Review Article

The role of microRNA in the resistance to treatment of hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related death with a limited efficacy of treatment for intermediate and advanced stages of the disease. Several therapeutic approaches such as trans-arterial chemoembolization (TACE) with anthracyclines, cisplatin and multikinase inhibitor sorafenib have been appealing choices of treatments yet failed to reach a satisfactory outcome mainly due to the numerous mechanisms that influence patient's response. MicroRNAs (miRNAs) are key regulators of many intracellular processes related to drug resistance. This phenomenon has been linked to the modulation of several complex pathways, ranging from the loss of ability of drug accumulation, protective mechanism of autophagy, adaptive mechanism of cancer cells towards the drugs-induced environment, decrease DNA damage and suppression of downstream events that transduce its signal into apoptosis. We summarize the recent findings on the involvement of miRNAs in various drug resistance-related mechanisms in the development of resistance to anthracyclines, cisplatin and sorafenib therapies. Furthermore, we describe the possible application of miRNAs as circulating biomarkers predicting therapy response in HCC. Thus, the undeniable potential and paramount role of miRNA in drug resistance may eventually lead to improved clinical strategies and outcomes for HCC patients.

Keywords: Anthracyclines; drug resistance; hepatocellular carcinoma (HCC); microRNA (miRNA); sorafenib

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Introduction

Over the years, there are significant developments in the therapeutic approaches of cancer after the introduction of several drugs able to improve the survival and overall prognosis of cancer patients. However, there are limitations regarding the efficacy of these drugs, mainly associated with the complicated nature of cancer cells and the multi-factorial mechanism of drug resistance.

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide (1). It is primarily known as the second most common cause of cancer-derived mortality, contributing to 746,000 deaths in 2012 (9.1% of the total) (1). The late occurrence, principally in advanced liver disease, make HCC as a "difficult to treat" cancer, as surgical approach and liver transplantation, the only radical treatment for the disease, are feasible only in the early stages (2).

For intermediate and advanced stages, several therapeutic approaches are available according to the degree and severity of cancer. Trans-arterial chemoembolization (TACE) is the most widely used primary treatment for unresectable HCC, as a palliative or downstaging purposes. TACE combines the cytotoxic effect of several drugs (cisplatin or doxorubicin) and ischemic effect from the embolization of the branches of a hepatic artery, inducing necrosis of the tumoral tissue (2,3). Even though

previous studies have established the survival advantage of TACE, recent data have challenged this conclusion (4). A complete response failed to reach significant value either in bland embolization method or drug-eluting bead (DEB)-TACE (5), in addition recurrence episodes are still significantly high (6-8).

Molecular-targeted therapies for HCC has been an appealing option since the development of the multikinase inhibitor sorafenib, firstly described with the SHARP trial in 2007 in a cohort of advanced HCC (2,9,10). Sorafenib is an inhibitor of Raf-kinase and several tyrosine kinases receptors implicated in tumorigenesis, tumor progression, and vascularization. In most tumor types, sorafenib induces apoptosis by down-regulating the anti-apoptotic protein Mcl-1 through a MEK/ERK-independent mechanism (11,12). Sorafenib is also described to target pro-angiogenic and pro-fibrotic players such as receptor tyrosine kinase (c-Kit), Fms-like tyrosine kinase (FLT-3), vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR-β), and other tyrosine kinases (13,14). Unfortunately, even though sorafenib was an achievement for HCC therapy, the outcomes are still far from being satisfactory (15).

The understanding towards refractory to either chemotherapeutic drugs or targeted therapy is needed to identify the key players involved in the resistance. Thus, it is essential to understand cellular pathways involved in the mechanism of resistance.

MicroRNA (miRNA) in cancer

Since their discovery in *C. elegans* in 1993, miRNAs have drawn a great deal of interest among researchers, especially in consideration of their role in post-transcriptional gene regulation (16). They are a class of highly conserved short non-coding RNAs, 18 to 25 nucleotides long, which regulate the expression of various genes. It is hypothesized that miRNAs can regulate more than 60% of the protein-coding genes in cells (16,17) with an existing overlap among miRNAs targeting the same mRNA, as well as among different mRNAs targeted by one single miRNA (18).

Only in 2002, miRNAs entered the medical research with the report of their involvement in pathological conditions, including cancer (19). Abundant miRNA profiling studies described the significant difference of miRNA expression in tumor samples when compared to healthy tissues. The relationship between decreased miRNA expression and the consequently increased expression of the oncogenic target genes became evident with the identification of RAS as a cellular target of let-7 in human cancer, suggesting a tumor-suppressor function for some miRNAs (20). Conversely, the increase of specific miRNAs in tumor cells indicate their role as oncogenes (21,22), as for the cluster miR-17-92 found to be frequently amplified in B-cell lymphomas (22). Following the first miRNA profile investigation in HCC in 2006 (23), more than 2,000 studies have focused on the role of miRNAs in this disease. Currently, several, although incomplete information exists about the abnormal regulation of miRNAs in HCC and several studies reported the involvement of miRNAs in multi-drug resistance linking them to poor prognosis.

miRNAs in drug resistance in HCC

In tumor resistant cells, several mechanisms participate in the development of drug resistance in HCC. Recently, it was shown that altered expression of subsets of miRNAs plays an essential role in regulating genes involved in cell apoptosis, cell proliferation, autophagy, epithelial-mesenchymal transition (EMT) and regulation of drug efflux.

Although these mechanisms have been previously investigated, the role of miRNAs remains ambiguous and unclear. However, there were several *in vitro* studies reporting the involvement of specific miRNAs in each different pathway related with drug resistance in different HCC cell lines, suggesting their important role as a critical player in both anthracyclines and sorafenib resistance (*Tables 1*,*2*). In this review, we will clarify the role of these regulatory RNAs in the onset of drug resistance in HCC, focusing on the widely used therapeutic drugs against the liver tumor.

miRNAs and their involvement in the mechanisms of drug resistance

It is hypothesized that doxorubicin (dox) resistance results from the loss of ability for the drug to accumulate in the nucleus, decrease DNA damage, and suppression of the downstream events that transduce the DNA damage signal into apoptosis (47). The mechanism of resistance to sorafenib involves several complex genetic and epigenetic alterations, and it is assumed that miRNAs play a role in regulating resistance *via* the involvement in some canonical drug-resistance pathways (14). Primary resistance is assumed to be derived from the genetic heterogeneity of HCC, even though the exact mechanism remains unclear (14).

Table 1 miRNAs involved in anthracyclines and cisplatin resistance-related pathways

Drugs	miRNA	miRNA expression in HCC	Target genes	References
Doxorubicin	miR-181b	Up	*TIMP3*	Wang *et al.*, 2010 (24)
	miR-122	Down	*ABCB1, ABCF2, PKM2*	Yahya *et al.*, 2018 (25)
	miR-101	Down	*Mcl-1*	He *et al.*, 2016 (26)
	miR-26a/b	Down	*ULK1*	Jin *et al.*, 2017 (27)
	miR-375	Down	*YAP1, AEG-1*	Xue *et al.*, 2017 (28)
	miR-223	Down	*ABCB1*	Yang *et al.*, 2013 (29)
	miR-520b	Down	*ATG7*	Gao *et al.*, 2018 (30)
Cisplatin	miR-130a	Up	*RUNX3*	Xu *et al.*, 2012 (31)
	miR-182	Up	*TP53INP1*	Qin *et al.*, 2014 (32)
	miR-122	Down	*ABCB1, Bcl2*	Pan *et al.*, 2016 (33)
	miR-340	Down	*Nrf2*	Shi *et al.*, 2014 (34)
	miR-199a-5p	Down	*ATG7*	Xu *et al.*, 2012 (35)

Table 2 miRNAs involved in sorafenib-induced resistance

Drugs	miRNA	miRNA expression in HCC	Target genes	References
Sorafenib	miR-216a/217	Up	*PTEN, SMAD7*	Xia *et al.*, 2013 (36)
	miR-222	Up	*PTEN*	Liu *et al.*, 2015 (37)
	miR-21	Up	*AKT*	Tang *et al.*, 2016 (38)
	miR-153	Up		
	miR-216a	Up		
	miR-217	Up		
	miR-494	Up		
	miR-10a-5p	Up		
	miR-494	Up	*PTEN, P27, PUMA*	Pollutri *et al.*, 2018 (39)
	miR-181a	Up	*RASSF1*	Azumi *et al.*, 2016 (40)
	miR-142-3p	Down	*ATG5, ATG16L1*	Zhang *et al.*, 2018 (41)
	miR-122	Down	*IGF-1R*	Xu *et al.*, 2015 (42)
	miR-137	Down	*ANT2*	Lu *et al.*, 2017 (43)
	miR-34a	Down	*Bcl2*	Yang *et al.*, 2014 (44)
	MiR-338-3p	Down	*HIF-α*	Xu *et al.*, 2014 (45)
	miR-193b	Down	*Mcl-1*	Mao *et al.*, 2014 (46)

Multidrug resistance transporters

One of the most important mechanisms of dox resistance consists of drug elimination by the transporter family known as the ATP-binding cassette (ABC) transporters, known to be highly abundant in hepatocytes (47). ABCB1, also known as multidrug resistance 1 (MDR1) or P-glycoprotein (P-gyp), is expressed in 80–90% of HCC cases (25,48,49)

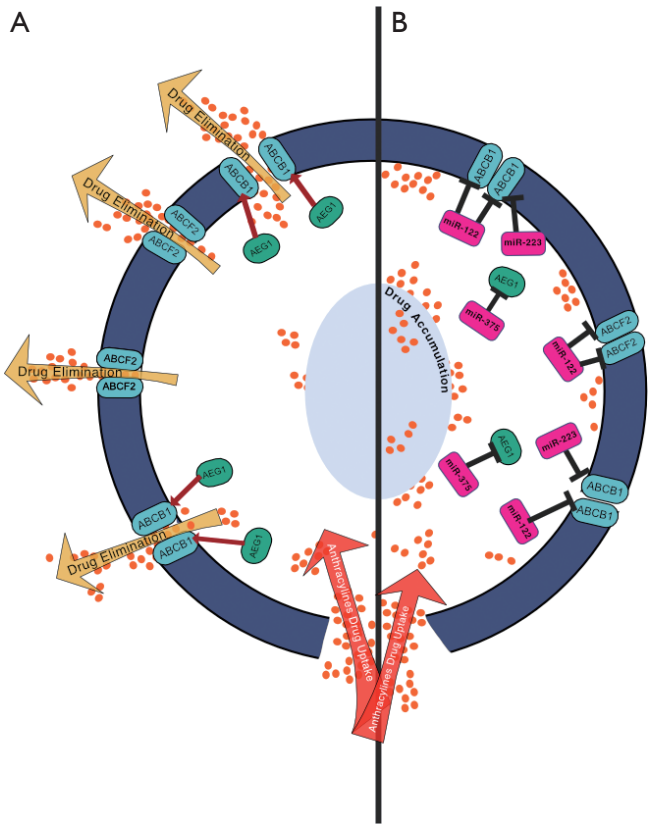

Figure 1 Mechanisms of drug accumulation in the cell. (A) miRNAs intracellular drug concentration by working as pump actively extruding drug compounds leading to resistance of HCC cells; (B) miRNAs involved in inhibiting MDR genes, leading to the accumulation of anthracyclines inside of HCC cells. HCC, hepatocellular carcinoma; MDR, multi-drug resistance transporter.

where it lowers the intracellular drug accumulation by working as a pump actively extruding exogenous compounds out of cells (25,48,50). Other ABC family members, such as ABCB1, ABCC1, ABCC2, and ABCG2 are also expressed in HCC, and the overlap in the substrate recognition makes those transmembrane transporters as one of the primary causes of drug failure (*Figure 1*).

Although several miRNAs are known to target different ABC transporters in HCC (51), only few of them were experimentally linked to anthracyclines resistance in this tumor (*Figures 2,3*). miR-122, one of the most abundant miRNA expressed in the liver, is known to be downregulated in HCC (52). This miRNA plays an essential role in hepatic lipid metabolism, hepatocyte development, and differentiation (53,54). miR-122 suppresses the anti-apoptotic gene Bcl-2 and regulates the expression of pyruvate kinase M2 (PKM2), involved in the proliferation and ability for the tumor cells to preserve ATP, that is needed for chemo-resistant cells to survive under stress (33,55). The overexpression of miR-122 induces cell cycle arrest that leads to the down-regulation of multidrug-resistant (MDR) genes, ABCB1, and ABCF2 (25,55). ABCB1 gene is also directly targeted by miR-223 in both mRNA and protein levels, and the overexpression of miR-223 can sensitize HCC cells to dox (29). Moreover, miR-375 is also able to decrease the expression of ABCB1 by targeting astrocyte elevated gene-1 (AEG-1) and thus participating in dox resistance (28,56,57). Recent evidence suggests the involvement of miR-590-5p on dox resistance. miR-590-5p have been proven to target yes-associated protein 1 (YAP1) that promotes cancer resistance by up-regulating ABC transporters (58). The overexpression of this miRNA chemosensitizes resistant cells both *in vitro* and *in vivo* interfering with the YAP1 network (58). This miR-590-5p/YAP1 axis was correlated with chemotherapy response in HCC patients. Although the few clinical

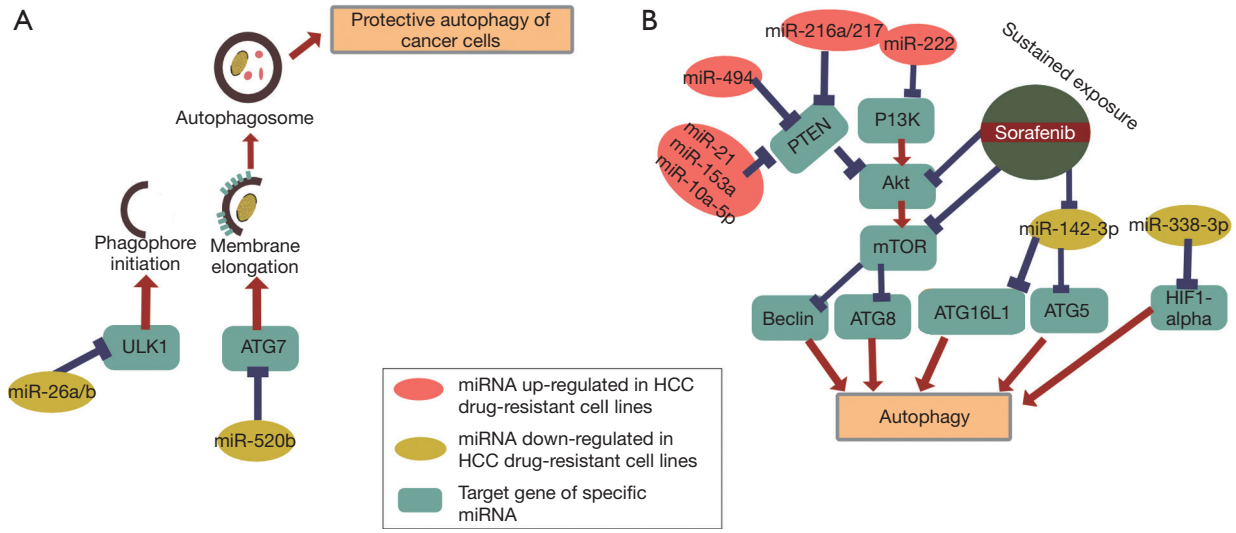

Figure 2 miRNAs involved in the multiple pathways of autophagy in cancer cells that develop resistance to anthracyclines and cisplatin (A) and sorafenib (B).

specimens analyzed, the YAP1 was higher in drug-resistant patients while miR-590-5p was downregulated, providing new insights into the identification of molecular targets for co-adjuvant therapies. Similar with miR-509-5p, the involvement of miR-122, miR-223, and miR-375 in the MDR1 drug-resistance related pathway (29,33,56) show a remarkable potential to re-sensitize cells to dox- resistance, becoming a new strategy combining miRNA therapeutics with chemotherapy.

Autophagy

Autophagy is an adaptive mechanism promoting cell homeostasis by the degradation of useless and damaged proteins or other cytoplasmic components in the lysosomal system (59,60). It is assumed to provide a protective effect against cancer, as it functions to prevent neoplastic transformation by removing damaged organelles and proteins complexes from cells exposed to stressful and pro-oncogenic conditions (61). However, in tumor cells, autophagy will also maintain metabolic homeostasis when cancer cells are subjected to stressful environments such as nutrient deprivation, hypoxia, or drug-induced damages during chemotherapy or targeted therapy (62). Several pathways, such as the mammalian target of rapamycin (mTOR) pathway, EFGR/Ras/MAPK pathway, p53 pathway, and hypoxia-inducible factor-1 (HIF-1) signaling pathway are primary regulators of autophagy in cancer cells (63). Furthermore, the hypoxic-environment after an anti-angiogenic therapy promotes HIF-1-mediated autophagy (63). However, depending on the type of anticancer treatment and genetic context of cancer, autophagy might also induce different effect and conversely work as an inducer of autophagic cell death, a mechanism that remains poorly explored and controversial (62).

Recently, the Unc-51 like autophagy activating kinase (ULK1), a serine/threonine protein kinase responsible for autophagy initiation (64) was identified as a target of the miR-26 family (miR-26a and miR-26b) (27). In dox-treated HepG2 cells, ULK1 was increased highlighting the protective role of autophagy in cancer cells. Interestingly, the overexpression of miR-26a/b decreased the expression of the ULK1 protein and sensitizing cancer cells to dox both *in vitro* and *in vivo* (27).

Several miRNAs involved in two protein conjugation systems that are required for autophagosome elongation and maturation in autophagy (65). One of the systems involved autophagy-related gene 7 (ATG7) that is targeted by miR-375, preventing the maturation of authophagosome thus inhibiting autophagy (66). Even though miR-375 was not directly involved in drug resistance, it is the inhibition of its target ATG7 that linked this miRNA to drug resistance (30). Another important miRNA in this pathway is miR-520b that is known to regulate ATG7 in both clinical and experimental settings. The down-regulation of miR-520-b and the up-regulation of its target ATG7 was

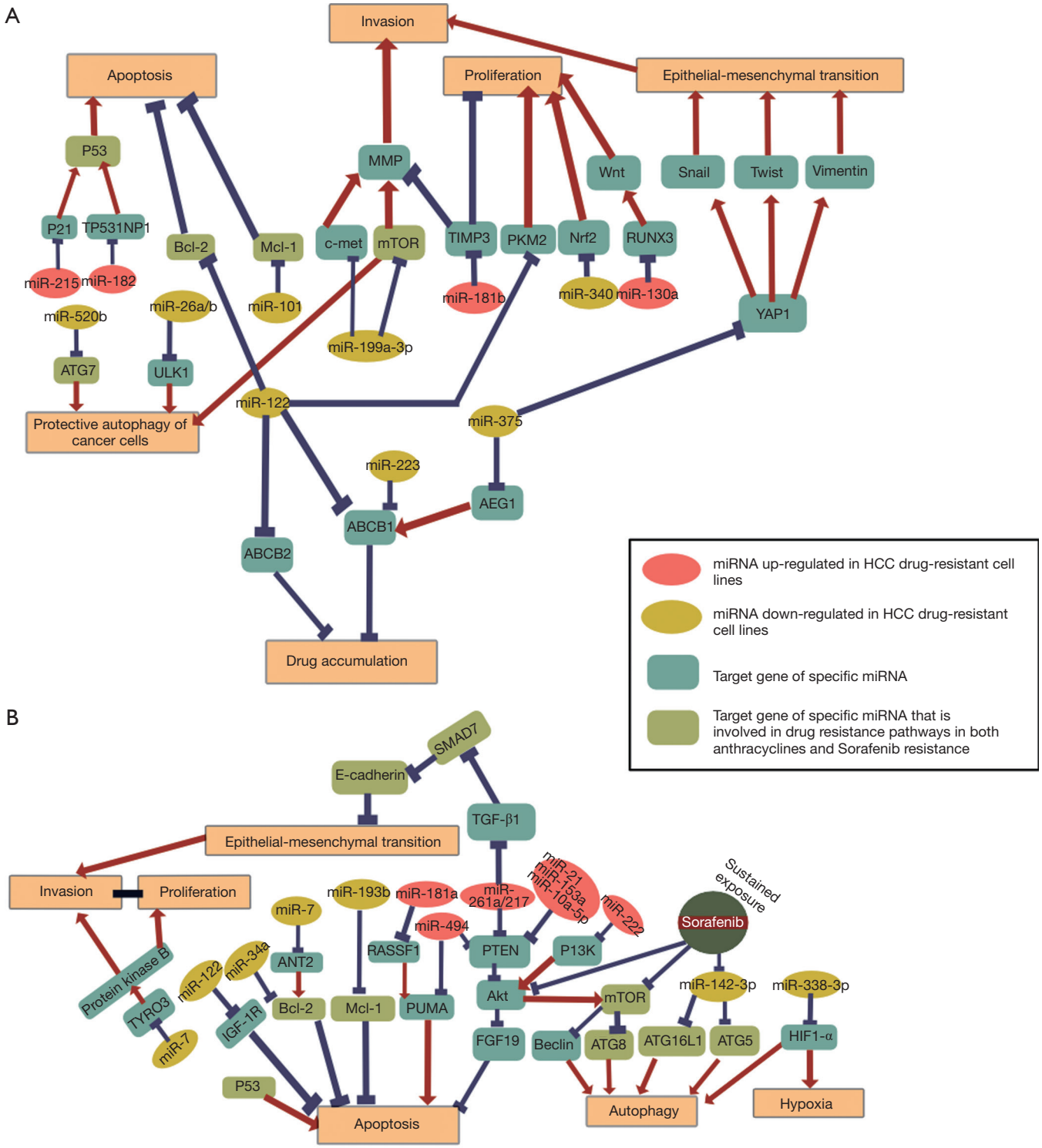

Figure 3 Multiple interpolated pathways regulated by miRNAs in the resistance to anthracyclines (A) and sorafenib (B) in HCC. Furthermore, several miRNAs target numerous genes while several genes are known to be involved either in both anthracyclines and sorafenib resistance. HCC, hepatocellular carcinoma.

observed both in human HCC tissues and dox-resistant HCC cell lines (30); indeed the resistance was reversed after restoring the expression of miR-520b using ectopic miR-520b mimic (30).

The role of autophagy during treatment with sorafenib is paradoxical. Some studies have established that sorafenib-induced autophagy is a cellular adaptive mechanism that promotes the survival of cancer cells (67). Thus inhibition of autophagy will enhance the effect of the drug (68). On the contrary, sorafenib was reported to inhibit the pro-death role of dox-induced autophagy in Hep3B cells (69-71). In sorafenib-resistant HCC cells, miR-142-3p was able to sensitize cells to the drug by inhibiting the drug-induced autophagy (72). It was demonstrated that sorafenib significantly reduced miR-142-3p levels by acting on the transcription factor PU.1. However, the overexpression of miR-142-3p was able to prevent the cytoprotective autophagy that protects cancer cells from sorafenib by targeting two key autophagy-related proteins, ATG5, and ATG16L1 (72). This mechanism might be one prospective therapeutic strategy to prevent cytoprotective autophagy and overcome sorafenib resistance (72).

Conversely, other evidence showed the opposite role of autophagy in inducing cell death. The inhibition of crucial autophagy regulators such as PTEN and AKT/mTOR pathway in HCC cells was described on decreasing the sensitivity of cells to sorafenib (73). PTEN is a tumor-suppressing phosphatase that inhibits Akt activation and thus preventing mTOR upregulation and the consequent regulation of the autophagic proteins LC3 and Beclin-1 (74). One study described that miR-21 is highly expressed in sorafenib-resistant cell lines and inhibiting autophagy by targeting PTEN (73). Furthermore, *in vivo* experiment by injecting anti-miR-21 oligonucleotides along with Sorafenib reduced the size of the tumor, pointing to the potential of anti-miR-21 as a prospective adjuvant therapy to overcome sorafenib resistance (73). Despite the findings in cell models, serum miR-21 was not able to predict sorafenib response in HCC patients (75).

Additional studies described the correlation between miRNA and autophagy-related sorafenib resistance; miRNAs such as miR-153, miR-216a, miR-217, miR-10a-5p, miR-222, negatively regulate PTEN, leading to the overexpression of AKT and mTOR, regulating autophagy, proliferation, and apoptosis (36-38). miR-494 also regulates the AKT/mTOR pathway and increases cell survival during stress condition induced by sorafenib, suggesting its involvement in the mechanism of autophagy activated during stress condition (39,65).

Collectively, the role of autophagy in promoting or preventing Sorafenib resistance is not yet fully clarified and needs more investigation. Disclosing the miRNA networks that participate in the regulation of autophagy (*Figures 2*,*4*) gives further hints to alternative therapeutically strategies.

Hallmarks of cancer

EMT

EMT is known to be an essential mechanism in the initial steps of cancer invasion and metastasis, as well as in the acquired resistance to cell senescence and apoptosis (76). It is a process where epithelial cells lose the polarity and cell-cell adhesion turning into an invasive mesenchymal cells' phenotype. Cells undergoing EMT are characterized by the activation of zinc-finger transcriptional repressors that are responsible of the decreased expression of E-cadherin, an epithelial gene that functions as a full EMT inducer and crucial step in the progression of cancer invasion (77,78). The down-regulation of transforming growth factor-β (TGF-β) found in up to 40% of HCC, is recognized as a crucial event that promotes EMT and enhances the migratory and invasive properties of cancer cells by involving the transcriptional regulators such as SMAD family (79,80). Moreover, EMT results in the dysregulation that involves TGF-β 1 and Yes-Associated Protein 1 (YAP1) (81). Recently, the identification of miR-375 as a YAP1 antagonist in cell models has suggested a complex network connecting EMT, apoptosis and ABC transporters modulation in drug resistance (*Figure 3*, *Figure 5A*) (28,56,57).

The long-term exposure to sorafenib leads to EMT in sorafenib-resistant HepG2 and HuH7 cells resulting in the loss of E-cadherin and a concomitant increase in the cell growth and metastatic potential (82). E-cadherin is also suppressed by the transcription factor SNAIL induced by the serum response factor (SRF), a member of the MADS-box family resulting in a reduction of cytotoxic effects of sorafenib in cancer cells (83). Furthermore, both SRF and SNAIL were overexpressed in advanced HCC (83).

The miR-216a/217 cluster was significantly upregulated in HCC tissues from patients with recurrent disease (36). Further experiments in HCC cell lines demonstrated the association of this miRNA cluster with EMT phenotype by targeting SMAD7, an antagonist of TGF-B type 1 receptor (36). The over-expression of these miRNAs in cellular models leads to TGF-β pathway activation

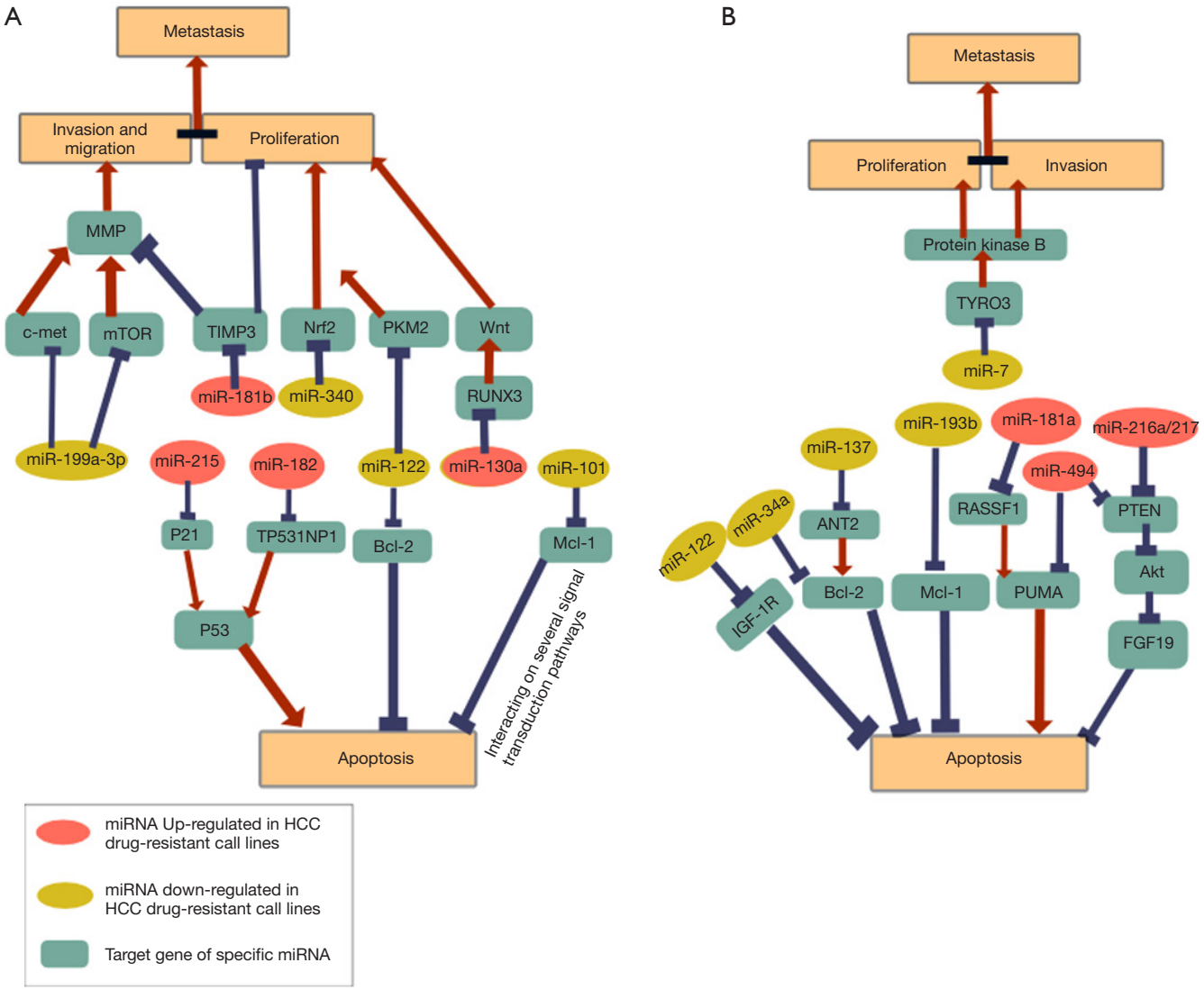

Figure 4 miRNAs involved in multiple pathways contributing to the hallmark of cancer cells in anthracyclines, cisplatin (A) and sorafenib resistance (B).

through the down-regulation of SMAD7, resulting in an EMT phenotype associated with sorafenib resistance (36). This fact points to the involvement of the miR-216a/217-SAMD7-TGF-β axis in sorafenib resistance in HCC (*Figure 3*, *Figure 5B*). Indeed, TGF-β has emerged as a potent secreted factor that drives epithelial plasticity leading to EMT, leading to tumor cell dissemination and cancer cell invasion by combining both Smad and non-Smad signaling pathways (36,84).

Apoptosis

Apoptosis is one of the main pathways involved in the MDR. In this pathway, one of the key regulators is the tumor suppressor p53 transcription factor that is activated in response to various cell stress and behavior, including DNA damage. The induction of DNA damage from chemotherapeutic agents may lead to cell cycle arrest, DNA repair, or apoptosis through the p53 pathway (85). The mutation of tp53 gene is frequently reported in HCC patient and associated with resistance to chemotherapeutics and poor prognosis (85) due to the alteration of several pro-apoptotic elements involved in the resistance such as Bax and TRAIL, as well as anti-apoptotic factors including Bcl-2 and Survivin (86-88). Qin *et al.* reported that the up-

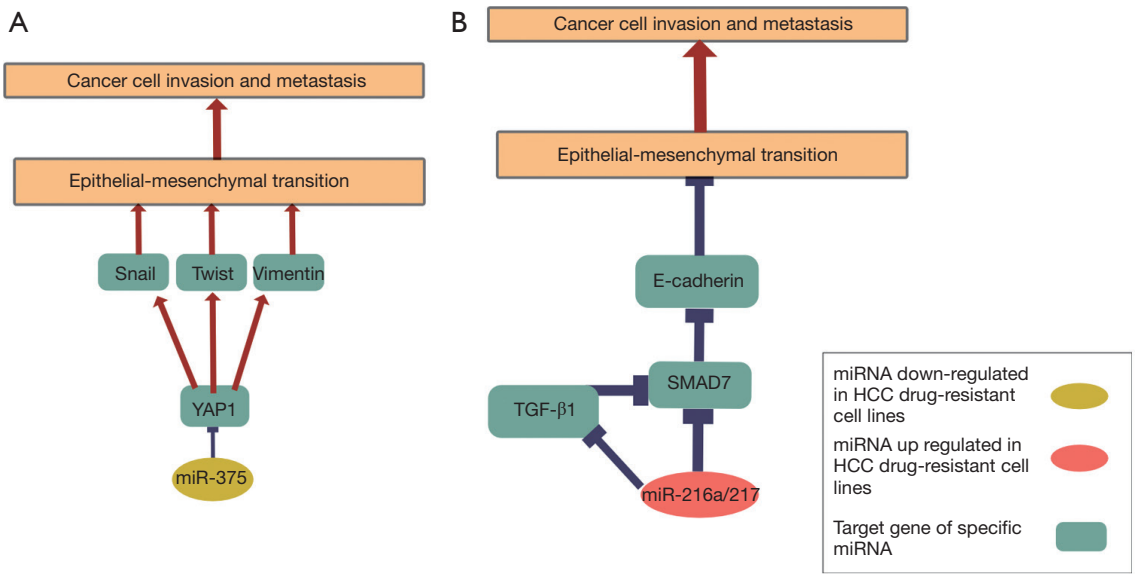

Figure 5 The role of miRNAs in EMT pathway. (A) Down-regulated of miR-375 and miR-145 in anthracyclines and cisplatin-resistant HCC cell lines and their involvement in several pathways related to EMT; (B) up-regulation of mir-216a and miR-217 to SMAD-related pathways of EMT. EMT, epithelial-mesenchymal transition

regulation of miR-182 increased resistance to cisplatin, by inhibiting the expression tumor protein P53 inducible nuclear protein 1 (TP53INP1), a pro-apoptotic gene of the p53 pathway, leading to the increase of cell viability during treatment with cisplatin (32). This is the only study connecting miRNA, P53 pathway, and drug-resistance, thus more studies on cellular models and clinical specimens are needed to further confirm the hypothesis.

Another miRNA involved in the anti-apoptotic mechanism is miR-101 that have a tumor suppressor role in HCC cell lines. miR-101 acts as a negative regulator of Mcl-1, a critical anti-apoptotic protein in cancer cells that maintains critical element in tumor environments such as growth factor and cellular stress (26). Mcl-1 has been already associated with several drug-resistance phenomenon in various cancers (89) and its downregulation by miR-101 suggests potential application for this miRNA in a Dox resistance setting (26).

During sorafenib treatment, several miRNAs were described to dysregulate the apoptotic pathway contributing to the resistance (*Figures 3*,*4*). miR-122 for example, was also significantly reduced in sorafenib-resistant cell lines (42). The mechanism by which this liver-specific miRNA reverses drug resistance involves the downregulation of insulin-like growth factor 1 receptor (IGF-1R) that was proven to inhibits apoptosis and disrupt tolerance to sorafenib *in vitro* (42).

Two other miRNAs affect apoptosis by regulating the anti-apoptotic proteins Bcl-2 and Mcl-1 in drug resistance. In sorafenib-resistant cell lines, the down-regulation of miR-193b resulted in the overexpression of its target Mcl-1. The transfection with miR-193 mimic suppresses Mcl-1, promoting cell apoptosis, and the co-exposure of miR-193b along with sorafenib had a synergistic effect in treating resistant cell lines (46). The down-regulation of miR-34 and the overexpression of Bcl-2 were concomitantly observed in HCC tissues. Ectopic expression of miR-34a sensitized HCC cells to sorafenib-induced apoptosis through the regulation of Bcl-2 of Bcl2 and Mcl-1 expression, suggesting an underlying mechanism between miR-34a and anti-apoptotic proteins (44).

The involvement of other miRNAs in the disruption of the apoptotic pathway, a significant event leading to drug resistance, is also known after sorafenib treatment. The down-regulation of miR-137 in sorafenib-resistant cell lines is known to alter adenine nucleotide translocator 2 (ANT2). Its upregulation activates the anti-apoptotic mechanism through the interaction with Bcl2-Bax proteins, ensuring cell survivals (43,90). The restoration of miR-137 was able to reverse the resistance in HCC cell lines showing the potential of this miRNA as anticancer molecule (43). On the other side, the up-regulation of miR-181a in HCC

cell lines reduced apoptosis by targeting RASSF1, tumor suppressor gene that induces apoptosis through induction of PUMA. Considering that sorafenib inhibits Raf kinase, that is a downstream regulator of RASSF1, the low expression of miR-181 may enhance RASSF1 apoptotic signaling and increase the sensitivity of sorafenib towards cancer cells (40). Considering this evidence, the additional use of these group of miRNAs along with sorafenib might re-sensitize cancer cells to the therapy in drug-resistant settings.

Proliferation, migration, and invasion

Several miRNAs play an essential role in the mechanisms of tumorigenesis like the invasion and proliferation of drug-resistant cells (*Figure 4*). Up-regulation of miR-181b in dox-resistant HCC cell lines leads to the inhibition of TIMP metallopeptidase inhibitor 3 (TIMP3), an inhibitor of cells migration, invasion, and angiogenesis in cancer cells (24). In addition, miR-199-3p represses the translation of mTOR and c-Met, suggested as a major activator of MMP, resulting in a reduced cell invasion and metastasis of dox-resistant cells (91). MTOR is also known to play a pivotal role in HCC regulating several other genes involved in major cancer pathways such as STAT3, AKT, HIF-α, or ULK1, suggesting its predominant role in multi-drug resistance (92).

Two studies described resistance of cisplatin in HCC cells. Xu *et al.* reported that the up-regulation of miR-130a in HuH7-resistant cells directly inhibit the expression of tumor suppressor gene, runt related transcription factor 3 (RUNX3), that results in the activation of Wnt/β-catenin, leading to cisplatin resistance (31). In cisplatin-resistant cells, it was reported that miR-340 modulates the expression of nuclear factor, erythroid 2 (Nrf2), a gene involved in drug resistance of various solid tumors and playing a role in cells proliferation and metastasis (34). This study was the first to describe the role of Nrf2, a cytoprotective transcription factor, in the acquisition of chemo-resistance to cancer cells.

Several miRNAs are also regulating the P13K/AKT pathway that is involved in various regulatory mechanism in HCC such as cell proliferation, invasion, apoptosis, metastasis, and autophagy during cancer progression. Kabir *et al.* identified miR-7 as a potent tumor suppressor targeting tyrosine-protein kinase 3 (TYRO3) that regulates proliferation, migration, and invasion through the P13/AKT pathway in sorafenib-resistant Huh-7 cells model (93). Another study showed that the overexpression of miR-494 in HCC leads to the decrease of PTEN, a multifunctional tumor suppressor that will activate P13/AKT signaling pathway and promoting anti-apoptosis and anti-proliferation in HCC (39,94). miR-494 transfection in HepG2 cells decreases the sensitivity to sorafenib and inversely correlates with the expression of PTEN and apoptosis level. This evidence highlights the importance of miR-494/PTEN/P13K/AKT axis in the hallmark of cancer (94). Further, the overexpression of miR-494 decreases also other pro-apoptotic factors such as P27 and PUMA proteins in both cell lines and sorafenib-resistant animal model (39).

Hypoxia

Hypoxia plays a significant role in enhancing the resistance of cancer cells to cytotoxic drugs (95), particularly relevant in the setting of concurrent induction of acute hypoxia during TACE (50). The hypoxic environment, to which surviving cells are exposed during and soon after chemoembolization, lead to the adaptive mechanisms affecting tumor proliferation, metabolism, and angiogenesis, that involve HIF-1 (96). HIF-1 directly activates various pro-survival genes, such as vascular endothelial growth factor (VEGF), insulin-like growth factor-2 (IGF-2), TGFα and β, and p53 (96).

Although hypoxia is considered as another leading cause of drug-resistance in cancer, only a single study exists regarding the involvement of miRNAs in this pathway. Indeed, the dysregulation of HIF-1 was associated with sorafenib resistance, being overexpressed in sorafenib-resistant HCC specimens (97). In the study, miR-338-3p was observed to be down-regulated in HCC patients and strongly associated with resistance to sorafenib. The re-exposure of miR-338-3p to resistant-cell models re-sensitizes cells by targeting HIF-1α, known to elicit the hypoxic environment in resistant tissue, by enhancing angiogenesis, metabolism, and resistance to apoptosis (45).

Circulating miRNAs as a biomarker to predict resistance to HCC therapy

The involvement of miRNAs on numerous regulatory networks and mechanisms of drug resistance, and the remarkable specificity of the circulatory expression patterns in cancer settings have raised provocative questions regarding their potential as markers to predict resistance in HCC. Circulating miRNA have been extensively studied as predictive or prognostic biomarkers in several cancer types, including HCC (35,41). Exosomal miRNAs are believed to represent a sort of cell-to-cell communication in

which parental cells can transfer molecules, including small RNAs to recipient cells influencing their phenotype (98). In the recent years, evidence pointed out the exosomal transfer of miRNAs to induce chemoresistance in different cancers (99,100). miR-21 was identified as one of the principal players in this setting, with an intriguing role in chemoresistance acquisition in ovarian cancer, neuroblastoma, and lung cancer (100-102). This evidence makes exosomal miRNAs as an essential source of a biomarker for therapy response. Several other small-scale studies have evaluated the role of miRNA as a biomarker for therapy response in several types of cancer. One particular example is found in colon cancer where blood miR-296 predicts tumor progression and metastasis post-chemotherapy (103). The serum level of miR-451 in two groups of breast cancer patients that were either sensitive or resistant to neoadjuvant chemotherapy showed a significant difference of expression level, similar with miR-345 taken from the serum of advanced rectal cancer patient treated with preoperative chemoradiotherapy (CRT) (104,105). These studies open the question whether miRNAs might also show a similar potential in predicting therapeutic response in HCC.

However, only a few studies reported evidence on the role of circulating miRNAs as therapy response biomarker in HCC. Liu *et al.* evaluated serum samples from 136 HCC patient undergoing TACE (a mixture of dox, lipiodol, and contrast agent) describing the potential role of miR-200a as an independent prognostic factor associated with disease outcome (106). Serum miR-335 is also significantly correlated with the therapeutic response after TACE in 125 HCC patients (107) in which the low miR-335 levels were significantly associated with inadequate treatment response and decreased survival. Conversely, higher expression of miR-590-5p had been related to a better response after TACE in patients with HCC (58). However, the latter study is a retrospective study with a small sample size that needs further validation to assess its potential and its regulatory role in the mechanism of multi-drug resistance in HCC.

Among the several limitations of the study in this area, one particular miRNA was assessed in regard to the expression in HCC patients, its association with therapy response, and its mechanism for regulating the drug-resistance. Kim *et al.* described the association of high expression of plasma miR-122 with early TACE (dox) refractoriness in 177 HCC patients (108). This is in line with the role of miR-122 in multi-drug resistance as this miRNA is strongly associated with multiple pathways regulating apoptosis, drug accumulation, invasion, and proliferation in drug-resistant HCC cells.

Meanwhile, in, there is only one small-scale study addressing the potential of miRNA as a predictor of response to sorafenib. High expression of serum miR-181a-5p in HCC patients treated with the drug was associated with better disease control and response to treatment (109). These findings, although limited by the small-sized cohort, are in line with the participation or miR-181 in cellular mechanisms leading to sorafenib resistance through EMT (110).

Conclusions

Numerous studies addressed the highly complex mechanism(s) of multi-drug resistance in HCC, which remains the main obstacle to achieving the success of chemotherapies. The development of MDR involves multiple pathways and networks, with diverse mechanisms and subtle key players. In the present review, we summarized the regulatory role of miRNAs in modulating drug resistance to anthracycline, cisplatin, and the multikinase inhibitor sorafenib. miRNAs play a significant role in targeting specific genes involved in the resistance, as many of them target multiple genes involved in different but interpolated pathways. Various studies prove that counterbalance the expression of miRNAs in resistant cells can re-sensitize cancer cells to chemotherapeutic agents, strengthening the potential of miRNAs as co-adjuvants in anticancer therapy.

However, there are still several technical obstacles to translate these results into a convincing clinical application. A homogenous methodological protocol to process and analyze sample has not yet been reached, making the comparison of different studies at least tenuous. In addition, the small cohorts and the heterogeneity of the disease limit the transposition of the results from bench to bedside. Indeed, the potential of miRNA is undeniable, and their paramount role in drug resistance could be utilized as alternatives in the future as well as a promising circulatory biomarker predicting therapy response. However, several additional evidence must be provided before miRNA will enter the routine clinical scenario.

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Footnote

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