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# Roles and Mechanisms of Ferroptosis in Sorafenib Resistance for Hepatocellular Carcinoma

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**Abstract:** Hepatocellular carcinoma (HCC) is the most prevalent malignant tumor, characterized by a poor prognosis. In recent decades, both the incidence and mortality rates of HCC have risen sharply. Sorafenib has emerged as the first conventional drug approved by the US Food and Drug Administration for first-line treatment in advanced HCC patients due to its favorable safety profile. However, its effectiveness is severely hindered by acquired drug resistance, which leads to only approximately 30% of HCC patients benefited from sorafenib therapy. Sorafenib resistance involves various mechanisms that inhibit cellular uptake of iron and reactive oxygen species (ROS). Consequently, ferroptosis a novel form of cell death contingent upon the accumulation of intracellular iron and ROS plays a critical role in mediating sorafenib resistance through the Hippo YAP pathway or Keap1-Nrf2 system. This review aimed to comprehensively elucidate the mechanisms underlying sorafenib resistance in HCC, particularly focusing on ferroptosis and its pathways, to provide valuable insights into targeting ferroptosis or its pathways for sorafenib-resistant HCC treatment.

**Keywords:** ferroptosis, sorafenib resistance, hepatocellular carcinoma, molecular mechanism

## Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of global cancer-related deaths, often occurring in patients with chronic liver inflammation associated with viral infections, alcohol abuse, or metabolic syndrome.[1,2] Significant progress has been made in the prevention, diagnosis, and treatment of HCC. However, over 50% of HCC patients are diagnosed at an advanced stage (Barcelona Clinic Liver Cancer B stage or higher), with 70% experiencing recurrence within the first five years of initial treatment. Early-stage HCC is typically resectable, whereas late-stage HCC often requires systemic treatment with sorafenib in addition to local therapies such as ablation, transarterial chemoembolization, or external irradiation.[3,4]

In a groundbreaking study, sorafenib, a multi-targeted tyrosine kinase inhibitor, demonstrated anti-angiogenic and anti-proliferative effects, prolonging the overall median survival of advanced HCC patients. Sorafenib inhibits tumor cell proliferation by targeting Raf-1, B-Raf, and kinase activity in the Ras/Raf/MEK/ERK signaling pathway.[5] Moreover, sorafenib targets platelet-derived growth factor receptor (PDGFR-β), vascular endothelial growth factor receptor 2 (VEGFR2), hepatocyte cytokine receptor (c-KIT), and other proteins to inhibit tumor angiogenesis.[6] In the Asia-Pacific and Sorafenib HCC Assessment Randomized Protocol (SHARP) trials, sorafenib significantly improved the prognosis of advanced HCC patients, initiating a robust period of clinical research.[7,8]

However, only about 30% of HCC patients benefited from sorafenib, and this group typically developed resistance within six months. Adverse events observed in patients taking sorafenib primarily included gastrointestinal, systemic, and dermatologic conditions. In severe cases, sorafenib could cause hypertension and abdominal pain, leading to treatment

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cessation.[9] Therefore, the search for sorafenib alternatives is emerging. Since 2017, a large Phase III trial has shown that lenvatinib is non-inferior to sorafenib for first-line treatment. Additionally, regorafenib, cabozantinib, and ramucirumab have been approved as second-line treatment options following sorafenib.[10–13] Results from the IMbrave150 study (NCT03434379) indicate that the combination of atezolizumab and bevacizumab may broaden treatment options for first-line therapy in HCC.[14–16]

Though sorafenib and its alternatives remains the cornerstone of HCC treatment, their effectiveness is severely hindered by acquired drug resistance. Therefore, elucidating the resistance mechanisms of sorafenib in HCC is crucial. Ferroptosis refers to a form of non-apoptotic regulated cell death involving abnormal metabolism of lipid oxidation products catalyzed by iron ions or iron enzymes. Increasingly, studies indicate a complex relationship between ferroptosis and sorafenib resistance, suggesting ferroptosis as a potential novel treatment for sorafenib-resistant HCC.

## Resistance Mechanisms of Sorafenib in HCC

Research has demonstrated that multiple factors significantly contribute to the initiation and progression of sorafenib resistance in HCC. These include epigenetic modifications (such as non-coding RNAs and DNA methylation), transport mechanisms (including ABC transporters and exosomes), the tumor microenvironment (characterized by hypoxic conditions, alterations in the immune landscape, and viral reactivation), and regulated forms of cell death (notably autophagy and ferroptosis).

### Epigenetics

Epigenetic modifications play a pivotal role in modulating gene expression without altering the underlying DNA sequence. Non-coding RNAs can regulate critical signaling pathways involved in cell survival, proliferation, and apoptosis, thereby influencing cancer cell responses to therapeutic agents such as sorafenib. Similarly, patterns of DNA methylation can silence tumor suppressor genes or activate oncogenes, complicating treatment outcomes.

Increasing evidence indicates that non-coding RNAs, including long noncoding RNAs (lncRNAs) and microRNAs (miRNAs), are critical for the development of sorafenib resistance in HCC.[17] Small nucleolar RNA host gene 3 (SNHG3) and SNHG16 can regulate sorafenib resistance in various HCC cell lines through over-expression or inhibition of miR-128/CD151,[18] miR-140-5p,[19] miR-591,[20] and miR-622.[21] Hsa_circ_0088036 promoted sorafenib resistance of HCC by activating the PI3K/Akt and Notch pathways through regulating miR-140-3p/KIF2A signaling.[22] The absence of miR-936 could increase the expression of Pim-3 to enhance sorafenib resistance of HCC by inhibiting cell ferroptosis.[23] Current research primarily examines the role of lncRNAs as miRNA sponges in sorafenib resistance associated with HCC; however, their additional mechanisms and the urgent need for clinical trials to investigate ncRNA-based therapeutic strategies require further exploration.[17]

Moreover, DNA methylation profoundly impacts gene expression modifications associated with sorafenib resistance in HCC. This encompasses the differential methylation patterns of the MORC2-NF2/KIBRA axis,[24] alterations in the methylation profiles of oncogenes and tumor suppressor genes,[25] as well as the prospective targeting of lncRNA H19 to alleviate this resistance.[17,26] Ten-eleven translocation protein 1 (TET1) and Yes-associated protein 1 (YAP1) synergistically regulate the promoter methylation and gene expression of DNA repair-related genes in sorafenib-resistant HCC cells.[27] HDAC11 overexpression, resulted from its promoter hypomethylation and miR-145-5p downregulation, is found to enhance sorafenib resistance of HCC cells.[28]

All these studies indicate significant effects of epigenetics in HCC cells to elucidate the molecular mechanisms of sorafenib resistance, which will provide diagnostic markers and develop new tumor-specific inhibitors for HCC.

### Transport Processes

Transport mechanisms are equally significant; ABC transporters facilitate the efflux of chemotherapeutic agents from cancer cells, diminishing their intracellular concentrations and efficacy. Exosomes serve as vehicles for intercellular communication within the tumor microenvironment, carrying proteins or RNA molecules that promote drug resistance by modifying the behavior of neighboring cells.

Sorafenib resistance is mediated by ATP-binding cassette (ABC) transporters, which reduce the efficacy of chemotherapy by extruding the drug from cancer cells, adversely affecting the outcomes of anti-cancer therapies. Research has identified a subfamily derived from human HCC cells that exhibits increased multidrug resistance (MDR) associated with the upregulation of multidrug resistance proteins 1 (MRP1) and 2 (MRP2), resulting from ABC transporter inhibition.[29]

Exosomes are essential for cellular communication and serve as therapeutic targets by modulating lncRNA-VLDLR upregulation in drug-resistant tumors, thereby affecting the efficacy of chemotherapeutic agents like sorafenib.[30] Additionally, miR-122-transfected adipose tissue-derived mesenchymal stem cells (AMSCs) encapsulate miR-122 in secreted exosomes, potentially facilitating interactions with HCC cells and altering the expression of miR-122 target genes to enhance their sensitivity to sorafenib.[31]

## Tumor Microenvironment

The tumor microenvironment is a dynamic entity that profoundly influences cancer progression. Hypoxic conditions often induce metabolic adaptations in tumors, enhancing their survival under low oxygen levels while promoting angiogenesis through factors such as HIF-1α. The immune landscape within tumors may also be altered due to immunosuppressive signals emanating from both cancer cells and stromal components, which can impede effective anti-tumor immunity.

HCC promotes angiogenesis via a vascular endothelial growth factor (VEGF)-dependent mechanism and HIF-1α activation. Prolonged sorafenib treatment inhibits anti-angiogenic activity in the tumor microenvironment, leading to hypoxia that selects for drug-resistant cell clones adapted to low oxygen and nutrient levels. These resistant cells are activated by HIF-1α and NF-κB, compromising sorafenib efficacy.[32] Additionally, it has been documented that sorafenib facilitates viral reactivation by markedly diminishing the population of natural killer (NK) cells and compromising their responsiveness to HCC cells.[33]

## Ferroptosis

In recent years, research on ferroptosis in cancer has rapidly expanded, underscoring its potential role in anticancer therapies. Ferroptosis is characterized by iron-dependent lipid peroxidation and is recognized as a distinct form of regulated cell death, differentiating it from apoptosis and necrosis. This unique mechanism offers new avenues for therapeutic intervention, particularly in challenging malignancies such as HCC.

Research has revealed that ferroptosis is primarily regulated by the cysteine glutamate reverse transporter (System Xc-) and glutathione peroxidase 4 (GPX4).[34,35] System Xc- is a membrane-bound, $Na^+$-dependent cysteine-glutamate exchange transporter composed of a light chain subunit (xCT, SLC7A11) and a heavy chain subunit (CD98hc, SLC3A2), linked by disulfide bridges. It exchanges intracellular glutamate for extracellular cysteine, converting it into cysteine to synthesize the antioxidant glutathione (GSH). The common inducer of ferroptosis, erastin and subsequent discussed stem from sorafenib's inhibition of System Xc-, which results in the accumulation of intracellular ROS, thereby initiating ferroptosis. GPX4 is recognized as a protein enzyme that suppresses lipid peroxidation. It degrades hydrogen peroxide and other common small molecule peroxides, as well as complex lipid peroxides, with GSH serving as an essential cofactor in its activation. Typically, GPX4 prevents ferroptosis by inhibiting the accumulation of intracellular lipid peroxides; when GPX4 is inhibited, it can lead to the accumulation of intracellular ROS, thus initiating ferroptosis (Figure 1). Therefore, two key molecules in the ferroptosis pathway are xCT and GPX4.[36,37] Inhibiting the xCT system and GPX4 may help eliminate cancer cells resistant to conventional chemotherapy or radiotherapy.[38]

Changes in mitochondrial metabolism, including mitochondrial stress responses, metabolic reprogramming, and mitochondrial protease abnormalities, are highly associated with HCC occurrence and metastasis.[39] The primary role of mitochondria is to supply energy to cells through oxidative phosphorylation (OXPHOS).[40] Mitochondrial defects can lead to OXPHOS damage, mitochondrial dysfunction, and increased ROS production. Given that a significant cytological hallmark of ferroptosis is changes in mitochondrial morphology,[41] and ROS are major byproducts of ferroptosis,[42] it is speculated that ferroptosis also significantly impacts the progression of HCC and its drug resistance. Sorafenib induces ferroptosis, and ferroptosis promotes sorafenib resistance, complicating the understanding of sorafenib's antitumor

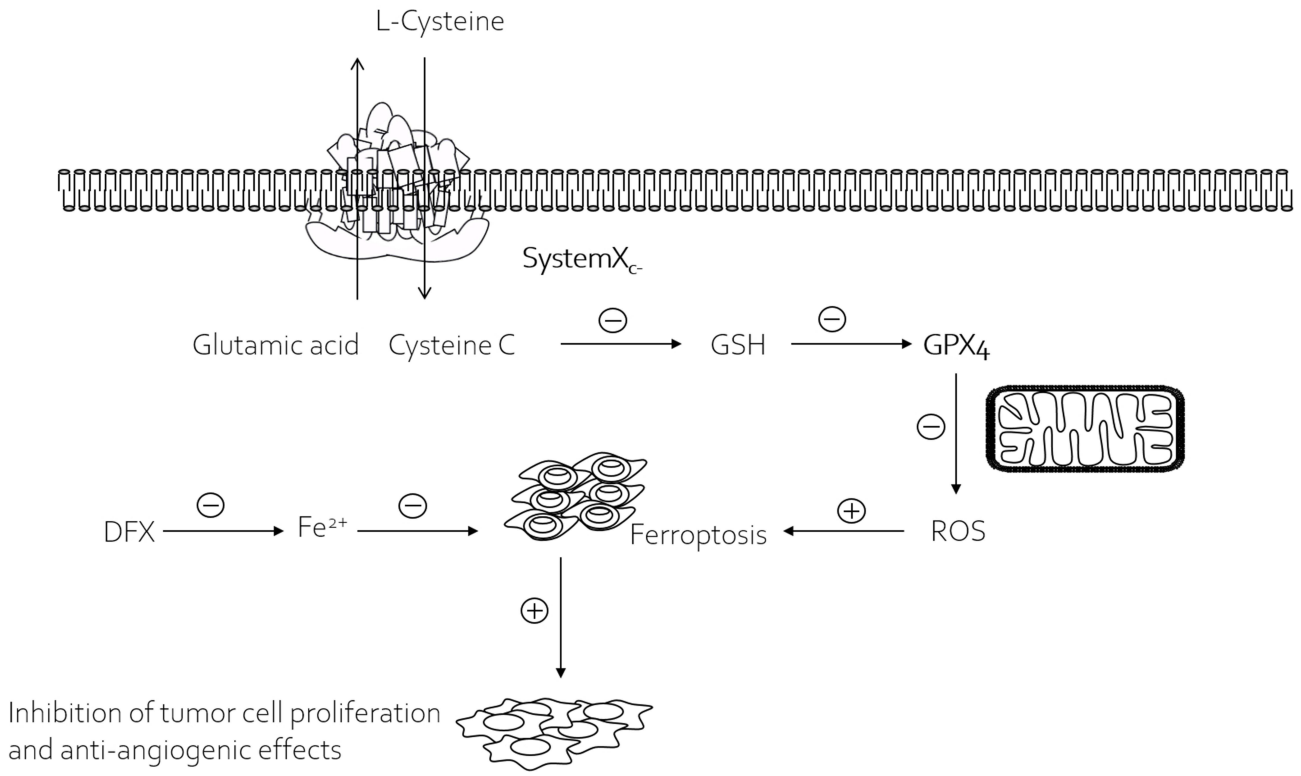


**Figure 1** Fundamental mechanisms of ferroptosis.
**Abbreviations**: GSH, glutathione; GPX4, glutathione peroxidase 4; DFX, deferoxamine; ROS, reactive oxygen species.

mechanisms in HCC. Recent research suggests that the depletion of intracellular iron reserves, achieved through the iron chelating agent deferoxamine (DFX), significantly protects HCC cells from the cytotoxic effects of sorafenib. Depletion of cellular iron reserves prevents sorafenib from inducing oxidative stress in HCC cells (Figure 1).[43]

Furthermore, Lachaier et al reported sorafenib-induced ferroptosis in various cancer cell lines.[44] Compared to other kinase inhibitors, sorafenib is the only drug demonstrating efficacy in inducing ferroptosis. From a mechanistic perspective, Sun et al found that metallothionein-1G (MT-1G) acts as a key modulator of human HCC sorafenib resistance and a promising therapeutic target.[45] The activation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), rather than p53 and HIF-1α, is crucial for sorafenib-induced MT-1G expression post-treatment. The molecular mechanism of MT-1G in sorafenib resistance involves the inhibition of ferroptosis. RNA interference or knocking down MT-1G increases glutathione consumption and lipid peroxidation, resulting in sorafenib-induced ferroptosis. According to another study, primary human hepatic stellate cells (HSCs) in collected human liver tissues exhibited upregulation of ELAV-like RNA binding protein 1 (ELAVL1), activation of ferritin autophagy, and induction of ferroptosis.[46] In conclusion, sorafenib-induced ferroptosis may be an effective mechanism for inducing cell death in HCC.

The mechanism by which sorafenib induces ferroptosis in HCC also depends on the retinoblastoma (Rb) gene status. By silencing the Rb gene in HCC using short hairpin RNA, the ferroptosis and tumor-suppressive effects of sorafenib can be significantly enhanced. Further research has confirmed that these effects are associated with the exposure of mitochondrial respiratory chains to sorafenib, leading to the production of significant amounts of ROS.[47]

## Ferroptosis Pathways Associated with HCC Sorafenib Resistance

### Hippo YAP Pathway

The Hippo pathway has been identified as a critical regulator of tissue growth, responsible for controlling transcription processes and managing cell proliferation, differentiation, and migration within developing organs.[48] Disruption of the Hippo pathway can lead to abnormal cell growth and tumor formation.[49]

YAP/TAZ, as transcriptional co-activators of the Hippo signaling pathway, not only promote tissue growth and regulate cell dynamics, but also participate in various pathophysiological processes by modulating the activity of TEAD and SMAD.[50] In most solid tumors, YAP/TAZ is crucial for tumor occurrence and growth. The activation of YAP/TAZ can induce the production, proliferation, drug resistance, and metastasis of cancer stem cells.[51]

Recent studies have indicated that YAP/TAZ serves as a novel regulatory factor for the expression of the solute carrier family 7 member 11 (SLC7A11). In sorafenib-sensitive HCC cells, YAP/TAZ and ATF4 are not activated and are not localized in the nucleus, thereby failing to initiate the expression of SLC7A11 or elevate intracellular glutathione levels. However, in sorafenib-resistant liver cancer cells, YAP/TAZ and ATF4 are activated in the nucleus, where YAP or TAZ bind to the SLC7A11 promoter DNA segment containing a TEAD binding motif, inducing the expression of SLC7A11, increasing intracellular GSH levels, and reducing ROS levels. This process inhibits ferroptosis in HCC cells and implies their resistance to sorafenib treatment. Recent histological studies exploring the expression and localization of YAP using immunohistochemistry have shown that total YAP and nuclear YAP staining are more prevalent in HCC tissues than in non-tumor areas.[52] Additionally, YAP/TAZ maintains the stability, nuclear localization, and transcriptional activity of the ATF4 protein, aiding in the cooperative induction of SLC7A11 protein expression (Figure 2).[53]

## LIFR-NF-κB-LCN2 Pathway

Leukemia inhibitory factor (LIF) is the most pleiotropic member of the IL-6 cytokine family, exhibiting broad activity.[54] The LIF receptor (LIFR) is present in various organs and tissues, and is expressed in both fetal and adult hepatic cells.[55]

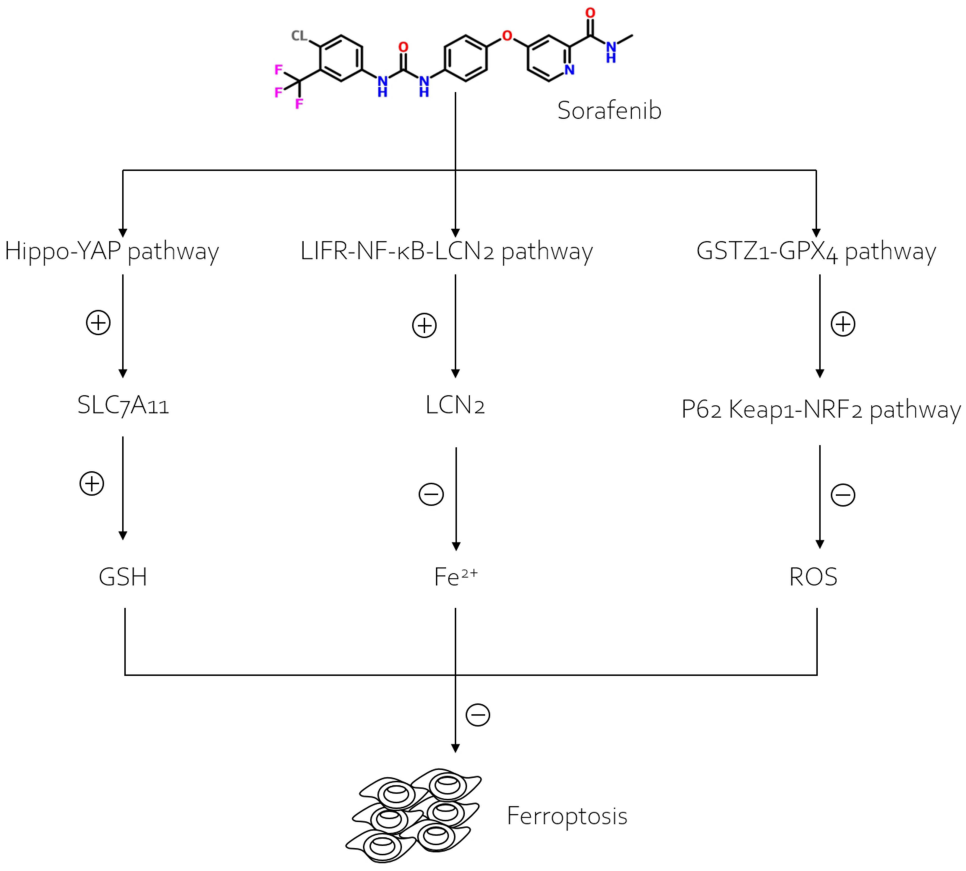


**Figure 2** Mechanisms of sorafenib resistance across Hippo YAP pathway, LIFR-NF-κB-LCN2 pathway, and GSTZ1-GPX4 pathway.
**Abbreviations**: YAP, yes-associated protein; LIFR, leukemia inhibitory factor receptor; LCN2, lipocalin 2; GSTZ1, glutathione S-transferase zeta 1; GPX4, glutathione peroxidase 4; SLC7A11, solute carrier family 7 member 11; Keap1, kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid 2-related factor 2; GSH, glutathione; ROS, reactive oxygen species.

Lipocalin 2 (LCN2) is an innate immune protein,[56] that has been observed to be highly expressed in various diseases, including nasopharyngeal carcinoma, neurodegenerative diseases, glioblastoma, and liver diseases.[57–60] LCN2 does not directly bind to iron but interacts with it as a helper factor by forming a ternary complex with iron transport proteins, playing an important role in iron homeostasis and inflammation.[60]

LIFR serves as a negative regulator of the hepatic LCN2-NF-κB signaling pathway.[61] According to studies, decreased LIFR expression in HCC cell lines may be associated with DNA methylation. LIFR deficiency activates the NF-κB signaling pathway via src homology 2 (SH2) domain-containing phosphatase 1 (SHP1), resulting in increased LCN2 expression, reduced intracellular iron entry, and decreased sensitivity to iron-induced cell death, thereby promoting liver tumor formation. Tumors may exhibit increased sensitivity to radiation and immunotherapy when neutralizing LCN2 antibodies are applied. High LIFR expression levels and low LCN2 expression levels can be used to estimate the efficacy of sorafenib, while low LIFR expression levels and high LCN2 expression levels can help identify HCC patients who may benefit from sorafenib monotherapy or combination therapy.

In conclusion, these studies provide a new direction for targeting ferroptosis optimization in HCC cancer treatment strategies (Figure 2).

## Keap1-Nrf2 System

Throughout evolution, mammals have developed sophisticated cryoprotective mechanisms to safeguard cells against oxidative stress and exogenous substances. The kelch-like ECH-associated protein 1 (Keap1)-Nrf2 pathway is one of the most crucial mechanisms in this regard. Nrf2 acts as a potent transcriptional activator, regulating the expression of detoxifying enzymes and genes encoding antioxidant proteins by binding to antioxidant response elements (ARE) and electrophile response elements (EpRE). Keap1 is a component of the cullin 3 (CUL3)-based E3 ubiquitin ligase complex, responsible for maintaining Nrf2 stability and promoting its accumulation.

Previous studies have elucidated the complex molecular mechanisms underlying Nrf2 activation in response to stress and its connection to numerous human diseases (Figure 2). Activation of Nrf2 via the cystathionase pathway is essential for sorafenib-induced MT-1G expression in HCC cells. Genetic and pharmacological inhibition of MT-1G expression enhances the antitumor sensitivity of sorafenib in thses cells.[44] Nrf2-regulated genes such as oxidoreductase-1 (NQO1), heme oxygenase-1 (HO-1), and ferritin heavy chain 1 (FTH1) can confer ferroptosis resistance by modifying iron metabolism and lipid peroxidation. Recent research have highlighted that the p62-Keap1-Nrf2 axis is a central pathway in inhibiting ferroptosis. Upregulation of MT-1G, quinone, NQO1, HO-1, and FTH1, ABCC5, and other downstream Nrf2 target genes can induce resistance to sorafenib by impeding the development of intracellular ferroptosis.[62] Suppression of Nrf2 expression leads to increased sorafenib-induced depletion of glutathione (GSH) in HCC cells, rendering the cancer more sensitive to sorafenib through the induction of GSH depletion and ferroptosis in vivo.[63]

## Ferroptosis

Viral reactivation particularly in cases involving hepatitis viruses can exacerbate liver inflammation while altering cellular signaling pathways associated with drug metabolism or response. Regulated forms of cell death such as autophagy enable cancer cells to survive under stress conditions by recycling cellular components for energy production. Conversely, ferroptosis refers to a form of non-apoptotic regulated cell death involving abnormal metabolism of lipid oxidation products catalyzed by iron ions or iron enzymes. Various inducers disrupt cellular redox balance, generating large amounts of lipid peroxidation products, thereby triggering cell death. Increasingly, studies indicate a complex relationship between ferroptosis and cancer, suggesting ferroptosis as a potential novel cancer treatment method.[64]

### Nrf2-MT-1G

Metallothioneins (MT) are a class of low molecular weight proteins characterized by high cysteine levels (15–30%), provides them with optimal metal coordination capabilities.[65,66] They play a crucial role in heavy metal detoxification and antioxidant activities. Mammalian MT consists of four main members: MT-1, MT-2, MT-3, and MT-4, with MT-1 and MT-2 being the most widely expressed. While MT-2, MT-3, and MT-4 are encoded by a single gene, MT-1 comprises

various subtypes encoded by 13 genes, including active genes such as MT-1A, MT-1B, MT-1E, MT-1F, MT-1G, MT-1H, MT-1M, and MT-1X.[67]

In the presence of sorafenib, the expression of MT-1G is significantly upregulated in HCC cells, whereas other MT types remain unaffected. This upregulation of MT-1G is mediated by the transcription factor Nrf2, rather than p53 or HIF-1α. Nrf2 is a critical regulator of cellular antioxidant responses, directing the expression of genes involved in antioxidant defense and electrophile stress, thereby neutralizing ROS and restoring cellular redox balance.[68] Nrf2 plays a crucial role in protecting HCC cells from ferroptosis. The upregulation of MT-1G can induce resistance to sorafenib in these cells by inhibiting lipid peroxidation without altering intracellular iron levels. Conversely, blocking MT-1G expression can enhance the antitumor activity of sorafenib by inducing ferroptosis both in vitro and in vivo. Sorafenib-induced ferroptosis is inhibited not only by the suppression of MT-1G but also by the upregulation of other Nrf2 target genes such as NQO1, HO-1, and FTH1.[44]

### Nrf2-ABCC5

ABC transporters are a class of membrane proteins that facilitate various ATP-controlled transport processes, playing a crucial role in the transmembrane transportation of substances.[69] Based on the arrangement of molecular structural elements, including the nucleotide-binding domain and transmembrane domain topology, human ABC proteins can be classified into seven subfamilies (A-G). The C subfamily contains 13 members, among which the ABCC5 gene is frequently upregulated in various cancers, including breast, esophageal, head and neck, renal, liver, and lung cancers, and has been implicated in cancer progression.[70]

A 2015 study suggested that ABCC5 may influence the metabolism of endogenous metabolites, toxins, and drugs.[71] Research using Huh-7, HepG2, and SK-Hep-1 cell lines indicates that prolonged exposure to sorafenib activates the PI3K/AKT/Nrf2 pathway in HCC, which is essential for the sorafenib-induced expression of ABCC5. High ABCC5 expression downregulates ferroptosis by stabilizing the SLC7A11 protein, reducing GPX4 consumption, inhibiting lipid peroxidation, and increasing mitochondrial membrane potential (MMP). These mechanisms collectively contribute to the development of sorafenib resistance in HCC cells. Conversely, blocking ABCC5 expression significantly enhances the in vivo anti-tumor activity of sorafenib by inducing ferroptosis both in vitro and in vivo. Therefore, modulating ABCC5 expression and inducing ferroptosis present a promising therapeutic strategy to overcome acquired sorafenib resistance in HCC cells (Figure 3).

### GSTZ1-GPX4 Pathway

Glutathione S-transferase zeta 1 (GSTZ1), also known as maleylacetoacetate isomerase (MAAI), is part of the glutathione S-transferase (GST) superfamily.[72] GSTZ1 deficiency leads to oxidative stress, which activates the Keap1/Nrf2/GPX4 signaling pathway, promoting the progression of HCC.[73]

Recent studies have shown that downregulation of GSTZ1 expression in sorafenib-resistant liver cancer cells can inhibit sorafenib-induced cell death by activating the Nrf2 pathway. This results in increased expression levels of genes associated with ferroptosis, such as GPX4, SLC7A11, and FTL, thereby preventing iron accumulation, lipid peroxidation, and reducing ROS levels. Furthermore, the re-expression of GSTZ1 enhances the sensitivity of HCC cells to sorafenib treatment, indicating that GSTZ1 negatively regulates sorafenib resistance.[74] Consequently, targeting the Nrf2/GPX4 pathway and enhancing the anticancer effects of sorafenib through the induction of ferroptosis may serve as a promising therapeutic strategy for HCC (Figure 2).

## ETS1-miR-23a-3p-ACSL4 Pathway

In addition to the previously discussed pathways, certain miRNAs play a significant role in the development of sorafenib resistance in HCC cells. Notably, miR-23a-3p acts as a negative regulator of ferroptosis by targeting the downstream 3'-UTR of acyl-CoA synthetase long-chain family member 4 (ACSL4), thus reducing ROS production and decreasing cell ferroptosis. ETS oncogene 1 (ETS1) is a key transcription factor that directly stimulates the transcription of miR-23a-3p in response to sorafenib treatment.[75] The ETS1-miR-23a-3p-ACSL4 pathway contributes to the resistance of HCC cells to sorafenib through the regulation of ferroptosis (Figure 3).

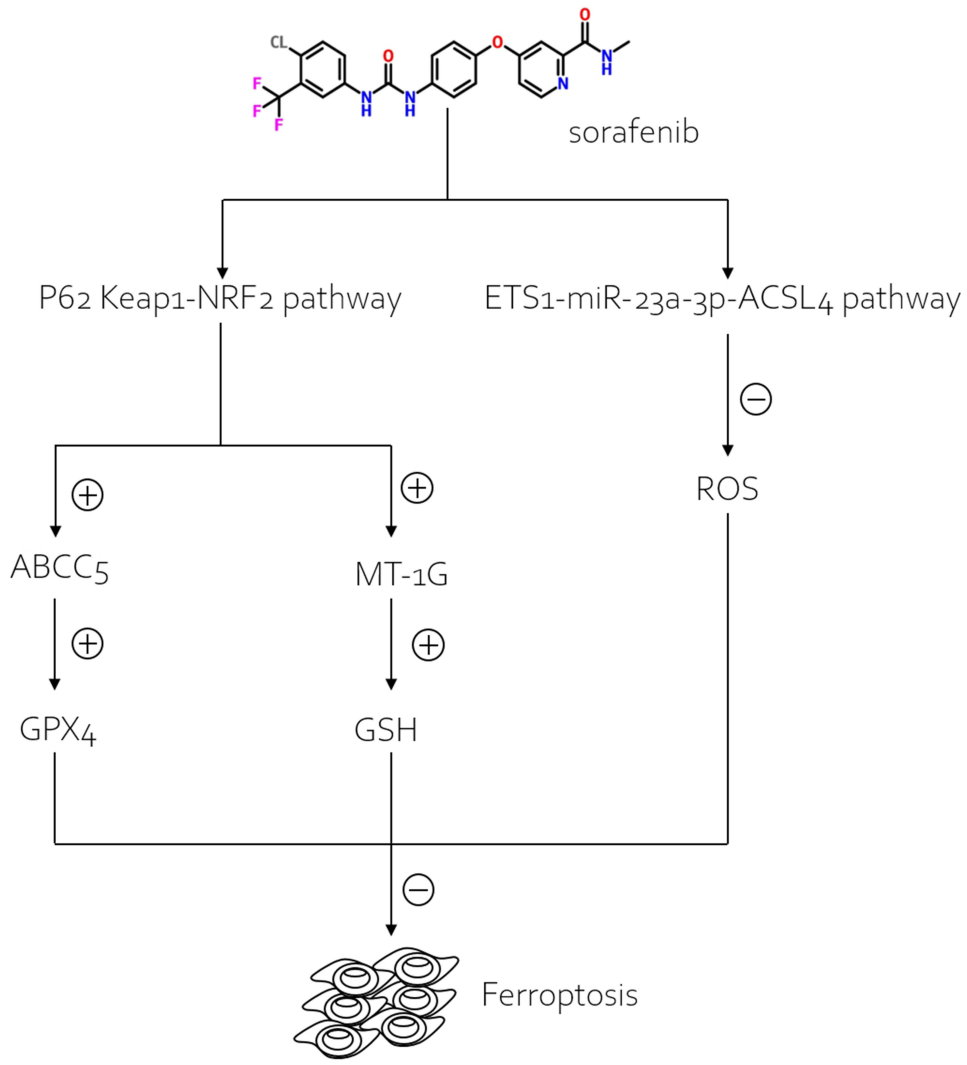


**Figure 3** Mechanisms of sorafenib resistance across Keap1-Nrf2 system and ETS1-miR-23a-3p-ACSL4 pathway.
**Abbreviations**: Keap1, kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid 2-related factor 2; ETS1, ETS oncogene 1; ACSL4, acyl-CoA synthetase long-chain family member 4; ABCC5, ATP-binding cassette transporter C5; MT-1G1, metallothionein 1G; GPX4, glutathione peroxidase 4; GSH, glutathione; ROS, reactive oxygen species.

## Conclusion

Sorafenib primarily acts as a multi-kinase inhibitor targeting pathways involved in HCC growth and angiogenesis; however, its effectiveness to HCC can be compromised by various factors including drug resistance. Further investigations indicate that enhancing intracellular ferroptosis can significantly improve the therapeutic efficacy of sorafenib. This enhancement is particularly relevant in cases where HCC cells have developed resistance to this chemotherapy agent, often due to adaptive responses that enable cancer cells to survive despite pharmacological pressure. Consequently, inducing ferroptosis within HCC cells has emerged as a promising strategy for overcoming sorafenib resistance.

The mechanism of sorafenib resistance in the ferroptosis pathway mainly resides in its influences on intracellular iron level and ROS level. The pathway that affects iron level encompasses the LIFR-NF-κB-LCN2 pathway, which can diminish intracellular iron uptake to reduce the sensitivity to iron-induced cell death. The main pathway influencing ROS level is the Hippo YAP pathway, within which YAP/TAZ and ATF4 are activated in the resistant HCC cells and induce the expression of SLC7A11 to increase intracellular glutathione level and decrease ROS level. Nrf2-ABCC5, Nrf2-MT -1G, and GSTZ1-GPX4 pathways decrease GPX4 consumption and inhibit lipid peroxidation. And ETS1-miR-23a-3p-ACSL4 pathway reduces ROS production and cellular ferroptosis (Figure 4).

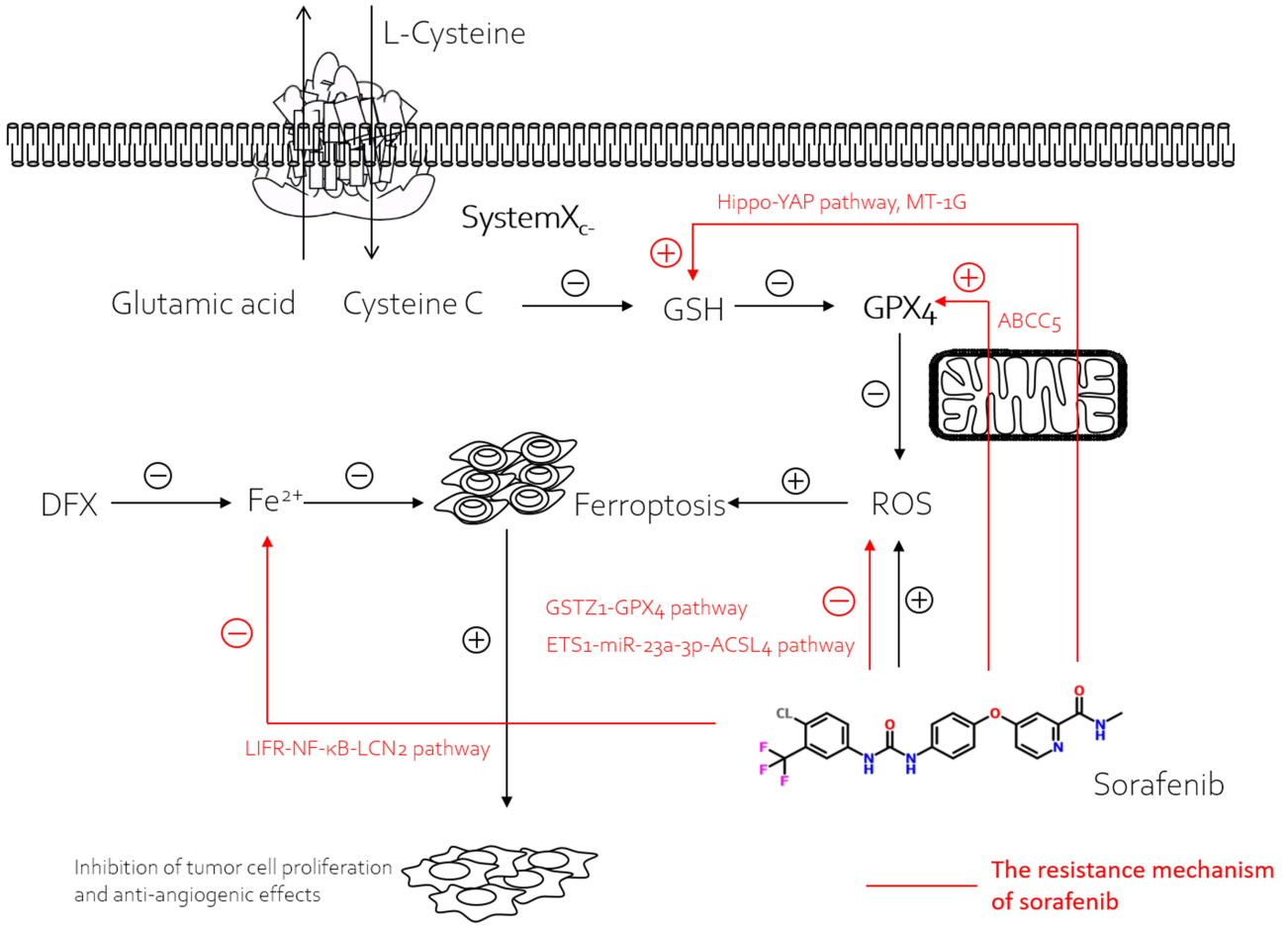


**Figure 4** The resistance mechanism of sorafenib.
**Abbreviations**: GSH, glutathione; GPX4, glutathione peroxidase 4; DFX, deferoxamine; ROS, reactive oxygen species; YAP, yes-associated protein; LIFR, leukemia inhibitory factor receptor; LCN2, lipocalin 2; GSTZ1, glutathione S-transferase zeta 1; ETS1, ETS oncogene 1; ACSL4, acyl-CoA synthetase long-chain family member 4; ABCC5, ATP-binding cassette transporter C5; MT-1G1, metallothionein 1G.

Research efforts continue to explore potential combinatory strategies for HCC involving ferroptosis inducers alongside sorafenib treatment. These strategies aim not only to sensitize sorafenib-resistant HCC but also to exploit vulnerabilities specific to HCC metabolism related to iron homeostasis and lipid peroxidation processes. As our understanding deepens regarding the interplay between ferroptosis induction and existing therapeutic modalities, it may pave the way for novel interventions specifically designed for patients suffering from advanced stages of HCC, who currently face limited options due to treatment resistance.

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## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Llovet JM, Zucman-Rossi J, Pikarsky E, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. 2016;2(1):16018. doi:10.1038/nrdp.2016.18

2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87–108. doi:10.3322/caac.21262
3. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet*. 2018;391(10127):1301–1314. doi:10.1016/S0140-6736(18)30010-2
4. Li L, Wang H. Heterogeneity of liver cancer and personalized therapy. *Cancer Lett*. 2016;379(2):191–197. doi:10.1016/j.canlet.2015.07.018
5. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359(4):378–390. doi:10.1056/NEJMoa0708857
6. Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res*. 2004;64(19):7099–7109. doi:10.1158/0008-5472.CAN-04-1443
7. Cheng AL, Kang YK, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific 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
8. Vogel A, Saborowski A. Current strategies for the treatment of intermediate and advanced hepatocellular carcinoma. *Cancer Treat Rev*. 2020;82:101946. doi:10.1016/j.ctrv.2019.101946
9. Colagrande S, Regini F, Taliani GG, Nardi C, Inghilesi AL. Advanced hepatocellular carcinoma and sorafenib: diagnosis, indications, clinical and radiological follow-up. *World J Hepatol*. 2015;7(8):1041–1053. doi:10.4254/wjh.v7.i8.1041
10. Abou-Alfa GK, Meyer T, Cheng AL, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N Engl J Med*. 2018;379(1):54–63. doi:10.1056/NEJMoa1717002
11. Bruix J, Qin S, Merle P, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, Phase 3 trial. *Lancet*. 2017;389(10064):56–66. doi:10.1016/S0140-6736(16)32453-9
12. Kudo M, Finn RS, Qin S, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet*. 2018;391(10126):1163–1173. doi:10.1016/S0140-6736(18)30207-1
13. Zhu AX, Park JO, Ryoo BY, et al. Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol*. 2015;16(7):859–870. doi:10.1016/S1470-2045(15)00050-9
14. Kudo M, Finn RS, Cheng AL, et al. Albumin-bilirubin grade analyses of atezolizumab plus bevacizumab versus sorafenib in patients with unresectable hepatocellular carcinoma: a post hoc analysis of the phase III IMbrave150 Study. *Liver Cancer*. 2023;12(5):479–493. doi:10.1159/000529996
15. Kudo M, Finn RS, Galle PR, et al. IMbrave150: efficacy and safety of atezolizumab plus bevacizumab versus sorafenib in patients with barcelona clinic liver cancer stage B unresectable hepatocellular carcinoma: an exploratory analysis of the phase III study. *Liver Cancer*. 2023;12(3):238–250. doi:10.1159/000528272
16. Kudo M, Tsuchiya K, Shao YY, et al. Impact of bevacizumab being skipped due to adverse events of special interest for bevacizumab in patients with unresectable hepatocellular carcinoma treated with atezolizumab plus bevacizumab: an exploratory analysis of the phase III IMbrave150 study. *Liver Cancer*. 2024;13(4):401–412. doi:10.1159/000535501
17. Tang W, Chen Z, Zhang W, et al. The mechanisms of sorafenib resistance in hepatocellular carcinoma: theoretical basis and therapeutic aspects. *Signal Transduct Target Ther*. 2020;5(1):87. doi:10.1038/s41392-020-0187-x
18. Zhang T, Cao C, Wu D, Liu L. SNHG3 correlates with malignant status and poor prognosis in hepatocellular carcinoma. *Tumour Biol*. 2016;37(2):2379–2385. doi:10.1007/s13277-015-4052-4
19. Ye J, Zhang R, Du X, Chai W, Zhou Q. Long noncoding RNA SNHG16 induces sorafenib resistance in hepatocellular carcinoma cells through sponging miR-140-5p. *Onco Targets Ther*. 2019;12:415–422. doi:10.2147/OTT.S175176
20. 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):173–186. doi:10.1159/000501162
21. 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):1328–1341. doi:10.1136/gutjnl-2017-315402
22. Wang Z, Wang L, Yin G, et al. Hsa_circ_0088036 promotes tumorigenesis and chemotherapy resistance in hepatocellular carcinoma via the miR-140-3p/KIF2A axis. *Histol Histopathol*;2024. 18849. doi:10.14670/HH-18-849
23. Li X, Cui M, Xu L, Guo Q. Low miR-936-mediated upregulation of Pim-3 drives sorafenib resistance in liver cancer through ferroptosis inhibition by activating the ANKRD18A/Src/NRF2 pathway. *Front Oncol*. 2024;14:1483660. doi:10.3389/fonc.2024.1483660
24. Wang T, Qin ZY, Wen LZ, et al. Epigenetic restriction of Hippo signaling by MORC2 underlies stemness of hepatocellular carcinoma cells. *Cell Death Differ*. 2018;25(12):2086–2100. doi:10.1038/s41418-018-0095-6
25. Abeni E, Salvi A, Marchina E, Traversa M, Arici B, De Petro G. Sorafenib induces variations of the DNA methylome in HA22T/VGH human hepatocellular carcinoma-derived cells. *Int J Oncol*. 2017;51(1):128–144. doi:10.3892/ijo.2017.4019
26. Schultheiss CS, Laggai S, Czepukojc B, 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
27. Mo C, You W, Rao Y, et al. Epigenetic regulation of DNA repair gene program by Hippo/YAP1-TET1 axis mediates sorafenib resistance in HCC. *Cell Mol Life Sci*. 2024;81(1):284. doi:10.1007/s00018-024-05296-y
28. Wang W, Ding B, Lou W, Lin S. Promoter hypomethylation and miR-145-5p downregulation- mediated HDAC11 overexpression promotes sorafenib resistance and metastasis of hepatocellular carcinoma cells. *Front Cell Dev Biol*. 2020;8:724. doi:10.3389/fcell.2020.00724
29. Di Giacomo S, Briz O, Monte MJ, et al. Chemosensitization of hepatocellular carcinoma cells to sorafenib by beta-caryophyllene oxide-induced inhibition of ABC export pumps. *Arch Toxicol*. 2019;93(3):623–634. doi:10.1007/s00204-019-02395-9
30. Takahashi K, Yan IK, Wood J, Haga H, Patel T. Involvement of extracellular vesicle long noncoding RNA (linc-VLDLR) in tumor cell responses to chemotherapy. *Mol Cancer Res*. 2014;12(10):1377–1387. doi:10.1158/1541-7786.MCR-13-0636
31. Shi S, Rao Q, Zhang C, Zhang X, Qin Y, Niu Z. Dendritic cells pulsed with exosomes in combination with PD-1 antibody increase the efficacy of sorafenib in hepatocellular carcinoma model. *Transl Oncol*. 2018;11(2):250–258. doi:10.1016/j.tranon.2018.01.001

32. Liang Y, Zheng T, Song R, 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):1847–1857. doi:10.1002/hep.26224
33. Lin X, Li AM, Li YH, et al. Silencing MYH9 blocks HBx-induced GSK3beta ubiquitination and degradation to inhibit tumor stemness in hepatocellular carcinoma. *Signal Transduct Target Ther*. 2020;5(1):13. doi:10.1038/s41392-020-0111-4
34. Song X, Zhu S, Chen P, et al. AMPK-Mediated BECN1 phosphorylation promotes ferroptosis by directly blocking system X(c)(-) Activity. *Curr Biol*. 2018;28(15):2388–2399e2385. doi:10.1016/j.cub.2018.05.094
35. Zou Y, Palte MJ, Deik AA, et al. A GPX4-dependent cancer cell state underlies the clear-cell morphology and confers sensitivity to ferroptosis. *Nat Commun*. 2019;10(1):1617. doi:10.1038/s41467-019-09277-9
36. Liu MR, Zhu WT, Pei DS. System Xc(-): a key regulatory target of ferroptosis in cancer. *Invest New Drugs*. 2021;39(4):1123–1131. doi:10.1007/s10637-021-01070-0
37. Seibt TM, Proneth B, Conrad M. Role of GPX4 in ferroptosis and its pharmacological implication. *Free Radic Biol Med*. 2019;133:144–152. doi:10.1016/j.freeradbiomed.2018.09.014
38. Shin D, Kim EH, Lee J, Roh JL. Nrf2 inhibition reverses resistance to GPX4 inhibitor-induced ferroptosis in head and neck cancer. *Free Radic Biol Med*. 2018;129:454–462. doi:10.1016/j.freeradbiomed.2018.10.426
39. Lee HY, Nga HT, Tian J, Yi HS. Mitochondrial metabolic signatures in hepatocellular carcinoma. *Cells*. 2021;10(8). doi:10.3390/cells10081901
40. Wang H, Liu C, Zhao Y, Gao G. Mitochondria regulation in ferroptosis. *Eur J Cell Biol*. 2020;99(1):151058. doi:10.1016/j.ejcb.2019.151058
41. Xie Y, Hou W, Song X, et al. Ferroptosis: process and function. *Cell Death Differ*. 2016;23(3):369–379. doi:10.1038/cdd.2015.158
42. Li X, He S, Ma B. Autophagy and autophagy-related proteins in cancer. *Mol Cancer*. 2020;19(1):12. doi:10.1186/s12943-020-1138-4
43. Lachaier E, Louandre C, Godin C, et al. Sorafenib induces ferroptosis in human cancer cell lines originating from different solid tumors. *Anticancer Res*. 2014;34(11):6417–6422.
44. Sun X, Niu X, Chen R, et al. Metallothionein-1G facilitates sorafenib resistance through inhibition of ferroptosis. *Hepatology*. 2016;64(2):488–500. doi:10.1002/hep.28574
45. Zhang Z, Yao Z, Wang L, et al. Activation of ferritinophagy is required for the RNA-binding protein ELAVL1/HuR to regulate ferroptosis in hepatic stellate cells. *Autophagy*. 2018;14(12):2083–2103. doi:10.1080/15548627.2018.1503146
46. Ma S, Meng Z, Chen R, Guan KL. The hippo pathway: biology and pathophysiology. *Annu Rev Biochem*. 2019;88(1):577–604. doi:10.1146/annurev-biochem-013118-111829
47. Louandre C, Marcq I, Bouhlal H, et al. The retinoblastoma (Rb) protein regulates ferroptosis induced by sorafenib in human hepatocellular carcinoma cells. *Cancer Lett*. 2015;356(2 Pt B):971–977. doi:10.1016/j.canlet.2014.11.014
48. Meng Z, Moroishi T, Guan KL. Mechanisms of Hippo pathway regulation. *Genes Dev*. 2016;30(1):1–17. doi:10.1101/gad.274027.115
49. Hong W, Guan KL. The YAP and TAZ transcription co-activators: key downstream effectors of the mammalian Hippo pathway. *Semin Cell Dev Biol*. 2012;23(7):785–793. doi:10.1016/j.semcdb.2012.05.004
50. Zanconato F, Cordenonsi M, Piccolo S. YAP/TAZ at the Roots of Cancer. *Cancer Cell*. 2016;29(6):783–803. doi:10.1016/j.ccell.2016.05.005
51. Qin Y, Pei Z, Feng Z, et al. Oncogenic activation of YAP signaling sensitizes ferroptosis of hepatocellular carcinoma via ALOXE3-mediated lipid peroxidation accumulation. *Front Cell Dev Biol*. 2021;9:751593. doi:10.3389/fcell.2021.751593
52. Gao R, Kalathur RKR, Coto-Llerena M, et al. YAP/TAZ and ATF4 drive resistance to Sorafenib in hepatocellular carcinoma by preventing ferroptosis. *EMBO Mol Med*. 2021;13(12):e14351. doi:10.15252/emmm.202114351
53. Nicola NA, Babon JJ. Leukemia inhibitory factor (LIF). *Cytokine Growth Factor Rev*. 2015;26(5):533–544. doi:10.1016/j.cytogfr.2015.07.001
54. Hilton DJ, Nicola NA, Metcalf D. Distribution and comparison of receptors for leukemia inhibitory factor on murine hemopoietic and hepatic cells. *J Cell Physiol*. 1991;146(2):207–215. doi:10.1002/jcp.1041460204
55. Xu WX, Zhang J, Hua YT, Yang SJ, Wang DD, Tang JH. An integrative pan-cancer analysis revealing LCN2 as an oncogenic immune protein in tumor microenvironment. *Front Oncol*. 2020;10:605097. doi:10.3389/fonc.2020.605097
56. Liu R, Wang J, Chen Y, et al. NOX activation in reactive astrocytes regulates astrocytic LCN2 expression and neurodegeneration. *Cell Death Dis*. 2022;13(4):371. doi:10.1038/s41419-022-04831-8
57. Asimakopoulou A, Weiskirchen S, Weiskirchen R. Lipocalin 2 (LCN2) expression in hepatic malfunction and therapy. *Front Physiol*. 2016;7:430. doi:10.3389/fphys.2016.00430
58. Du Z, Wu B, Xia Q, et al. LCN2-interacting proteins and their expression patterns in brain tumors. *Brain Res*. 2019;1720:146304. doi:10.1016/j.brainres.2019.146304
59. Xiao F, Li J, Huang K, et al. Macropinocytosis: mechanism and targeted therapy in cancers. *Am J Cancer Res*. 2021;11(1):14–30.
60. Yao F, Deng Y, Zhao Y, et al. A targetable LIFR-NF-kappaB-LCN2 axis controls liver tumorigenesis and vulnerability to ferroptosis. *Nat Commun*. 2021;12(1):7333. doi:10.1038/s41467-021-27452-9
61. Taguchi K, Yamamoto M. The KEAP1-NRF2 system in cancer. *Front Oncol*. 2017;7:85. doi:10.3389/fonc.2017.00085
62. Sun X, Ou Z, Chen R, et al. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology*. 2016;63(1):173–184. doi:10.1002/hep.28251
63. Tong R, Feng X, Sun J, et al. Co-Delivery of siNRF2 and sorafenib by a “click” dual functioned hyperbranched nanocarrier for synergistically inducing ferroptosis in hepatocellular carcinoma. *Small*. 2024;20(21):e2307273. doi:10.1002/smll.202307273
64. Louandre C, Ezzoukhry Z, Godin C, et al. Iron-dependent cell death of hepatocellular carcinoma cells exposed to sorafenib. *Int, J, Cancer*. 2013;133(7):1732–1742. doi:10.1002/ijc.28159
65. Atrian S, Capdevila M. Metallothionein-protein interactions. *Biomol Concepts*. 2013;4(2):143–160. doi:10.1515/bmc-2012-0049
66. Coyle P, Philcox JC, Carey LC, Rofe AM. Metallothionein: the multipurpose protein. *Cell Mol Life Sci*. 2002;59(4):627–647. doi:10.1007/s00018-002-8454-2
67. Dai H, Wang L, Li L, Huang Z, Ye L. Metallothionein 1: a new spotlight on inflammatory diseases. *Front Immunol*. 2021;12:739918. doi:10.3389/fimmu.2021.739918
68. Xue D, Zhou X, Qiu J. Emerging role of NRF2 in ROS-mediated tumor chemoresistance. *Biomed Pharmacother*. 2020;131:110676. doi:10.1016/j.biopha.2020.110676
69. Toyoda Y, Hagiya Y, Adachi T, Hoshijima K, Kuo MT, Ishikawa T. MRP class of human ATP binding cassette (ABC) transporters: historical background and new research directions. *Xenobiotica*. 2008;38(7–8):833–862. doi:10.1080/00498250701883514

70. Chen L, Yang Z, Cao Y, et al. Pan-cancer analysis and single-cell analysis revealed the role of ABCC5 transporter in hepatocellular carcinoma. *Channels (Austin)*. 2021;15(1):541–554. doi:10.1080/19336950.2021.1968592
71. Jansen RS, Mahakena S, de Haas M, Borst P, van de Wetering K. ATP-binding cassette subfamily C member 5 (ABCC5) functions as an efflux transporter of glutamate conjugates and analogs. *J Biol Chem*. 2015;290(51):30429–30440. doi:10.1074/jbc.M115.692103
72. Guo W, Zhou BP. Oncometabolite modification of Keap1 links GSTZ1 deficiency with cancer. *Genes Dis*. 2019;6(4):333–334. doi:10.1016/j.gendis.2019.10.005
73. Li J, Wang Q, Yang Y, et al. GSTZ1 deficiency promotes hepatocellular carcinoma proliferation via activation of the KEAP1/NRF2 pathway. *J Exp Clin Cancer Res*. 2019;38(1):438. doi:10.1186/s13046-019-1459-6
74. Wang Q, Bin C, Xue Q, et al. GSTZ1 sensitizes hepatocellular carcinoma cells to sorafenib-induced ferroptosis via inhibition of NRF2/GPX4 axis. *Cell Death Dis*. 2021;12(5):426. doi:10.1038/s41419-021-03718-4
75. Lu Y, Chan YT, Tan HY, et al. Epigenetic regulation of ferroptosis via ETS1/miR-23a-3p/ACSL4 axis mediates sorafenib resistance in human hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2022;41(1):3. doi:10.1186/s13046-021-02208-x