, X. T. Pei: Hepatocellular carcinoma-associated mesenchymal stem cells promote hepatocarcinoma progression: role of the S100A4-miR155-SOCS1-MMP9 axis. *Hepatology,* 57 (6), 2274-86 (2013)
DOI: 10.1002/hep.26257

48. X. Yang, L. Liang, X. F. Zhang, H. L. Jia, Y. Qin, X. C. Zhu, X. M. Gao, P. Qiao, Y. Zheng, Y. Y. Sheng, J. W. Wei, H. J. Zhou, N. Ren, Q. H. Ye, Q. Z. Dong, L. X. Qin: MicroRNA-26a suppresses tumor growth and metastasis of human hepatocellular carcinoma by targeting interleukin-6-Stat3 pathway. *Hepatology,* 58 (1), 158-70 (2013)
DOI: 10.1002/hep.26305

Send correspondence to: Deepak Kumar, Julius L. Chambers Biomedical/Biotechnology Research Institute (BBRI), North Carolina Central University, 1801 Fayetteville St. Durham NC 27707, Tel: 919-530-7017, Fax: 919-530-7998, E-mail: dkumar@nccu.edu