Effect of the Hypoxia Inducible Factor on Sorafenib Resistance of Hepatocellular Carcinoma

Zhi Zeng1,2†, Qiliang Lu1,2†, Yang Liu1,2†, Junjun Zhao2,3†, Qian Zhang1, Linjun Hu1,2, Zhan Shi4, Yifeng Tu4, Zunqiang Xiao4, Qiuran Xu5* and Dongsheng Huang5*

1 The Medical College of Qingdao University, Qingdao, China, 2 Zhejiang Provincial People’s Hospital (People’s Hospital of Hangzhou Medical College), Hangzhou, China, 3 Graduate Department, Bengbu Medical College, Bengbu, China, 4 The Second Clinical Medical College of Zhejiang Chinese Medical University, Hangzhou, China, 5 The Key Laboratory of Tumor Molecular Diagnosis and Individualized Medicine of Zhejiang Province, Zhejiang Provincial People’s Hospital (People’s Hospital of Hangzhou Medical College), Hangzhou, China

Sorafenib a multi-target tyrosine kinase inhibitor, is the first-line drug for treating advanced hepatocellular carcinoma (HCC). Mechanistically, it suppresses tumor angiogenesis, cell proliferation and promotes apoptosis. Although sorafenib effectively prolongs median survival rates of patients with advanced HCC, its efficacy is limited by drug resistance in some patients. In HCC, this resistance is attributed to multiple complex mechanisms. Previous clinical data has shown that HIFs expression is a predictor of poor prognosis, with further evidence demonstrating that a combination of sorafenib and HIFs-targeted therapy or HIFs inhibitors can overcome HCC sorafenib resistance. Here, we describe the molecular mechanism underlying sorafenib resistance in HCC patients, and highlight the impact of hypoxia microenvironment on sorafenib resistance.

Keywords: sorafenib, hepatocellular carcinoma, HIF-1α, HIF-2α, sorafenib-resistance

INTRODUCTION

The globally cancer statistics of 2018 show that liver cancer is the sixth most commonly diagnosed form of cancer, and a fourth cause of cancer-related deaths worldwide (1). Despite significant progress being made in development of therapies for early diagnosis and treatment therapies for HCC in recent years, over 50% of all HCC cases are still diagnosed at an advanced stage. Additionally, approximately 70% of all HCC patients relapse within five years of initial treatment (2). Current treatment options for HCC include radiotherapy, chemotherapy, local ablation and molecular targeted therapy (3). Several targeted inhibitors have also been developed and applied in clinical practice. For example, sorafenib, which acts as a multiple-target tyrosine kinase inhibitor (TKI), was the first systematic drug to be approved for advanced HCC patients based on results of two randomized clinical trials. Functionally, sorafenib inhibits proliferation and angiogenesis of tumor cells, thereby delaying HCC progression while effectively prolonging the survival time of patients (4). A previous Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) trial confirmed that the drug was safe and efficacious in patients with advanced HCC. In fact, these similar results were corroborated by findings in Asia-Pacific clinical trials (5, 6), in which
sorafenib improved the clinical symptoms of about 30% of HCC patients. However, this group of patients reportedly develop resistance to sorafenib within 6 months of treatment (7). Previous studies indicate that sorafenib inhibits activity of various kinases, including Ras, Raf, MEK, and ERK, among others, and further targets VEGFR, c-KIT, PDGFR-β, and FLT-3, as well as other proteins that suppress tumor angiogenesis (8, 9). Moreover, sorafenib plays an anti-tumor role in HCC and other types of cancer, such as desmoid tumor, renal cell carcinoma, lung cancer and thyroid cancer (10–13). Although the drug effectively prolongs survival rates of HCC patients, its efficacy is significantly limited by development of drug-acquired resistance. The underlying mechanism of sorafenib resistance is complex. Previous studies have shown that the drug activates c-Jun, Akt pathway, epidermal growth factor receptor (EGFR), cancer stem cells enrichment, epithelial-mesenchymal transition (EMT) enhancement and reduces autophagy. Recently, other factors, such as dysregulation of miRNAs and IncRNAs in HCC have been implicated in sorafenib resistance (9, 14). Moreover, sorafenib reportedly induces hypoxia response in HCC, with dysregulation of hypoxia microenvironment and HIF expression shown to contribute to poor prognosis of HCC patients. In addition, sorafenib has also been implicated in effective inhibition of the HIF-1α/VEGFA signaling pathway (15). Weinberg et al. described six hallmarks of cancer, namely evasion of growth suppression, sustained proliferative signaling, induction of angiogenesis, resistance to cell death, replicative immortality, as well as activation of invasion and metastasis, and further demonstrated that these biological behaviors influence the degree of malignancy (16). Hypoxia, a common event that plays important roles in development and progression of malignant tumors, has been implicated in development of drug resistance and activation of tumor metastasis (17, 18). In the present study, we sought to clarify the underlying mechanism of sorafenib resistance, its relationship with the hypoxia microenvironment and the effect of targeting HIFs on sorafenib resistance in hepatocellular carcinoma.

SORAFENIB RESISTANCE IN HCC

Drug resistance is divided into the primary and acquired resistance, based on the time and sequence of exposure to the drug. Although both categories involve a complex chemical resistance mechanism, signaling pathways, characterized by up-/down-regulation and changes in molecular targets, represent the two most important factors (19). Elucidating the underlying mechanism of drug resistance is imperative to development of effective strategies to prevent or overcome its development (Figure 1).

**c-Jun** also known as AP-1 transcription factor subunit. It’s located at 1p32-p31, Deletion and translocation of this chromosomal region has been associated with development of malignant tumors. A previous study reported that regulation of mitotic signals can activate AP-1 (20). while others have shown demonstrated its significance in hepatocyte activity and liver regeneration, as well as in development of hepatocellular

---

**FIGURE 1** | Molecules and signal pathways related to sorafenib resistance in hepatocellular carcinoma. Sustained sorafenib treatment will affect the expression of the molecules and activate pathways, leading to sorafenib resistance in hepatocellular carcinoma. VEGFA, vascular endothelial growth factor A; EGFR, epidermal growth factor receptor; PDGFR, Platelet-derived growth factor receptor; c-kit, tyrosine kinase receptors hepatoctye factor receptor; EMT, epithelial-mesenchymal transition; CSCs, cancer stem cells; PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; ERK, extracellular signal-regulated kinase; JAK, janus tyrosine kinase; STAT, signal transducer and activator of transcription.
cancerous (21). Previous studies have also shown that c-Jun was remarkably activated in sorafenib-treated HCC cells, with its downregulation found to significantly elevate apoptosis of HCC cells induced by sorafenib (22). Another in vitro study found that sorafenib treatment could activate expression of c-Jun, while its inhibition significantly enhanced sorafenib-induced apoptosis in HCC cells (23). Results from a clinical trial revealed that HCC patients approached with sorafenib, the expression of Phosphorylation C-Jun was remarkably higher in the non-responder group than in the responder group (24). Therefore, c-Jun is probably one of the molecules that causes of HCC resistance to sorafenib.

The PI3K/AKT signaling pathway plays a role in regulation of apoptosis and chemotherapeutic resistance in malignant tumors. Previous studies have shown that sorafenib-mediated inhibition of AKT expression enhanced apoptosis induction in HCC cells (25). Moreover, Zhang et al. found that long term exposure of HCC cells to sorafenib could activate the PI3K/Akt signaling pathway, thereby whereas inhibiting PI3K using LY294002 could reverse sorafenib resistance (26). In another study, sorafenib effectively promoted AKT phosphorylation but did not significantly affect that of other proteins in the PI3K/AKT/mTOR signaling pathway (27). Therefore, activation of the PI3K/AKT signaling pathway is considered a compensatory mechanism for acquired sorafenib resistance. In fact, numerous studies have demonstrated that HCC cells with acquired sorafenib resistance exhibit significantly higher levels of phosphorylation AKT than parental cells, although suppression of AKT can reverse acquired sorafenib resistance (27, 28). The activation of AKT pathway has an important impact in sorafenib resistance in HCC.

Epidermal growth factor receptor (EGFR), which belongs to the protein kinase superfamily, can induce receptor dimerization and regulate autophosphorylation of tyrosine thereby causing cell proliferation. This phenomenon has been shown to be a potential indicator of sorafenib resistance in HCC cells. In HCC cells with higher EGFR expression, the efficacy of sorafenib is significantly weakened. A previous study demonstrated that sensitizing cells to sorafenib could be increased by downregulating EGFR expression or inhibiting its kinase activity (29). In, Moreover, the EGFR pathway is overexpressed in HCC cells with acquired resistance to sorafenib, where it acts as the driving force for maintaining HCC cell proliferation under sorafenib (30).

Epithelial–mesenchymal transformation (EMT) contributes to migration and drug resistance, and is therefore an important cellular program (31). Previous studies have reported that EMT is associated with cancer chemotherapeutic resistance, with its inhibition found to reverse this drug-resistant outcome (32). For example, Fisher et al. established a genetically engineered mouse model and demonstrated the relationship between cancer drug resistance and EMT (33). Moreover, induction of EMT reportedly promotes tumor progression and sorafenib resistance in HCC. Although EMT development is inhibited by sorafenib, it has also been reported to promote chemotherapy resistance to sorafenib in HCC cells (34, 35). Previous studies have demonstrated that cancer stem cells (CSCs) are also involved in development of chemoresistance in HCC. In addition, there is crosstalk between EMT and CSCs, as evidenced by the fact that acquired EMT cells exhibit CSCs-like characteristics, while CSCs show mesenchymal phenotype (36), EMT activation is usually associated with enrichment of CSCs subsets in sorafenib resistance cells (37, 38). Thus CSCs markers have been used as predictors of sorafenib reaction. Notably, upregulation of CSC markers, CD90 and CD133, was shown to be a predictor for sorafenib resistance in HCC cells. Moreover, PTK2 activated CSC characteristics to promote tumor progression by inducing β-catenin nuclear accumulation in HCC cells thereby inducing sorafenib resistance (39). CD44 can be used as a marker for evaluating efficacy of sorafenib in HCC cells, as evidenced by its role in development of drug resistance (40). Other markers, such as CD13, EpCAM, and CD24 are also considered helpful for CSCs enrichment in HCC (41).

Autophagy, a self-degrading system that directs cells to eliminate abnormal proteins and dysfunctional organelles, plays an essential role in maintaining homeostasis in cells under stress, such as nutritional deficiency or hypoxia (42). In fact, autophagy plays a double-edged sword in different cancers by suppressing initiation of tumors but also supporting their progression. This mechanism further plays an important role in drug resistance, enabling tumor cells to maintain cell activity under metabolic and therapeutic stress. In fact, autophagy is often activated in radiotherapy, chemotherapy, and targeted therapy (43). In HCC, elevated autophagy reportedly regulated sorafenib resistance (44). Patients treated with sorafenib were found to overexpress Atg7 and had elevated autophagy activity, indicative of poor prognosis (45). However, another study demonstrated that sorafenib induced autophagy and further enhanced the drug’s effect on HCC, contrary to previous results (46). Furthermore, different HCC cell lines showed varied sensitivities to sorafenib, possibly due autophagy (47). The mechanism of autophagy has not been fully elucidated, and further research in this field is worthy of further study.

Numerous studies have reported that non-coding genes can also play an important role in development of chemotherapeutic resistance in cancers. For example, some miRNAs associated with sorafenib resistance have been identified, and can be used as biomarkers for predicting sorafenib treatment in HCC (Table 1). miRNAs play various functions like mediate proliferation, invasion and metastasis, angiogenesis, induction of hypoxia, and et al. For example, low expression of some miRNAs had been found to promote sorafenib-resistance of HCC cells, miRNAs although others may have an opposite effect. A previous study reported that miR-486-3p inhibits cell proliferation and induces apoptosis, however, it was downregulated in sorafenib-resistant HCC cell lines by up-regulating FGFR4 and EGFR activity (67). In contrast, miR-216a/217 cluster was significantly upregulated in HCC compared to normal cells. although this upregulation could activate the TGF-β and PI3K/AKT signaling pathways, thereby contributing to acquired sorafenib resistance in HCC cells (75). miRNAs functional mechanism is complicated and still controversial. Different miRNAs have different effects on
HCC, moreover, the same miRNA could have different effects on different cancers. There is still plenty of room for research in this field. Apart from miRNAs, many lncRNAs have also been implicated in sorafenib resistance (Table 2).

### HYPOXIC AND SORAFENIB-RESISTANCE IN HCC

Hypoxia, which often occurs in many solid tumors, including HCC, is caused by faulty vascularity and vigorous metabolic activity, and has been associated with chemoresistance, increased invasiveness, and poor prognosis (100). Thus, suppressing hypoxia is considered a feasible approach for overcoming drug resistance. HIFs are transcription factors related to regulating angiogenesis, proliferation, glucose metabolism, tumor invasion and metastasis (100). Particularly, HIF-1α, -2α, -3α and -β are key factors that play a role in regulating a range of genes to control the hypoxia-induced signaling pathway. Since expression of the α-subunit is sensitive to oxygen, while the β-subunit is constitutively expressed, this review focuses on the α-subunit of HIFs (101). Among known α-subunits, HIF-1α and HIF-2α have been shown to regulate occurrence of hepatocellular carcinoma, while HIF-3α has generally been associated with inhibition of HIF-1α and HIF-2α activities (102, 103). Previous studies have shown that multiple factors are involved in hypoxia

---

**Table 2**

| miRNA and sorafenib resistance in HCC cells. |
|---------------------------------------------|
| Name | Cell line/animal models | Target | Reference |
| miR-21† | HepG2, HuH7/BALB/c nude mice subcutaneous HCC model | AKT† (49) |
| miR-161| Huh7/BALB/c nude mice subcutaneous HCC model | 14-3-3η†, HIF-1α† (49) |
| miR-494| HuH7, SNU182, HepG2/DEN-treated rats | AKT†, mTOR†, P271, PUMA† (50) |
| miR-212† | HepG2, Hep3B, PLCC/PFF/5, SNU398, SNU449, SNU182, SNU475, HuH7/DEN-treated rats, NOD/SCID mice hydrodynamic tail vein injection | Caspase-3† (51) |
| miR-222† | HepG2, HL-7702/− | AKT† (52) |
| miR-233 | HuH7, SNU387, SNU449/− | FBW7† (53) |
| miR-622† | PLCC, Hep3B, HepG2, HuH7/male mice orthotopic tumor injected with HCC cells | KRAF† (54) |
| miR-347b† | Hep3B, HepG2, HCCLM3/male SCID mice subcutaneous HCC model | PKM2† (55) |
| miR-181a† | Hep3B, HepG2/− | RASSF1† (56) |
| miR-122† | HuH7, PLCC, T1115/NOD/SCID mice subcutaneous HCC model | IGF-1R† (57) |
| miR-122† | HepG2, Hep3B, HuH7/DEN-HCC rat | SerpinB3† (58) |
| miR-7441 | LO2, HepG2, MMC-7721/− | PAX2† (59) |
| miR-1371 | Hep7/− | ANTI† (60) |
| miR-7† | HuH7, Hep3B/mice orthotopic liver cancer model and tail vein injection | TYRO3† (61) |
| miR-142† | HepG2, SMMC-7721/BALB/c nude mice subcutaneous HCC model | PTEN† (62) |
| miR-3163† | MHC97-H, LM-3, HepG2, HuH7, BEL-7402, SMMC-7722, MHC97-L/nude mice subcutaneous HCC model and tail vein injection | ADAM-17† (63) |
| miR-140-3p | MHC97-H, HepG2/nude mice subcutaneous HCC model and hepatic portal vein injection | PXR† (64) |
| miR-30e| HepG2, Hep3B, HuH7, SNU449, SNU475/DEN-HCC rat | MDM2†, TP53† (65) |
| miR-19a| PLCC/PFF/5, BEL-7402, Hep3B and HepG2/− | PTEN† (66) |
| miR-486| SK-HEP-1, HepG2, HuH7/BALB/c nude mice orthotopic HCC model and Subcutaneous HCC model | FGFR4†, EGFR† (67) |
| miR-591| HepG2, Hep3B, SK-HEP1, HuH7/BALB/c nude mice subcutaneous HCC model | FBP2†, AKT† (68) |
| miR-194| HuH7, HCCLM3/NOD-SCID mice subcutaneous HCC model | RAC1† (69) |
| miR-613| HuH7, HCCLM3/NOD-SCID mice subcutaneous HCC model | SOX9† (70) |
| miR-365| HCCLM3, SMMC7721/− | RAC1† (71) |
| miR-29a| HuH7, HepG2/NOD-SCID mice subcutaneous HCC model | BCL-2† (72) |
| miR-344a| HuH-7, MHC97H/− | BCL-2† (73) |
| miR-219† | HCCLM3, HepG2/NOD-SCID mice subcutaneous HCC model | E-cadherin† (74) |
| miR-216a| HepG2, Hep3B, HuH7, PLCC/PFF/5, HCCLM3, Bel-7404, HLE, SK-HEP-1, SNU-449/BALB/c nude mice 217† | PTE1†, SMAD7† (75) |
| miR-378a| HuH7, HCCLM3, SK-HEP-1/BALB/c nude mice orthotopic HCC model, NOD/SCID mouse | IGF-1R† (76) |
| miR-522† | HepG2, HCCLM3/NOD-SCID mice subcutaneous HCC model | PTEN1 (77) |
| miR-494| HuH7, HepG2/− | PTEN1 (78) |
| miR-375† | Hep3B, HepG2, HuH1, HuH7/BALB/c nude mice subcutaneous HCC model | AEG-1, PDCPC1 (79) |
| miR-338| HepG2, SMMC-7721, BEK-7402, Hep3B, HuH-7/BALB/c nude mice subcutaneous HCC model | HIF-1α† (80) |
| miR-193b† | HepG2 and HepG2.2.15 (derived from HepG2 cells and stably integrated with the entire HBV genome) | Bcl-xl† (81) |
| miR-195† | Mcl-1† (HBe infection induce sorafenib resistance) (82) |
Notably, several proteins have been shown to affect this pathway by reversing sorafenib induced hypoxia. Bort et al. reported that galectin-1 is elevated in sorafenib-resistant HCC cells, while its upregulation AMPK restores the sensitivity of HCC cells to sorafenib (111, 112). In addition, Yeh et al. showed that galectin-1 is elevated in sorafenib-resistant HCC cells, both in vitro and in vivo, promoting tumor metastasis and increasing tumor invasion, suggesting that galectin-1 plays a role in downstream regulation of the AKT/mTOR/HIF-1 signaling pathway (113). Hypoxia induces overexpression of AQP3 in hypoxic HCC cells, thereby altering sensitivity of these cells to sorafenib by activating the PI3K/Akt signaling pathway (114).

Other proteins have also been reported to alter resistance of HCC cells to sorafenib by acting on HIFs. For example, sorafenib was found to inhibit TIP30, thereby promoting EMT, which caused resistance to the drug (115, 116). Overexpression of HIF-2α was shown to downregulate TIP30 and promote EMT, although its down-regulation could reverse these effects (117). Bnip3 is also a hypoxic-regulated protein. Blanco et al. showed that HIFs stability and overexpression not only silenced the Bnip3 promoter, but also inhibited sorafenib-mediated apoptosis, thereby contributing to acquired drug resistance in HCC cells (118). On the other hand, RIT1, which belongs to the Ras superfamily, was shown to induce overexpression of RIT1 in HCC cells under HIF-1α-mediated hypoxia. Notably, sorafenib treatment could upregulate RIT1, while downregulating RIT1 in HCC cells could restore sensitivity of the cells to sorafenib (119). In addition, Long et al. found that PFKFB3 was upregulated in sorafenib-treated HCC cells. Notably, overexpressing PFKFB3 significantly enhanced sorafenib resistance in these cells by downregulating expression of apoptosis-related molecules, while blocking HIF-1α inhibited the enhancement of PFKFB3 (120). Apart from proteins, miRNAs have also been shown to play an important role in hypoxia. For example, 14-3-3η could stabilize HIF-1α and maintain resistance to sorafenib in HCC by increasing tumorigenesis, inducing and enhancing the CSCs of HCC cells, while its upregulation AMPK restores the sensitivity of HCC cells to sorafenib (111, 112). In addition, Yeh et al. showed that galectin-1 is elevated in sorafenib-resistant HCC cells, both in vitro and in vivo, promoting tumor metastasis and increasing tumor invasion, suggesting that galectin-1 plays a role in downstream regulation of the AKT/mTOR/HIF-1 signaling pathway (113). Hypoxia induces overexpression of AQP3 in hypoxic HCC cells, thereby altering sensitivity of these cells to sorafenib by activating the PI3K/Akt signaling pathway (114).

Other proteins have also been reported to alter resistance of HCC cells to sorafenib by acting on HIFs. For example, sorafenib was found to inhibit TIP30, thereby promoting EMT, which caused resistance to the drug (115, 116). Overexpression of HIF-2α was shown to downregulate TIP30 and promote EMT, although its down-regulation could reverse these effects (117). Bnip3 is also a hypoxic-regulated protein. Blanco et al. showed that HIFs stability and overexpression not only silenced the Bnip3 promoter, but also inhibited sorafenib-mediated apoptosis, thereby contributing to acquired drug resistance in HCC cells (118). On the other hand, RIT1, which belongs to the Ras superfamily, was shown to induce overexpression of RIT1 in HCC cells under HIF-1α-mediated hypoxia. Notably, sorafenib treatment could upregulate RIT1, while downregulating RIT1 in HCC cells could restore sensitivity of the cells to sorafenib (119). In addition, Long et al. found that PFKFB3 was upregulated in sorafenib-treated HCC cells. Notably, overexpressing PFKFB3 significantly enhanced sorafenib resistance in these cells by downregulating expression of apoptosis-related molecules, while blocking HIF-1α inhibited the enhancement of PFKFB3 (120). Apart from proteins, miRNAs have also been shown to play an important role in hypoxia. For example, 14-3-3η could stabilize HIF-1α and maintain resistance to sorafenib in HCC by

### TABLE 2 | IncRNA and sorafenib resistance in HCC cells.

| Name | Cell line/animal models | Target | Reference |
|------|------------------------|--------|-----------|
| SNGI1 | HepG2, Huh7/BALB/c nude mice subcutaneous HCC model | AKT↑ | (63) |
| SHG31 | PLo/PRF/5, Hep3B, HepG2, MMCC97L, Huh7, HCCLM3-7721, HCCLM3/→ | EMT↑ | (64) |
| SHG16 | Huh7, Hep3B, HCC1954, HCCLM3, LO2/nude mice subcutaneous HCC model | – | (65) |
| FOXD2-AS1 | Huh7, Huh7/→ | – | (66) |
| NEAT1 | Huh7, Huh7/→ | – | (67) |
| DANCR1 | HEK-293T, Huh7, Hep3B/BALB/c nude mice subcutaneous HCC model | HOTAIR↑ | (68) |
| RAR1 | Huh7, Hep3B, SNU-387, SNU-449/→ | – | (69) |
| HE1 | Huh7, HCCLM3/→ | – | (70) |
| MALAT1 | HePG2, MMCC-7721/nude mice subcutaneous HCC model and tail veins injection | Aurora-A↑ | (71) |
| ROR1 | LO2, Huh7, MMCC-7721, Huh7, MMCC97H, Hep3B, HCCLM3/BALB/c nude mice subcutaneous HCC model | FOXM1↑ | (72) |
| Yor1 | HCCLM3, MMCC7721/ → | β-catenin↑ | (73) |
| Adic5-5 | Huh7, Huh7/BALB/c nude mice subcutaneous HCC model | AKT↑ | (74) |
| HOXA13 | SNU-449, HepG2/→ | – | (75) |
| TUSC38 | Huh7, MMCC-7721, bEK-7402, Hep3B, Huh7, LO2/nude mice subcutaneous HCC model | RASAL1↑ | (76) |
| HANR1 | Huh7, Hep3B, 293T/BALB/c nude mice subcutaneous HCC model | ATG9A↑ | (77) |
| H19 | Huh7, Hep3B, SNU-449, SNU-387/→ | EMT↑ | (78) |
| H19 | Huh7, Hep7, Plc/DEN-treat HCC mouse model | – | (79) |

Previous studies have reported that FOXD2-AS1 is downregulated in sorafenib-resistant HCC cells. Moreover, targeting FOXD2-AS1 was associated with inhibition of the NRf2 signaling pathway by regulating expression of TMEM99 and reversing resistance to sorafenib in HCC (63). Fan et al. found that MALAT1 was significantly up-regulated in sorafenib-resistant HCC cells, suggesting that it regulates Aurora A to promote cell proliferation, migration and EMT formation, thereby promoting the observed resistance (91). The expression levels of IncRNAs were significantly different in different tissues, and their functions were also different, the mechanisms that mediate the generation of functions are complex and diverse. LncRNA-mediated cell drug resistance is an emerging field, and in many current studies on IncRNA, their roles are also different.
inhibiting degradation of ubiquitin-proteasome-dependent protein, thereby maintaining CSCs. In addition, miR-16 was shown to reverse sorafenib resistance by inhibiting the 14-3-3ζ/HIF-1α/CSCs axis (49).

Taken together, the findings of these studies affirm the relationship between HIF expression disorder and sorafenib resistance, suggesting that hypoxia may significantly affect the therapeutic effect of sorafenib. Therefore, targeting these factors holds promise to future development of effective therapies to overcome drug resistance (Table 3).

### STRATEGIES TO OVERCOME SORAFENIB RESISTANCE IN HCC BY TARGET HIFs

Considering that HIFs participate in a variety of cancer-promoting pathways and regulate the biological behavior of HCC cells, targeting HIFs may be an effective treatment strategy. For example, since HIFs play a key role in development of HCC resistance to chemotherapy drugs, inhibiting them could be a feasible strategy to manage drug development of HCC resistance to chemotherapy drugs, thereby promoting and lowering apoptosis and proliferation of HCC cells, respectively. In addition, the drug also enhanced the effect of sorafenib in HCC cells (124). Another drug, 2-Methoxyestradiol, was shown to significantly downregulate HIF-1α and HIF-2α expression as well as that of downstream molecules such as VEGF, cyclin D1, and LDHA. Notably, its synergistic interaction with sorafenib reportedly inhibited proliferation of HCC cells and induced apoptosis both in vivo and in vitro, thereby inhibiting tumor angiogenesis (109).

Certain natural compounds have also shown efficacy in improving sorafenib-mediated treatment in drug-resistant liver cancer cells. For example, flavonoid procyandin B2 was shown

---

**TABLE 3 | Hypoxia and sorafenib resistance in HCC cells.**

| HIFs after sorafenib treat in HCC | Cell line/animal models | Target | Reference |
|-----------------------------------|------------------------|--------|-----------|
| HIF-1α† | HepG2, Huh7/- | AKT† | (114) |
| HIF-1α† | HepG2, SMMC-7721, BEK-7402, Hep3B, Huh-7/BALB/c nude mice subcutaneous HCC model | VEGF/ cyclinD1 | (109) |
| HIF-1α† | HepG2, Huh7/BALB/c mice subcutaneous HCC model | AKT† | (121) |
| HIF-1α† | Huh7/BALB/c mice subcutaneous HCC model | PCNA/β-catenin | (122) |
| HIF-1α† | HepG2, Huh7/BALB/c mice subcutaneous HCC model | AMPK | (112) |
| HIF-1α† | Huh7/BALB/c mice subcutaneous HCC model | Galectin mTOR | (113) |
| HIF-2α† | Hep3B/- | mTOR† | (123) |
| HIF-1α† | Huh3/BALB/c mice subcutaneous HCC model | PPAR-γ | (124) |
| HIF-1α† | MHC-97H/BALB/c mice subcutaneous HCC model | TIP30/EMT | (117) |
| HIF-2α† | MHC-97H/BALB/c mice subcutaneous HCC model | GULT1/HK2 | (125) |
| HIF-1α† | MHC-97H/BALB/c mice subcutaneous HCC model | PKM2 | (128) |
| HIF-2α† | HepG2/- | BNIP3 | (118) |
| HIF-1α† | Huh7/BALB/c mice subcutaneous HCC model | 14-3-3ζ | (49) |
| HIF-2α† | Hep2, Hep3B, SK-Hep-1/BALB/c mice subcutaneous HCC model | ATPase | (127) |
| HIF-2α† | Hep2, Hep3B, SK-Hep-1/BALB/c mice subcutaneous HCC model | androgen receptor | (128) |
| HIF-1α† | Hep2, Hep3B, PLC/5/BALB/c mice subcutaneous HCC model | VEGF/MDR/PPAR-γ | (129) |
| HIF-2α† | Hep2, Hep3B, SK-Hep1/BALB/c mice subcutaneous HCC model | GULT1/NF-kB | (107) |
| HIF-1α† | Hep3B, Hep2, PLC/PRF-5/BALB/c mice orthotopic model | RIT1† | (119) |
| HIF-1α† | SK-Hep-1, SMMC-7721, HepG2, Huh7, MHC-97H, LM3/- | PFKB3† | (120) |
to downregulate PKM2 expression, thereby affecting the PKM2/HSP90/HIF-1α axis, inhibiting aerobic glycolysis, as well as proliferation and induction of apoptosis in HCC cells. Notably, co-treatment of procyanidin B2 and sorafenib could effectively improve the latter’s sensitivity in HCC cells (126). Genistein, a natural isoflavone that inhibits glycolysis, was shown to induce apoptosis and down-regulate GLUT-1 and HK2 by suppressing HIF-1α, thereby enhancing the effect of sorafenib on drug-resistant HCC cells both in vitro and in vivo (125). In addition, saponins derived from Rhizoma Paridis significantly downregulated mRNA expression and protein levels of HIF-1α, and further exhibited their anti-tumor activity by regulating glycolysis and lipid metabolism. Notably, a combination of these saponins with sorafenib could improve the anti-tumor effect in vivo. Previous studies have further shown that sorafenib resistance in liver cancer cells can be overcome by preventing mitochondrial damage, inhibiting anaerobic glycolysis and suppressing lipid synthesis by targeting the PI3K/Akt/mTOR signaling pathway (121). For example, EF24 effectively reversed sorafenib resistance by degrading HIF-1α and inactivating NF-κB via a VHL tumor suppressor. A combination of EF24 with sorafenib was also found to generate a synergistic effect that enhanced the associated anti-tumor effect (129).

Previous studies have also reported that application of PT-2385 could specifically inhibit HIF-2α, to increase androgen receptors, suppress downstream factors such as STAT3, and activate the Akt and ERK signaling pathways, thereby improve sorafenib efficacy in HCC cells both in vivo and in vitro (128). In summary, inhibiting HIFs can effectively enhance the sensitivity of HCC cells to sorafenib and improve drug resistance.

CONCLUSION AND DISCUSSION

Although sorafenib is a safe and effective therapy for treating advanced HCC, development of drug resistance has been shown...
to reduce its benefits. The underlying mechanism of this resistance is complex and currently remains unclear. Primary drug resistance can be explained by the genetic heterogeneity of HCC. Elucidating the underlying mechanism of acquired drug resistance is important in guiding development of approaches to overcome or delay its development. Previous studies have demonstrated that sorafenib-acquired resistance involves multiple mechanisms, including crosstalk in the PI3K/Akt, MAPK, JAK-STAT, ERK and HIF signaling pathways, abnormal expression of proteins, such as PDGFR-β, c-KIT, FLT-3, VEGFR, EGFR, as well as EMT, cancer stem cells, and autophagy, among others. Abnormal regulation of miRNAs and lncRNAs, as well as development of hypoxia in HCC also play an important role in inducing acquired resistance to sorafenib.

For patients with advanced liver cancer, who have been exposed to sorafenib for a long time, the drug’s anti-angiogenic effect is expected to cause a decrease in microvessel density and enhance tumor hypoxia. Consequently, this induces the HIF-mediated cell adaptation mechanism to the hypoxic microenvironment. In other tumors, extensive researches have been done using gene therapy to target HIFs or adding HIFs inhibitors to current therapies, with a view to improve its effectiveness. Particularly, overexpression of HIFs in liver cancer has been reported, with sorafenib found to promote HIF activity. Notably, a combination of sorafenib with other drugs, to lower the level of or directly target HIFs, has been proven to improve efficacy of sorafenib, suppress the proliferation and promote apoptosis of HCC cells, as well as reduce the number of metastases and tumor volume both in vitro and in vivo. For patients with advanced HCC, who have failed sorafenib treatment, several drugs, such as lenvatinib, regorafenib, cabozantinib, and ramucirumab, have been approved for second-line treatment. However, sorafenib remains the mainstay for treating advanced HCC (131). The importance of overcoming sorafenib resistance in HCC cells cannot be overemphasized. For this, targeting HIFs and improving the tumor hypoxic microenvironment hold promise for future development of therapies to manage sorafenib resistance and improve prognosis of patients with advanced HCC.

**AUTHOR CONTRIBUTIONS**

ZZ, QL, YL, and JZ conceived the project and wrote the manuscript. QZ, LH, ZX, YT, and ZS participated in data analysis. QX participated in discussion and language editing. DH reviewed the manuscript. All authors contributed to the article and approved the submitted version.

**FUNDING**

This work was funded by the Co-construction of Provincial and Department Project (WKJ-ZJ-1919), the National Science and Technology Major Project for New Drug (No. 2017ZX093 02003004), and the Key Research and Development Project of Zhejiang Science and Technology Department (2020C03008).

**REFERENCES**

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A, et al. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2018) 68 (6):394–424. doi: 10.3322/caac.21492

2. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, et al. Hepatocellular Carcinoma. *Nat Rev Dis Primers* (2016) 2:16018. doi: 10.1038/nrdp.2016.18

3. Sia D, Villanueva A, Friedman SL, Llovet JM. Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis. *Gastroenterology* (2017) 152(4):745–61. doi: 10.1053/j.gastro.2016.11.048

4. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc J-F, et al. Sorafenib in Advanced Hepatocellular Carcinoma. *N Engl J Med* (2008) 359 (4):378–90. doi: 10.1056/NEJMoa0708857

5. Vogel A, Saborowski A. Corrigendum to “Current Strategies for the Treatment of Intermediate and Advanced Hepatocellular Carcinoma” [Cancer Treatm. Rev. 82 (2019) 101946]. *Cancer Treat Rev* (2020) 83:101962. doi: 10.1016/j.ctrv.2019.101962

6. Cheng AL, Kang Y-K, Chen Z, Tsao C-J, Qin S, Kim JS, et al. Efficacy and Safety of Sorafenib in Patients in the Asia-Pacitic Region With Advanced Hepatocellular Carcinoma: A Phase III Randomised, Double-Blind, Placebo-Controlled Trial. *Lancet Oncol* (2009) 10(1):25–34. doi: 10.1016/S1470-2045(08)70285-7

7. Ford R, Schwartz L, Dancey J, Dodd LE, Eisenhauer EA, Gwyther S, et al. Lessons Learned From Independent Central Review. *Eur J Cancer* (2009) 45 (2):268–74. doi: 10.1016/j.ejca.2008.10.031

8. Liu L, Cao Y, Chen C, Zhang X, McNabola A, Willie D, et al. Sorafenib Blocks the RAF/MEK/ERK Pathway, Inhibits Tumor Angiogenesis, and Induces Tumor Cell Apoptosis in Hepatocellular Carcinoma Model PLC/PRF/5. *Cancer Res* (2006) 66(24):11851–8. doi: 10.1158/0008-5472.CAN-06-1377

9. Zhu YJ, Zheng B, Wang HY, Chen L. New Knowledge of the Mechanisms of the RAF/MEK/ERK Pathway, Inhibits Tumor Angiogenesis, and Induces Factor-1alpha Synthesis: Implications for Antiangiogenic Activity in Hepatocellular Carcinoma. *Cancer Treat. Rev* 82 (2019) 101946. doi: 10.1016/j.ejca.2008.10.031

10. Gounder MM, Mahoney MR, Van Tine BA, Ravi V, Attia S, Deshpande HA, et al. Sorafenib for Advanced and Refractory Desmoid Tumors. *N Engl J Med* (2018) 379(25):2417–28. doi: 10.1056/NEJMoa1805055

11. Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, et al. Sorafenib in Radioactive Iodine-Refractory, Locally Advanced or Metastatic Differentiated Thyroid Cancer: A Randomised, Double-Blind, Phase 3 Trial. *Lancet* (2014) 384(9940):319–28. doi: 10.1016/S0140-6736(14)60421-9

12. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Staehler M, et al. Sorafenib for Treatment of Renal Cell Carcinoma: Final Efficacy and Safety Results of the Phase III Treatment Approaches in Renal Cancer Global Evaluation Trial. *J Clin Oncol* (2009) 27(20):3312–8. doi: 10.1200/JCO.2008.19.5511

13. Kim ES, Herbst RS, Wistuba II, Lee JJ, Blumenschein GR Jr, Tsao A, et al. The BATTLE Trial: Personalizing Therapy for Lung Cancer. *Cancer Discov* (2011) 1(1):44–53. doi: 10.1158/2159-8274.CD-10-0101

14. Niu L, Liu L, Yang S, Ren J, Lai PBS, Chen GG. New Insights Into Sorafenib Resistance in Hepatocellular Carcinoma: Responsible Mechanisms and Promising Strategies. *Biochim Biophys Acta Rev Cancer* (2017) 1868 (2):564–70. doi: 10.1016/j.bbcan.2017.10.002

15. Liu LP, Ho RLK, Chen GG, Lai PBS. Sorafenib Inhibits Hypoxia-Inducible Factor-α/Alpha Synthesis: Implications for Antiangiogenic Activity in Hepatocellular Carcinoma. *Clin Cancer Res* (2012) 18(20):5662–71. doi: 10.1158/1078-0432.CCR-12-0552
Zeng et al. Anti-HIFs Improve Sorafenib Drug-Resistance

28. Wu C, Zhang H, Wang Q, Liu J, Cao H. Inhibition of the PI3K/Akt Signaling Pathway Reverses Sorafenib Resistance in Hepatocellular Carcinoma Cells. J Hepatol (2012) 55(4):838–45. doi: 10.1016/j.jhep.2012.10.043

29. Fan Z, Duan J, Wang L, Xiao S, Li L, Yan X, et al. PTK2 Promotes Cancer Stem Cell Traits in Hepatocellular Carcinoma by Activating Wnt/beta-Catenin Signaling. Cancer Lett (2019) 450:132–43. doi: 10.1016/j.canlet.2019.02.040

30. Fernando J, Malfeitton A, Cepeda E, Vilarrasa-Blasi R, Bertran E, Raimondi G, et al. A Mesenchymal-Like Phenotype and Expression of CD44 Predict Lack of Apoptotic Response to Sorafenib in Liver Tumor Cells. Int J Cancer (2015) 136(4):E161–72. doi: 10.1002/ijc.29097

31. Liu LL, Fu D, Ma Y, Shen X-Z. The Power and the Promise of Liver Cancer Stem Cell Markers. Stem Cells Dev (2011) 20(12):2023–30. doi: 10.1089/scd.2011.0012

32. Tian Y, Kuo C-F, Sir D, Wang L, Govindarajan S, Petrovic L, et al. Autophagy Inhibits Oxidative Stress and Tumor Suppressors to Exert Its Dual Effect on Hepatocellular Carcinoma. Cancer Cell (2015) 22(6):305–34. doi: 10.1016/j.ccell.2015.02.011

33. Thorburn A, Thammin DH, Gustafson DL. Autophagy and Cancer Therapy. Cell (2014) 158(5):953–64. doi: 10.1016/j.cell.2014.06.021

34. Luo T, Fu J, Xu A, Su B, Ren Y, Li N, et al. PSMID10/Gankyrin Induces Autophagy to Promote Tumor Progression Through Cytoplasmic Interaction With ATG7 and Nuclear Transactivation of ATG7 Expression. Autophagy (2016) 12(8):1355–71. doi: 10.1002/aut.13440

35. Wei T-W, Shiu C-W, Chen H-L, Liu C-Y, Lin C-S, Chen C-A, et al. Mcl-1-Dependent Activation of Beclin 1 Mediates Autophagic Cell Death Induced by Sorafenib and SC-59 in Hepatocellular Carcinoma Cells. Cell Death Dis (2013) 4:e845. doi: 10.1038/cddis.2013.18

36. Fischer TD, Wang J-H, Vlada A, Kim J-S, Behrens K. Role of Autophagy in Differential Sensitivity of Hepatocarcinoma Cells to Sorafenib. World J Hepatol (2014) 6(10):752–8. doi: 10.4245/wjh.v6.i10.752

37. He C, Dong X, Zhai B, Jiang X, Dong D, Li B, et al. miR-21 Mediates Sorafenib Resistance of Hepatocellular Carcinoma Cells by Inhibiting Autophagy Via the PTEN/Akt Pathway. Oncotarget (2015) 6(30):28867–81. doi: 10.18632/oncotarget.4814

38. Qiu Y, Shan W, Yang J, Jin M, Dai Y, Yang R, et al. Reversal of Sorafenib Resistance in Hepatocellular Carcinoma Cells: Epigenetically Regulated Disruption of 4′-3′-beta-hypoxia-inducible Factor-1 alpha. Cell Death Discovery (2019) 5:120. doi: 10.1038/s41419-019-0200-8

39. Pollutri D, Patriarci C, Marinelli S, Giovannini C, Tombetta E, Giannone FA, et al. The Epigenetically Regulated miR-494 Associates With Stem-Cell Phenotype and Induces Sorafenib Resistance in Hepatocellular Carcinoma. Cell Death Dis (2018) 9(1):4. doi: 10.1038/s41419-017-0076-6

40. Fornari F, Pollutri D, Patriarci C, La Bella TL, Marinelli S, Gardini C, et al. In Hepatocellular Carcinoma Mir-221 Modulates Sorafenib Resistance Through Inhibition of Caspase-3-Mediated Apoptosis. Clin Cancer Res (2017) 23(14):3953–65. doi: 10.1158/1078-0432.CCR-16-1464

41. Liu K, Liu S, Zhang W, Ji B, Wang Y, Liu Y, miR–222 Regulates Sorafenib Resistance and Enhance Tumorogenicity in Hepatocellular Carcinoma. Int J Oncol (2014) 45(4):1537–46. doi: 10.3892/ijc.2014.2577

42. Tang X, Yang W, Shu Z, Shen X, Zhang W, Cen C, et al. MicroRNA223 Promotes Hepatocellular Carcinoma Cell Resistance to Sorafenib by Targeting FBW7. Oncol Rep (2019) 41(2):1231–7. doi: 10.3892/or.2018.6908

43. Dietrich P, Koch A, Fritz V, Hartmann A, Bosserhoff AK, Hellerbrand C. Wild Type Kirsten Rat Sarcoma Is a Novel microRNA-622-Regulated Therapeutic Target for Hepatocellular Carcinoma and Contributes to Sorafenib Resistance. Gut (2018) 67(7):1328–41. doi: 10.1136/gutjnl-2017-315402

44. Zhang M, Zhang H, Hong H, Zhang Z. MiR-374b Re-Sensitizes Hepatocellular Carcinoma Cells to Sorafenib Therapy by Antagonizing PKM2-Mediated Glycolysis Pathway. Am J Cancer Res (2019) 9(4):765–78.
Zeng et al.  Anti-HIFs Improve Sorafenib Drug-Resistance

60. Lu AQ, Lv B, Qiu F, Wang X-Y, Cao X-H. Upregulation of miR-137 Reverses Hepatocellular Carcinoma by Targeting Pae. Med Sci Mont (2019) 25:7209–17. doi: 10.12659/MSM.919219

61. Kabir TD, Ganda C, Brown RM, Beveridge DJ, Richardson KL, Chaturvedi V, et al. MicroRNA-217 Regulates Hepatocellular Carcinoma. Oncol Rep (2017) 37(4):2071–8. doi: 10.3892/or.2017.5498

62. Jiang Z-B, Ma B-Q, Liu S-G, Li J, Yang G-M, Hou Y-B, et al. miR-365 Inhibits Liver Cancer Stem Cell Expansion Via Bcl-2 Pathway. Cell Death Dis (2019) 10:3892/or.2015.4030

63. Yang B, Wang C, Xie H, Wang Y, Huang J, Rong Y, et al. MicroRNA-3163 Targets SerpinB3 and Is Involved in Sorafenib Resistance in Hepatocellular Carcinoma. J Clin Med (2019) 8(2). doi: 10.3390/jcm8020171

64. Li J, Zhao J, Wang H, Li X, Liu A, Qin Q, et al. MicroRNA-140-3p Enhances Sorafenib Resistance in Hepatocellular Carcinoma Cells by Degrading ANT2 in Hepatocellular Carcinoma. Oncol Rep (2017) 37(4):2071–8. doi: 10.3892/or.2017.5498

65. Kabir TD, Ganda C, Brown RM, Beveridge DJ, Richardson KL, Chaturvedi V, et al. MicroRNA-7/9 growth Arrest Specific 6/7TRO3 Axis Regulates the Growth and Invasiveness of Sorafenib-Resistant Cells in Human Hepatocellular Carcinoma. Hepatology (2018) 67(1):216–31. doi: 10.1002/hep.29478

66. Gramantieri L, Pollutri D, Gagliardi M, Giovannini C, Quarta S, Ferracin M, et al. miR-486-3p Mediates Sorafenib Resistance to Hepatocellular Carcinoma Cells by Targeting PTEN and Predicts Sorafenib Resistance in Hepatocellular Carcinoma by Targeting PTEN. Oncol Rep (2015) 34(2):1003–10. doi: 10.3892/or.2015.4030

67. Ji L, Lin Z, Wan Z, Xia S, Jiang S, Cen D, et al. miR-494 Promotes Cell Proliferation, Migration and Invasion, and Increased Sorafenib Resistance in Hepatocellular Carcinoma Cells by Targeting PTEN. Oncol Rep (2015) 34(2):1003–10. doi: 10.3892/or.2015.4030

68. Wang H, Tang Y, Yang D, Zheng L. MicroRNA-591 Functions as a Tumor Suppressor in Hepatocellular Carcinoma. Cell Death Dis (2018) 9(3):312. doi: 10.12659/419-018-0344-0

69. Yang B, Wang C, Xie H, Wang Y, Huang J, Rong Y, et al. MicroRNA-3163 Targets ADAM-17 and Enhances the Sensitivity of Hepatocellular Carcinoma Cells to Molecular Targeted Agents. Cell Death Dis (2019) 10(10):784. doi: 10.3892/cd.2019-2023-1

70. Li J, Zhao J, Wang H, Li X, Liu A, Qin Q, et al. MicroRNA-140-3p Enhances the Sensitivity of Hepatocellular Carcinoma Cells to Sorafenib by Targeting Pregnenolone X Receptor. Onco Targets Ther (2018) 11:5885–94. doi: 10.2147/OTT.S179509

71. Grampietri L, Pollutri D, Gagliardi M, Giovannini C, Quarta S, Ferracin M, et al. MiR-30e-3p Influences Tumor Phenotype Through MDM2/TP53 Axis and Predicts Sorafenib Resistance in Hepatocellular Carcinoma. Cancer Res (2020) 80(8):1720–34. doi: 10.1158/0008-5472.CAN-19-0472

72. Jiang XM, Yu XN, Liu TT, Zhu HR, Shi X, Bilesakihan E, et al. microRNA-19a-3p Promotes Tumor Metastasis and Chemoresistance Through the PTEN/Akt Pathway in Hepatocellular Carcinoma. BioMed Pharmacother (2018) 105:1147–54. doi: 10.1016/j.biopha.2018.06.097

73. Li L, Lin Z, Wu Z, Xia S, Jiang S, Cen D, et al. miR-486-3p Mediates Hepatocellular Carcinoma Sorafenib Resistance by Targeting FGFR4 and EGFR. Cell Death Dis (2020) 11(4):250. doi: 10.12659/419-020-2413-4

74. Wang H, Tang Y, Yang D, Zheng L. MicroRNA-591 Functions as a Tumor Suppressor in Hepatocellular Carcinoma by Lowering Drug Resistance Through Inhibition of Far-Upstream Element-Binding Protein 2-Mediated Phosphoinositide 3-Kinase/Akt/Mammalian Target of Rapamycin Axis. Pharmacology (2019) 104(3-4):173–86. doi: 10.1159/000501162

75. Lan RZ, Chen J, Cui LJ, Lin XL, Fan MM, Gong ZZ, et al. miR-194 Inhibits Liver Cancer Stem Cell Expansion by Regulating RAC1 Pathway. Exp Cell Res (2019) 378(1):66–75. doi: 10.1016/j.yexcr.2019.03.007

76. Li B, Liu D, Yang P, Li HY, Wang D, miR-613 Inhibits Liver Cancer Stem Cell Expansion by Regulating SOX9 Pathway. Gene (2019) 707:78–85. doi: 10.1016/j.gene.2019.05.015

77. Liang Z-B, Ma B-Q, Liu S-G, Li J, Yang G-M, Hou Y-B, et al. miR-365 Regulates Liver Cancer Stem Cells Via RAC1 Pathway. Mol Carcinog (2019) 58(1):53–65. doi: 10.1002/mc.22906

78. Song S, Sun K, Dong J, Zhao Y, Liu F, Liu H, et al. microRNA-29a Regulates Liver Tumor-Initiating Cells Expansion Via Bcl-2 Pathway. Exp Cell Res (2020) 387(2):111781. doi: 10.1016/j.yexcr.2019.111781

79. Yang F, Li Q-J, Gong Z-B, Zhou L, You N, Wang S, et al. MicroRNA-34a Targets Bcl-2 and Sensitizes Human Hepatocellular Carcinoma Cells to Sorafenib Treatment. Technol Cancer Res Treat (2014) 13(1):77–86. doi: 10.7785/tcrt.2012.500364
in Hepatocellular Carcinoma. *Mol Ther Nucleic Acids* (2019) 16:576–88. doi: 10.1016/j.mtna.2019.04.008

93. Cheng Z, Lei Z, Yang P, Sun L, Xiang D, Zhou J, et al. Long Non-Coding RNA THOR Promotes Liver Cancer Stem Cells Expansion Via Beta-Catenin Pathway. *Gene* (2019) 684:95–103. doi: 10.1016/j.gene.2018.10.051

94. Tang S, Tan G, Jiang X, Han P, Zhai B, Dong X, et al. An Artificial IncRNA Targeting Multiple miRNAs Overcomes Sorafenib Resistance in Hepatocellular Carcinoma Cells. *Oncotarget* (2016) 7(45):73257–69. doi: 10.18632/oncotarget.12304

95. Quagliata L, Quintavalle C, Matter MS, Novello MS, di Tommaso C, Pressiani L, et al. AMPK Is a Mediator of Glycolytic Flux in Cancer Progression. *Crit Rev Oncol Hematol* (2014) 92(3):312–21. doi: 10.1016/j.crirevonc.2014.05.007

96. Schultheiss CS, Laggai S, Czepukojc B, Hussein UK, List M, Barghash A, et al. The Long Non-Coding RNA H19 Suppresses Carcinogenesis and Chemoresistance in Hepatocellular Carcinoma. *Cell Stress* (2017) 1(1):37–54. doi: 10.15698/cst2017.10.105

97. Wilson GK, Tennant DA, McKeating JA. Hypoxia Inducible Factors in Liver Tumors. *FASEB J* (2008) 22(9):2267–80. doi: 10.1096/fj.08-138113

98. Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, et al. The Long Non-Coding RNA TUC338 Is Functionally Involved in Sorafenib-Sensitized Hepatocarcinoma Cells by Targeting RASAL1. *Oncol Rep* (2017) 37(1):275–80. doi: 10.3892/or.2016.5248

99. Shi Y, Yang X, Xue X, Sun D, Cai P, Song Q, et al. HnrNp Enhances Autophagy-Associated Sorafenib Resistance Through MIR-29b/ATG9A Axis in Hepatocellular Carcinoma. *Oncology Targets Ther* (2020) 13:2127–37. doi: 10.2147/OTT.S229913

100. Zeng et al. Anti-HIFs Improve Sorafenib Drug-Resistance in Hepatocellular Carcinoma Stem Cells Induced by Long-Term Treatment With Sorafenib. *Mol Oncol* (2019) 13(5):3111–31. doi: 10.1002/1878-0261.12488

101. Mendez-Blanco C, Fondevila F, Fernandez-Palanca P, Garcia–Palomo A, van Pelt J, Verslype C, et al. Stabilization of Hypoxia-Inducible Factors and Regulatory RNAs Promotes Methylation Contribution to Acquired Sorafenib Resistance in Human Hepatocellular Carcinoma Cells. *Cancers (Basel)* (2019) 11(12). doi: 10.3390/cancers11121984

102. Song Z, Liu T, Chen J, Ge C, Zhao F, Zhu M, et al. HIF-1alpha-Induced RTI-3 Changes Hepatocellular Carcinoma Cell Sensitivity to Sorafenib by Activating the PI3K/Akt Signaling Pathway. *Cancer Manag Res* (2020) 12:4321–33. doi: 10.2147/CMA.S43918

103. Zhu M, Yin F, Fan X, Jing W, Chen R, Liu L, et al. Decreased TIP30 Promotes Snail-Mediated Epithelial-Mesenchymal Transition and Tumor-Initiating Properties in Hepatocellular Carcinoma. *Oncogene* (2013) 34(19):2410–21. doi: 10.1038/onc.2014.73

104. Zhang W, Sun HC, Wang WQ, Zhang QB, Zhuang YQ, et al. Sorafenib Down-Regulates Expression of HTATIP2 to Promote Invasiveness and Metastasis of Orthotopic Hepatocellular Carcinoma Tumors in Mice. *Gastroenterology* (2012) 143(6):1641–1649.e5. doi: 10.1053/j.gastro.2012.08.032

105. You A, Cao M, Guo Z, Zuo B, Gao J, Zhou H, et al. Metformin Sensitizes Sorafenib To Inhibit Postoperative Recurrence and Metastasis of Hepatocellular Carcinoma in Orthotopic Mouse Models. *J Hematol Oncol* (2016) 9:96. doi: 10.1186/s13045-016-0255-6

106. Xiong XX, Qiu XY, Hu DX, Chen XQ. Advances in Hypoxia-Mediated Mechanisms in Hepatocellular Carcinoma. *Mol Pharmacol* (2017) 92(3):246–55. doi: 10.1124/ml.116.107706

107. Murugesan T, Rajayajabalachandran G, Kumar S, Nagaraju S, Jegathesan SK. Targeting HIF-2alpha as Therapy for Advanced Cancers. *Drug Discov Today* (2018) 23(7):1443–51. doi: 10.1016/j.drudis.2018.05.003

108. Al Hasawi N, Alkandari MF, Luqmani YA. Phosphofructokinase: A Mediator of Glycolytic Flux in Cancer Progression. *Crit Rev Oncol Hematol* (2014) 92(3):312–21. doi: 10.1016/j.critrevonc.2014.05.007

109. Semenza GL. Targeting HIF-1 for Cancer Therapy. *Nat Rev Cancer* (2003) 3:10(2):721–32. doi: 10.1038/nrc1187

110. Zhao D, Zhai B, He C, Tan G, Jiang X, Pan X, et al. Upregulation of HIF-2alpha Induced by Sorafenib Contributes to the Resistance by Activating the TGFB-alpha/EGFR Pathway in Hepatocellular Carcinoma Cells. *Cell Signal* (2014) 26(5):1030–9. doi: 10.1016/j.cellsig.2014.01.026

111. Xu M, Zheng YL, Xie XY, Liang JY, Pan FS, Zheng SG, et al. Sorafenib Blocks the HIF-1alpha/VEGFA Pathway, Inhibits Tumor Invasion, and Induces Apoptosis in Hepatoma Cells. *DNA Cell Biol* (2014) 33(5):275–81. doi: 10.1089/dna.2013.2184

112. Ma L, Li G, Zhu H, Dong X, Zhao D, Jiang X, et al. 2-Methoxyestradiol Synergizes With Sorafenib to Suppress Hepatocellular Carcinoma by Simultaneously Dysregulating Hypoxia-Inducible Factor-1 and -2. *Cancer Lett* (2014) 355(1):96–105. doi: 10.1016/j.canlet.2014.09.011

113. Bort A, Spinola E, Rodriguez–Hench N, Divaz–Laviada I. Capsaicin Exerts Synergistic Antitumor Effect With Sorafenib in Hepatocellular Carcinoma Cells Through AMPK Activation. *Oncotarget* (2017) 8(30):28764–8. doi: 10.18632/oncotarget.21196

114. Fueht B, Boly G, Izevez S, Griss T, Samborska B, Dong Z, et al. AMPK Is a Negative Regulator of the Warburg Effect and Suppresses Tumor Growth In Vivo. *Cell Metab* (2013) 17(1):113–24. doi: 10.1016/j.cmet.2012.12.001

115. Bort A, Sanchez BG, Mateos–Gomez A, Vara–Ciruelos D, Rodriguez–Hench N, Diaz–Laviada I. Targeting AMP-activated Kinase Impacts Hepatocellular Cancer Stem Cells Induced by Long-Term Treatment With Sorafenib. *Mol Oncol* (2019) 13(5):3111–31. doi: 10.1002/1878-0261.12488
129. Liang Y, Zheng T, Song R, Wang J, Yin D, Wang L, et al. Hypoxia-Mediated Sorafenib Resistance can be Overcome by EF24 Through Von Hippel-Lindau Tumor Suppressor-Dependent HIF-1alpha Inhibition in Hepatocellular Carcinoma. *Hepatology* (2013) 57(5):1847–57. doi: 10.1002/hep.26224

130. Lin S, Hoffmann K, Gao C, Petrlutionis M, Herr I, Schemmer P. Melatonin Promotes Sorafenib-Induced Apoptosis Through Synergistic Activation of JNK/c-Jun Pathway in Human Hepatocellular Carcinoma. *J Pineal Res* (2017) 62(3). doi: 10.1111/jpi.12398

131. Kudo M. Systemic Therapy for Hepatocellular Carcinoma: Latest Advances. *Cancers (Basel)* (2018) 10(11):412. doi: 10.3390/cancers10110412

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Zeng, Lu, Zhao, Zhang, Hu, Shi, Tu, Xiao, Xu and Huang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.