[Frontiers In Bioscience, Landmark, 24, 545-554, Jan 1, 2019]

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

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

[1]Julius L. Chambers Biomedical Biotechnology Research Institute, North Carolina Central University, 1801 Fayetteville St. Durham NC 27707, [2]Department of Pharmaceutical Sciences, North Carolina Central University, 1801 Fayetteville St. Durham NC 27707

TABLE OF CONTENTS

1. ABSTRACT

Hepatocellular Carcinoma (HCC) is a leading cause of cancer related death worldwide with a relatively poor survival rate. Aside from liver resection and subsequent transplant, the most effective and leading curative measure for HCC is the chemotherapeutic, sorafenib, a multi-kinase inhibitor used for treatment of late stage HCC. However, the effects of sorafenib are short lived due to the liver's rapid acquisition of Multi-Drug Resistance (MDR). MicroRNAs (miRNAs) have emerged as critical regulatory molecules for almost every biochemical pathway in an organism. The development and 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 development of HCC and their role in key signaling pathways for MDR in HCC.

2. INTRODUCTION

Hepatocellular Carcinoma (HCC) is among the leading causes of cancer-related death (s) worldwide (1, 2). According to the American Cancer Society, survival rate for HCC in the US is relatively poor, with 42,220 new cases being diagnosed in 2018, and 30,200 of these individuals dying of the disease. Many external and environmental factors, such as disease and drug usage, contribute to a rapid onset of liver damage and HCC. Liver resection and subsequent transplant is still the most effective and leading curative measure for HCC (2). The chemotherapeutic, sorafenib, remains the most effective and widely used multi-kinase inhibitor for treatment of late stage HCC. However, the effects of sorafenib are short lived due to the liver's rapid acquisition of drug resistance (1, 2).

miRNAs are small (~20bp) non-coding RNAs which target specific mRNA sequences for degradation, inhibiting protein translation (3). miRNAs have emerged as a critical regulatory molecule for almost every biochemical pathway in organisms (3). Due to their short-lived efficacy and immediate effects on multiple pathways and downstream targets, miRNAs make 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 diseases, making them an attractive target for biomarker development (4).

A plethora of new research has been presented on miRNAs' role in HCC and specifically sorafenib resistance (2, 4-8). This review will focus on current research driven towards miRNAs which are key to the liver, pertaining to HCC development, progression and drug resistance. Discovering mechanisms of HCC development and resistance can uncover new methods for circumventing development of multidrug resistance (MDR) and treatment of HCC.

We aim to compile data from the literature to present a more cohesive story on miRNAs role in HCC development and progression to disease resistance.

3. HEPATOCELLULAR CARCINOMA

3.1. Critical Pathways in HCC development

The general progression for development of HCC is initially steatosis, followed by fibrosis and subsequent cirrhosis of the liver. A multitude of factors, viral infections, liver disease, alcohol abuse, diabetes and others, cause chronic inflammation and damage to the organ and are primarily responsible for development of HCC (9). The HCC tumor itself is characterized as a highly vascularized tumor, in which angiogenesis is critical to development and metastatic potential (8, 10). Dysregulation in cell cycle progression and failure of apoptosis/autophagy are a classic hallmark of progression to HCC, as with many other types of neoplasia. However, unlike other cancers such as prostate or breast which are more hormonally driven, HCC is a dysregulation of key cellular proliferative and developmental pathways (11). This makes study and treatment of HCC particularly difficult, in that there are no tissue-related, hormone-specific components to target (11). A few of the major pathways involved in liver cell dysregulation and progression to HCC are PTEN, P13K/AKT, MAPK, Jak/STAT and EGFR/IGF. The EGFR/IGF pathway is of particular note due to its significant role in vascularization and angiogenesis of the liver, controlling VEGF and platelet derived growth factor (PDGF) (1, 10). The MAPK pathway is a major target of therapeutics and has strong linkage to the P13K/AKT pathway, which is also involved with VEGF and PDGF. Also, critical to liver regulation is the STAT pathway, which is involved with the regulation of cell proliferation, inflammation, differentiation, survival, motility and apoptosis (1, 12). STAT3 has demonstrated upregulation in tumor cell lines, inhibiting an immunogenic response (12). Figure 1 Illustrates the general progression of a healthy liver to HCC in addition to the pathways/proteins involved and the associated regulatory miRNAs. This review reports on some of the key miRNAs related to liver function and the roles they play in HCC progression. This information can be translated to describe how these miRNAs contribute to drug resistance, for example, in the case of the first line chemotherapeutic, sorafenib.

3.2. HCC treatments

Aside from the previously mentioned liver transplant, the major treatment for HCC is the first line chemotherapeutic, sorafenib. Sorafenib is a multikinase inhibitor developed as a primary treatment for advanced HCC, which inhibits VEGF and PDGF, and blocks the Mapk/Raf pathway, all critical to angiogenesis and involved with tumor growth (1). Sorafenib can also induce autophagy in HCC, reducing the size of the developed tumor (7). Unfortunately, HCC is particularly resistant to many chemotherapeutics, due to high genetic variability and ability to develop resistance to multiple drugs. Sorafenib is no exception to HCC's development of resistance, where some patients have a primary resistance upon treatment and a majority of others treated with sorafenib develop resistance within months (1, 13). The average time span for prolongation of life with sorafenib treatment is a little under a year, which is a relatively mild enhancement (12). In addition, sorafenib was initially approved by the FDA in 2007, making this drug a decade old. Recently in 2017, new therapies like ramucirumab, which is an anti-VEGFR-2 monoclonal antibody, have been in trial for patients with HCC who have already received sorafenib. Specifically, ramucirumab has demonstrated increased survival of patients who have baseline α-fetoprotein levels of 400 ng/mL or greater (14). α-fetoprotein expression in the liver, after damage or cirrhosis has occurred, is believed to be a risk factor of HCC (15).

In addition, pre-existing chemotherapeutics have emerged, such as regorafenib, also a multikinase inhibitor, which has been approved by the FDA (2017) for use on HCC. Regorafenib has a similar mechanism of action to sorafenib, blocking VEGF, however it is a more potent inhibitor of angiogenesis than its counterpart (16). Further need for new therapies has pushed the consideration of additional already existing multikinase inhibitors (dovitinib, sunitinib, cabozantinib, vectibix), with targets revolving around the VEGF pathway, for clinical trials and HCC approval (12). dovitinib's (which is currently under development) mechanism of action is downregulation of the STAT3 pathway through the induction of SPH-1 and subsequent apotosis. (12). sunitinib, which is also in clinical trials, has demonstrated activity against HCC (17). Further study and combinatorial use of these chemotherapeutics has been driven by sorafenib resistance and its moderate effectiveness against HCC.

3.3. HCC drug resistance

Development of drug resistance in carcinogenic tissue is a significant confounding factor to consider when attempting to treat the disease. In general, HCC is difficult to treat due to the lack of liver-specific targets available for therapeutic development. As mentioned previously, pathways key to HCC development involve EGFR, PDGFR, FGFR, MAPK IFG and TGF-b, which are vital to many other tissue types (11). MDR is a major mode of acquired HCC resistance through a multitude of mechanisms, including hypoxic environment, DNA damage, EMT, autophagy and epigenetic regulation, circumventing shut down of any single pathway (18). FoxM1 is a

Table 1. Role of different miRNA 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	5, 14,26; 39
miR-155	Involved in the tumorigenesis and clinical characteristics of HCC	SOX6, SOCS1, TNFa	
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	6
miR-19a	Involved in the hepatocarcinogenesis in HBV-related HCC	PTEN	26
miR-223	Involved in the hepatocarcinogenesis in HBV-related HCC	c-myc	26
miR-21	Involved in fibrosis and acquired sorafenib resistance by suppressing autophagy in HCC	TGF-beta, PTEN	7
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 sorafenibs 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; 26
miR-326	Role in MDR	MRP-1	41
miR-27b	Role in MDR	BAX, P53, FoxO1, KRAS	38

transcription factor involved in cell cycle progression and proliferation, found to be essential in tumorigenic metastasis potential (19). Mouse models in which FoxM1 is deleted demonstrate a significant resistance to developing HCC (11). Expression of FoXM1 in HCC is also linked to reoccurrence and poor survival in the disease (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, MTDH is believed to be linked to DNA repair through binding of RNA, and high levels of MTHD are observed to be 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 such example where STAT3 regulates calcium dependent MDR in HCC (18).

Resistance to sorafenib is particularly focused on interaction between the Jak/STAT and EMT pathways. As mentioned previously, advanced HCC development is characterized by vascularization and angiogenesis, which has proven to be a promising target for HCC chemotherapeutics. However, blocking the Mapk/Raf pathway is only a small part of the chemotherapeutic mechanistic pathway. The PI3K/Akt pathway crosstalks with Mapk/Raf and can be activated in response to sorafenib, in turn stimulating resistance (1, 7). Sorafenib has also highlighted an ability to induce expression of GADD45β, which is a regulator of autophagic induction in HCC (20). Sorafenib has demonstrated an inhibitory effect on STAT3, which is involved in metastatic potential of HCC through phosphorylation of the protein (21). HCC cells that have become resistance to sorafenib demonstrate an increase in STAT-3 levels, highlighting the involvement of the Jak/STAT pathway in resistance (1). However, dovitinib has been demonstrated to re-sensitize cells that have acquired sorafenib resistance (12). Dovitinib induces expression of SHP-1, which is a negative regulator of the previously mentioned STAT3 and inhibits HCC proliferation (12). miRNAs are emerging as a major factor contributing to drug resistance acquisition through regulation of these pathways (18). Developing a working knowledge of critical miRNAs involved in development of drug resistance in HCC can aid in promoting the efficacy and longevity of currently existing chemotherapeutic agents. A list of currently known miRNAs, their roles in liver function and HCC, and their corresponding protein targets are detailed in Table 1.

4. HCC and miRNAs

4.1. miRNAs involved in HCC development

A significant miRNA to liver function is miR-122, comprising 70% of the total miRNA in the liver due to its significant role in basic liver function and homeostasis (22, 23). Because of miR-122's abundance and importance in liver function and HCC development, there has been a significant

Table 2. Clinical trials involving miRNA and HCC

Status	Study Title	Conditions
Not yet recruiting (New)	Study of miRNAs as a Diagnostic Tool for HCV-related HCC	miRna in HCC
Not yet recruiting	miRNAs as Diagnostic Biomarkers in HCC Among Somali Patients	HCC
Recruiting	Clinical Significance of Hepatic and Circulating misRNAs miR-221 and miR-222 in HCC	HCC
Unknown	Impact of IL-28B rs12979860 and rs4803217 Gene Polymorphisms Associated With miRNAs Deregulation on HCV-related HCC	HCV Infection (Genotype 4)
Not yet recruiting	miRNA as a Diagnostic and Prognostic Biomarker of HCC	Carcinoma, Hepatocellular, Marker, Biological
Not yet recruiting (New)	Risk of HCC in Cirrhotic Patients Post DAAs Ttt	HCC
Unknown	Different Genetic Features Associated With Hepatic Carcinogenesis	HCC
Active, not recruiting	Genetic Profiling of Liver Cancer in Patients Undergoing Liver Transplantation	HCC
Recruiting (New)	A Study of Individualized Radiotherapy Based on a Prediction Model of Lymph Node Metastasis in HCC	Lymph Node Metastasis, HCC, Radiotherapy
Terminated, Has Results	Axitinib For The Treatment Of Advanced HCC	HCC

accumulation of literature and research dedicated to this topic (5, 24-26). miR-122 is relatively specific to the liver, showing almost no expression in other tissues (24). miR-122 is highly conserved between vertebrate animals and is regulated in a circadian-like cycle. Transcription factors in the liver, HNF1α, HNF3β, HNF4α and CCAAT/enhancer-binding protein (C/EBP)α, bind to the promotor of miR-122 and increase expression, which facilitates liver development (5). In addition, miR-122 plays a primary role in lipid and cholesterol metabolism (5).

Due to its vital role in liver regulation, it is common to see a dysregulation in miR-122 upon liver damage (24). Nearly every phase in the molecular progression to HCC involves miR-122 (24, 25). Repeated inflammation of the liver is a significant molecular perturbation which contributes to the development of HCC (27). During drug and alcohol related liver damage and subsequent inflammation, miR-122 shows upregulation in serum (22). Due to its specificity to liver and upregulation upon liver damage, it has been proposed as a biomarker for liver disease and progression to HCC (22). However, upon development of a HCC tumor, miR-122 has shown downregulation in the primary tissue (22, 23). The progression from upregulation in serum to a loss of expression in primary tumor, eludes to the idea of molecular pathways attempting an initial rescue of damaged tissue through overregulation. Subsequently, when cell rescue has failed, HCC demonstrates dysregulation of the pathway which leads to tumorigenesis. Low levels of miR-122 in HCC have been associated with fibrotic development (23). miR-122's role in liver fibrosis has been demonstrated when miR-122 knockout mice present an increase in infiltrating inflammatory cells (28).

Potent external factors can lend themselves to accelerated deterioration of the liver and are a strong contributing factor to chronic inflammation. Drug induced liver injury, alcoholic liver disease, non-alcoholic fatty liver disease and viral infection through Hepatitis B and C are major contributing external factors. Due to its ubiquity in the liver, miR-122 contributes to each of these forms of externally induced liver inflammation and subsequent damage (22). In the case of viral perturbation of the liver, miR-122 was found to directly interact with the HBV genome (22, 29). Two other miRs, 19a and 223, have been demonstrated to regulate PTEN and c-myc respectively, playing a role in regulation of HBx in HBV and promoting progression of HCC (26). In addition to miR-122, miR-146a and miR-155 have a strong linkage to liver inflammation. miR-155 and miR-146a demonstrate upregulation upon liver inflammation and have been implicated in Hepatitis C (HCV) and progression to HCC. Each of these miRNAs has a role in Toll-like receptor signaling and immune response, hence their involvement in inflammatory response (22, 23). miR-155 is a direct positive regulator of the inflammatory cytokine TNFα (30).

Chronic inflammation of the liver, due to either disease or exposure to drugs, stimulates fibrotic development as a response in order to repair the damaged tissue. A primary driver of fibrosis is the TGF-b pathway, which is responsible for development of extracellular matrices and cell structure (22, 27). TGF-b is a cytokine which directly stimulates the development of fibrosis in the liver through stimulation of ECM protein synthesis (27). Repeated inflammation triggering liver damage from chronic disease or other external factors leads to accumulated fibrosis in the liver, cirrhosis, and ultimate development of HCC. A few miRNAs – miR-21, miR-221/222 and miR-181b have been found to play a role in fibrosis of the liver through regulation of TGF-b. miR-181b levels have been demonstrated to be significantly upregulated in the presence of TGF-b in addition to promoting

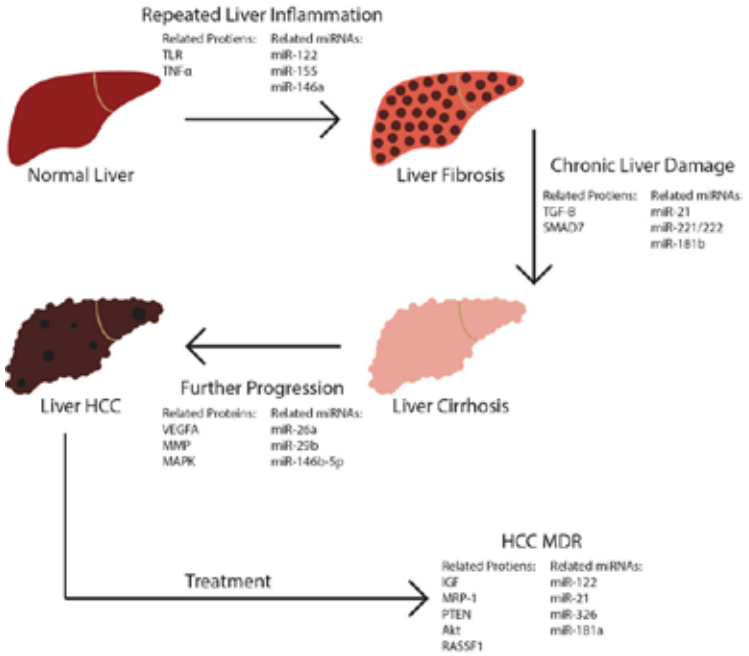

Figure 1. A Schematic depiction of liver disease progression listing the major proteins involved at each step and some of the major accompanying regulatory miRNAs involved.

increased proliferation of immortalized hepatic stellate cells. (31) miR-21 also demonstrates an enhancement of TGF-b signaling through negative regulation of SMAD7 (27).

EGFR has also shown linkage to TGF-b and could be a significant player in the progression of HCC. miRNAs which control TGF-b and EGFR are critical to observe and modulate, due to their downstream role in liver vascularization and angiogenesis, which play key roles in HCC development and metastasis. Repeated fibrosis caused by chronic damage to the liver has a clear impact on the miRNAs related to liver development and repair. Chronic stress on these pathways due to liver damage lead to their dysregulation and progression to HCC. As liver tissue accumulates more damage and dysregulation emerges as a function of this damage, development of early stage HCC has commenced.

4.2. Role of miRNA after HCC onset

miR-221/222 and miR-214 are both commonly involved in carcinogenic malfunctions and have a proven role in development of HCC. miR-221/222 are linked to cell growth and cell cycle across tissues while miR-214 is an important regulator of the PTEN/AKT, β -catenin, and tyrosine kinase receptor pathways (4, 32). In HCC, miR221/222 show significant upregulation while miR-214 demonstrates downregulation and is a marker of poor prognostic outcome and HCC metastasis. Expression of miR-214 directly downregulated b-catenin in HePG2 cells and demonstrated a decrease in cell proliferation, highlighting its potential role as a tumor suppressor (33). These miRNAs are key because of their broad carcinogenetic implication in many other tumor types, aside from HCC (4). It is critical to note that general carcinogenic miRNAs may have different roles in different tissues, however specific miRNAs that may be tissue selective must also be observed.

Further progression of HCC is characterized by significant vascularization and angiogenesis, leading to growth and metastasis of the tumor (6, 8). The PDGFR and VEGF pathways are critical to elucidating HCC tumor potentiation to aggressive development and metastasis through angiogenesis. Two miRNAs, miR-26a and miR-29b have been demonstrated as significant in angiogenesis of HCC (6, 8). Downregulation of miR-26a is prevalent in metastatic HCC tissue compared to normal and is linked to angiogenesis (6). miR-26a demonstrates the ability to inhibit angiogenesis in HCC through suppression of VEGA, in addition to its ability to inhibit Hepatocyte-Growth-Factor (HGF), both of which are critical for cell growth and motility (6). The second miRNA, miR-29b has also demonstrated significant downregulation in HCC tissue and is involved in HCC metastasis (8). miR-29b also suppresses angiogenesis, however through matrix etalloproteinase-2 (MMP) inhibition. miR-29b's repression of capillary tube structures is critical to inhibiting metastatic development of HCC (8). Down regulation of miR-29b has also demonstrated a relationship to poor survival of HCC patients (8). Along with miR-29b, miR-146b-5p, which has a previously

mentioned role in inflammation, also associates with poor prognostic outlook of HCC (34). Low expression of miR-146b-5p in HCC tissue is strongly linked to HCC proliferation and specifically, metastasis (34). miR-146b-5p is directly linked to suppression of TRAF6, which is a major regulator of MAPK signaling (35). Additionally, over expression of miR-99b in HCC promotes growth and potential metastasis through regulation of CLDN11 (36). HCC's proliferative and migratory abilities have been shown to be further modulated by miR-101 (37). Girdin, a protein providing support to actin filaments, exhibits elevated expression in tumors (37). miR-101 has shown inhibitory effects on girdin, in turn greatly reducing proliferation and migration of HCC (37).

Each miRNA discussed in the development, occurrence and progression of HCC is critical to assess as a biomarker in early stage HCC development and therapeutic consideration on the path to development of metastasis. Knowledge pertaining to the health of the liver and possible presence of disease 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 acquisition of MDR through regulation of key proteins in cell regulatory pathways (38). Vincristine and Doxorubicin are two chemotherapeutic agents to which HCC demonstrates an inherently high resistance and are used broadly to treat different types of cancer (39). miR-122, the primary player in liver function and HCC development, demonstrates enhancement of HCC cells to vincristine and doxorubicin chemotherapeutics when used in combination (39). In the presence of miR-122, HCC cell lines demonstrated a decrease in protein levels of Bcl-w and CCNG1, which are involved in apoptosis and cell cycle (39). In addition, exposure of miR-122 to HCC cell lines demonstrated reduction in expression of genes related to MDR, MDR1, MRP and 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 has also demonstrated interaction with MRP-1 through suppression of this protein (41). To further probe miRNAs key to HCC resistance, analysis of differentially regulated miRNAs in the HCC line Huh-7 has been conducted (38). Huh-7 cell lines resistant to adramycin, cisplatin, carboplatin, mitomycin C and vincristine were developed and a miRNA profile of the resistant cells, compared to the parental line, demonstrated significant differential regulation of a multitude of miRNAs (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 PTEN, P53 and KRAS, among others (38).

Treatment of HCC with one of the only FDA approved multikinase inhibitors for the disease, sorafanib, demonstrates primary and acquired disease resistance. miR-122 again has been found to play a role in sorafenib resistance (25). When sorafanib resistant cell lines are compared to the parental lines, a marked decrease in miR-122 is revealed (25). miR-122 has been shown to be essential to regulation of IGF, which is also a critical pathway in the development of HCC (10). IGF-1R is shown to be a direct target of miR-122, in which IGF-1R suppression resensitizes HCC cell lines to sorafenib through apopotosis (25). miRNA could be used preemptively in combination with therapeutics such as sorafenib in order to enhance their effectiveness. Urokinase-type plasminogen activator (uPA) is a common factor known to be upregulated in HCC (42). miR-193a, a negative regulator of uPA, when used in combination with sorafenib demonstrated an enhanced effect on inhibition of cell proliferation in HCC cell lines (42).

Progression and stage of HCC development can play a significant role in drug resistance. miR-181a demonstrates upregulation in more aggressive Hep3B HCC cells and plays a role in sorafenib resistance through repression of RASSF1 (2). Introduction of miR-181a demonstrated an inhibition of less aggressive HepG2 cells, while a reduction of miR-181a in Hep3B induced an increase in apoptosis. The miR-181 family's role in sorafenib resistance highlights the importance of HCC aggressiveness to acquired resistance, where more aggressive cell lines have differentially modulated miRNAs that attenuate HCC to resistance (2). Another key pathway in sorafenib resistant cells lines is PTEN, which leads to an upregulation of AKT when downregulated (7) and is a key player in cellular proliferation. miR-21, which demonstrates a significant upregulation in sorafenib resistant cell lines, is a significant inhibitor of PTEN. miR-21 demonstrated a linkage to autophagy through the PTEN/Akt pathway, and was demonstrated to inhibit sorafenib-induced autophagy in HCC lines (7). miR-10a-5p, miR-153, miR-216a, miR-217 and miR-494 demonstrate the most elevated expression in sorafenib-resistant cells, in addition to miR-21 (43). With a significant amount of miRNAs modulated by a single event, Tang *et al* make an important point that targeting one miRNA may have little effect on a given pathway (43). The redundant and overlapping functionality of miRNAs is a major facet of drug resistant HCC, making it key to adopt a multifaceted approach to researching personalized miRNA therapy. A combinatorial approach, modulating many miRNAs related to a signal pathway, may be critical. Important research by Tang (*et al*) targeted multiple miRNAs through usage of artificial long non-coding RNAs and demonstrated a decrease in cellular resistance to sorafenib through this method (43).

5. CONCLUSION

As highlighted in this review, regulating pathways to circumvent MDR in HCC through miRNA manipulation will prove complicated and will require molecular calibration and tuning. Development of HCC is an accumulation of molecular changes that occur through cellular inflammation and damages caused by disease and other external stimuli, leading ultimately to cellular dysregulation (44). To monitor liver health, inflammation, fibrosis and disease progression, which are integral to developing HCC, miRNAs integral to these processes such as miR-122, miR-21, miR-221/222, miR-181b and miR-146b-5p need to be assessed. miRNAs play a critical role in development of HCC MDR and can be a potent target for modulating the pathways related to developing resistance. According to the NIH website, clinical trials .gov, (accessed 2/12/18) there are 7 active trials for miRNAs across the world (Table 2). These primarily include diagnostic and prognostic biomarker development, miRNA clinical significance and circulating miRNAs. These are the early phases of miRNA clinical development, however, it is a critical step in the development of effective clinical strategies and therapies using miRNAs. Careful observation of changes in the miRNA expression profile and patterns is key to monitoring HCC and also tracking its development to a 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 need to continue to be continually assayed for throughout treatment. Monitoring and targeting these miRNAs to halt disease progression and overcome drug resistance is critical to the ever-changing landscape of HCC disease progression.

6. ACKNOWLEDGEMENTS

We would like to acknowledge Elena Arthur for her contributions in revision of the manuscript. We are grateful to everyone who has and continues to pioneer research in onset, development and treatment of HCC, which made this review possible. We gratefully acknowledge the grants 1U01CA194730, 1U54MD012392, 1R01MD012767, from the National Institutes of Health to DK. Research reported in this publication was also supported in part by the National Cancer Institute of the National Institutes of Health under Award Number U01CA185188 (Habtom Ressom).

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
PMid:23898367 PMCid:PMC3724962

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
PMid:27384977 PMCid:PMC5021022

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
PMid:25484347 PMCid:PMC4344861

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
PMid:25501033

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
PMid:22610888 PMCid:PMC4875471

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
PMid:24259426

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
PMid:26311740 PMCid:PMC4745697

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
PMid:21793034

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

DOI: 10.1002/hep.24577
PMid:21793034

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
PMid:26229392 PMCid:PMC4515831

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
PMid:22180308

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
PMid:22414764

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)

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
PMid:24081937

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)

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
PMid:21712406 PMCid:PMC3129416

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
PMid:21062976

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
PMid:22025881 PMCid:PMC3198022

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
PMid:26927063 PMCid:PMC4813144

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
PMid:23689081 PMCid:PMC4091636

24. C. Jopling: Liver-specific microRNA-122: Biogenesis and function. *RNA Biol*, 9 (2), 137-42 (2012)
DOI: 10.4161/rna.18827
PMid:22258222 PMCid:PMC3346312

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
PMid:26655273

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
PMid:27150195 PMCid:PMC4858919

27. R. Bataller and D. A. Brenner: Liver fibrosis. *J Clin Invest*, 115 (2), 209-18 (2005)
DOI: 10.1172/JCI24282
PMid:15690074 PMCid:PMC546435

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
PMid:22820288 PMCid:PMC3408748

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
PMid:21903935 PMCid:PMC3236624

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
PMid:22684891 PMCid:PMC3486954

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
PMid:22446332

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
PMid:27156077 PMCid:PMC4943968

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
PMid:28404923 PMCid:PMC5438683

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
PMid:23954936

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
PMid:26134929

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
PMid:26743900 PMCid:PMC4757808

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
PMid:23229111

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
PMid:19883630 PMCid:PMC2880168

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)

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
PMid:27689326 PMCid:PMC5341977

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)

Key Words: Hepatocellular Carcinoma, microRNAs, Drug Resistance, Cancer Progression, Cell Survival, Review

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, E-mail: dkumar@nccu.edu