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Resistance of first-line targeted drugs in hepatocellular carcinoma: the epigenetic regulation mechanisms

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Abstract

Targeted therapy has revolutionized the treatment landscape of hepatocellular carcinoma (HCC), offering unprecedented hope to patients. However, despite its promise, significant challenges arise in the form of drug resistance. Only a fraction of HCC patients respond to targeted therapy, and even those who respond often develop resistance over time. Sorafenib and lenvatinib, the sole first-line targeted therapeutic drugs for HCC, face severe clinical limitations due to drug resistance. Understanding the mechanisms underlying sorafenib/lenvatinib resistance is crucial for maximizing treatment efficacy. Recent studies have highlighted the pivotal role of epigenetic regulation in drug resistance. Cancer is recognized as both a genetic and epigenetic disease, with epigenetic factors influencing various aspects of tumor cell biology, especially drug resistance. This review systematically summarizes the mechanisms of epigenetic-mediated sorafenib/lenvatinib resistance,

encompassing non-coding RNA (ncRNA) regulation, DNA methylation, RNA methylation, and histone modification. These abnormal epigenetic processes typically influence biological activities including: escaped programmed cell death, tumor metabolic reprogramming, formation and maintenance of drug-resistant cells, uncontrolled cell proliferation signaling pathways and abnormal transport processes, ultimately culminating in profound drug resistance. By comprehensively summarizing the latest discoveries in epigenetic regulation mechanisms, this review highlights potential strategies to overcome drug resistance, paving the way for future advancements in HCC treatment.

Keywords:

Hepatocellular carcinoma, Sorafenib resistance, Lenvatinib resistance, Epigenetic regulation, Epigenetic therapy, Clinical translation

FACTS

1. Epigenetic regulation plays a significant role in sorafenib/lenvatinib resistance.
2. Epigenetic drug resistance outcomes involve programmed cell death evasion, metabolic reprogramming, the formation and maintenance of drug-resistant cells, uncontrolled cell proliferation signaling pathways and abnormal transport processes.
3. Combining epigenetic therapy with targeted drugs demonstrates potential in reversing drug resistance.
4. Personalized therapy represents a promising strategy to enhance the effectiveness of clinical drug resistance treatments.

Open questions

1. Whether the currently reported drug resistance mechanisms show significant differences in clinical patients?
2. Can resistance-overcoming therapies validated in animal studies achieve comparable efficacy in clinical patients?

3. Could targeting the shared resistance mechanisms/targets of sorafenib and lenvatinib simultaneously reverse resistance to both drugs?
4. How can we address the challenges in overcoming resistance posed by the complexity of epigenetic drug resistance?

1. Introduction

Primary liver cancer is the third most common cause of cancer-related death worldwide, with HCC accounting for over 80% of all cases ¹. In stark contrast to other prevalent cancers, such as breast, lung, and prostate, which are experiencing declining mortality rates, HCC-related mortality continues to climb at a rate of 2-3% annually ². Projections indicate that the global tally of liver cancer instances will surpass 1 million by 2025, with the highest incidence rates reported in East and Southeast Asia, as well as Central and West Africa ^{2,3}

To address the substantial impact of HCC, modern medicine offers various therapeutic options. Surgical resection and liver transplantation have the potential to completely cure early-stage HCC with 5-year survival rates exceeding 60% for both treatments⁴. For HCC patients who do not qualify for surgery, interventional techniques such as ablative therapy⁵ and transcatheter arterial chemoembolization (TACE) ⁶ provide some survival benefit. However, the eligibility criteria for these treatments often clash with the characteristics of advanced HCC. When the aforementioned treatments are unavailable, systemic therapy becomes the primary choice ⁷, encompassing chemotherapy, targeted therapy and immunotherapy ⁸

In the past decade, there has been significant progress in targeted therapy for the treatment of mid-stage and advanced HCC, prolonging patient survival. Targeted therapy drugs mainly include multikinase inhibitors and vascular endothelial growth factor (VEGF) receptor antibodies. Sorafenib and lenvatinib are currently the only first-line targeted drugs for HCC treatment ⁹. Importantly, sorafenib is efficacious in approximately 30% of patients ¹⁰; while lenvatinib demonstrates a similarly modest overall response rate of 24% ¹¹. High resistance rates to both sorafenib and lenvatinib pose significant impediments to achieving therapeutic success in the treatment of HCC. Available evidence suggests that numerous resistance cases arise due to aberrant epigenetic regulation. Consequently, it is

imperative to conduct thorough analyses of the underlying mechanisms responsible for this resistance and to identify effective strategies aimed at delaying or overcoming drug resistance, ultimately improving the prognosis for HCC patients.

Previous reviews have analyzed epigenetic mechanisms in HCC drug resistance. Oura et al. classified systemic therapy resistance into epigenetic regulation and tumor microenvironment (TME) modulation, mapping drug-specific resistance networks involving molecular targeted agents (MTAs) and immune checkpoint inhibitors (ICIs)¹². Sun et al. systematically categorized epigenetic resistance mechanisms by regulatory modalities (DNA methylation, RNA regulation, histone modification, chromatin remodeling), emphasizing epigenetic-targeted agents (e.g., DNMT/HDAC inhibitors) and their clinical potential¹³. While Oura et al. emphasize epigenetic-TME interplay in resistance networks, Sun et al. focus on mechanistic insights and clinical translation of epigenetic therapies, offering complementary frameworks to address HCC resistance. Our work attempts to both extend the reported epigenetic-mediated resistance mechanism and delve deeper into the clinical translational potential of epigenetic therapies in overcoming resistance, thereby providing novel insights into therapeutic development.

In this comprehensive review, we will delve into the epigenetic regulatory mechanisms that confer resistance to sorafenib and lenvatinib, and provide an in-depth analysis of the potential of epigenetic therapy and their clinical translation. Specifically, we will categorize the mechanisms underlying epigenetic modulation-induced drug resistance based on four primary epigenetic regulatory means: ncRNA regulation, DNA methylation, RNA methylation, and histone modification. These mechanisms will be grouped into the following categories: escaped programmed cell death, tumor metabolic reprogramming, formation and maintenance of drug-resistant cells, uncontrolled cell proliferation signaling pathways and abnormal transport processes (**Figure 1**).

2. Epigenetic modification

The term “epigenetics” first introduced by Conrad Waddington, refers to stable genetic phenotypes that arise from chromosomal alterations without altering the fundamental DNA sequence¹⁴. Epigenetic mechanisms determine the transcriptional availability of various parts of the genome, thereby shaping long-term cell behavior¹⁵. The most extensively studied epigenetic regulations encompass direct DNA methylation, post-translational modifications of histones, and regulations mediated by ncRNA,

collectively forming an intricate regulatory network; aberrant regulatory patterns of tumor cells within this network can directly lead to drug resistance¹⁶. In the past decade, dysregulated RNA methylation has also been identified in human cancers¹⁷. This dysregulation promotes tumor resistance by modifying the stability of crucial gene transcripts and influencing certain signaling pathways¹⁸. Below, we will briefly introduce the key modes of epigenetic regulation, DNA methylation, histone modification, ncRNA regulation, and RNA methylation. These regulatory mechanisms collectively contribute to the development of drug resistance against molecularly targeted therapies through intricate biological processes.

NcRNAs Over the past decades, ncRNAs have transitioned from being considered "junk products" to essential regulatory molecules that modulate gene expression. Despite not encoding proteins, ncRNAs exert influence on gene expression patterns through diverse mechanisms, encompassing chromatin remodeling, transcription, post-transcriptional modification, and signal transduction¹⁹. The three main types of ncRNAs are microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), each playing a distinct role in cancer. MiRNAs are short RNAs (~22 nucleotides [nt]); MiRNAs modulate gene expression through their association with the RNA-induced silencing complex (RISC), a multiprotein entity. This complex subsequently binds to the 3' untranslated region of the target mRNA, leading to the inhibition of translation, the shortening of the mRNA's poly (A) tail, and its eventual degradation²⁰. LncRNAs and circRNAs are longer (>200 nt); lncRNAs are linear, whereas circRNAs are cyclic²¹. The dysregulation of lncRNA is involved in tumor progression by regulating chromatin regulation and transcription, sponging miRNAs, and affecting structural functions.²² Some circRNAs function similarly to lncRNAs, but many of their mechanisms remain unclear²³. They are generated through back-splicing of pre-mRNA, forming a covalently closed loop without a 5'-cap or a 3'-poly (A) tail. Compared to linear RNA, circRNA is less susceptible to degradation by exogenous enzymes and exhibits higher stability. The physiological effects of circRNA are often mediated through binding to specific miRNAs (sponging), thereby eliminating the inhibitory effect of the miRNA on mRNA translation²⁴. These ncRNAs classes are closely linked to various cancer processes, including therapeutic resistance.

DNA methylation The core of DNA methylation involves transferring a methyl group from S-adenosylmethionine (SAM) to the C5 position of cytosine in cytosine-phosphate-guanine (CpG)

dinucleotides, a process facilitated by DNA methyltransferase (DNMT) enzymes, resulting in the formation of 5-methylcytosine; CpG dinucleotides are unevenly distributed in mankind genomes, predominantly clustering in CpG islands (CGIs), which are short, interspersed DNA sequences that deviate significantly from the typical genomic pattern and are characterized by their abundance of unmethylated CpG dinucleotides. It is plausible that the majority, or even all, of these CGIs function as sites for transcription initiation. In normal somatic promoters, methylation of CGIs can result in gene silencing, either directly by impeding the binding of transcription factors or indirectly through the interaction of 5-methylcytosine (5mC) with methyl-CpG binding domain (MBD) proteins²⁵. However, in tumor cells, abnormalities frequently arise in the DNA methylation process, contributing to tumorigenesis and progression. Disruptions to these processes or abnormalities in the participating enzymes can silence tumor suppressor genes^{26, 27}. Key enzymes involved in these processes include DNA methyltransferases (e.g., DNMT1, DNMT3A, and DNMT3B) and DNA demethylases such as ten-eleven translocation 1 (TET1), TET2, and TET3²⁸. Methyltransferase abnormalities usually result in hypermethylation of tumor suppressor gene promoters, silencing those genes. Conversely, demethylase abnormalities cause hypomethylation of oncogene promoters, leading to drug resistance²⁹.

Histone modification Histone is the core component of the nucleosome subunit, featuring a side chain or tail with a dense distribution of basic lysine and arginine residues. The histone tail undergoes extensive cooperative covalent post-translational modifications (PTMs) to regulate chromatin states. The best-characterized and most common types are acetylation and methylation. Chromatin-modifying enzymes are classified as histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), and histone demethylases (HDMs)³⁰. Generally, histone acetylation catalyzed by histone acetyltransferases (HATs) is associated with transcriptional activation, while hypoacetylation by histone deacetylases is linked to transcriptional repression. Histone methylation is relevant to both active and silent genes. For instance, trimethylation of lysine 27 on histone H3 (H3 K27) serves as a silencing mark, while methylation of lysine 4 on histone H3 (H3 K4) is found at the promoters of active genes. Histone demethylation, on the other hand, is usually associated with silent genes^{30, 31}. These modifications control gene expression via dynamic addition and removal, influencing processes such as transcriptional activation, chromosome folding, and DNA repair³².

RNA methylation Emerging evidence suggests that dysregulated RNA methylation is associated with cancer progression in humans ³³. Thus far, more than 100 types of RNA modifications have been identified ³⁴. The most prevalent type of methylation, N6-methyladenosine (m6A), occurs at the N6 position of adenosine and represents over 80% of RNA methylation in eukaryotes. Three types of proteins control m6A modification. The first type comprises m6A methyltransferases, including methyltransferase-like 3 (METTL3), METTL14, and Wilms tumor 1-associated protein (WTAP). METTL14 forms a stable complex with METTL3; WTAP ensures that the METTL3-METTL14 heterodimer is located in the nucleus and maintains catalytic activity. The second type comprises m6A demethylases, such as AlkB homolog H5 (ALKBH5) and fat mass and obesity-associated protein (FTO) binding proteins, which are mainly responsible for reversal of existing m6A modifications. The third type comprises methylation recognition proteins; notable examples are the YT521-B homology (YTH) domain proteins (YTH domain-containing family proteins 1 (YTHDF1), YTHDF2, YTHDF3, YTHDC1, YTHDC2). These proteins primarily recognize and bind m6A sites on RNA, thereby altering processes such as splicing, localization, translation, and stability ^{35, 36}.

3. Epigenetic modifications involved in sorafenib resistance

Sorafenib, an oral multikinase inhibitor targeting VEGF receptors, RAF, and platelet-derived growth factor (PDGF) receptors, exerts anti-angiogenic and antitumor effects ³⁷. The SHARPE trial revealed that patients treated with oral sorafenib exhibited significantly better overall survival (OS) compared to those receiving placebo, with OS extending to 10.7 months versus 7.9 months (hazard ratio [HR]: 0.69; confidence interval [CI]: 0.55–0.87; $P < 0.001$). Additionally, the time to radiographic progression was prolonged by nearly 3 months with sorafenib (5.5 months vs. 2.8 months; HR: 0.58; CI: 0.45–0.74; $P < 0.001$) ³⁸. A phase III randomized, double-blind, placebo-controlled trial conducted in the Asia-Pacific region further confirmed that sorafenib significantly improved survival in patients with advanced HCC ³⁹. Consequently, in 2008, sorafenib was approved by the FDA as a first-line targeted therapy for HCC ⁴⁰. This review specifically focuses on the role of epigenetic modifications in sorafenib resistance.

3.1 Epigenetic modification enables escaped programmed cell death

Tumor cell death pathways include unprogrammed cell death and programmed cell death (PCD) . Unprogrammed cell death is a passive process triggered by external stimuli and lacks cellular regulation. Conversely, PCD is a form of regulated cell death controlled by a complex gene network⁴¹. Recent advancements have significantly enhanced our understanding of how epigenetic modifications contribute to sorafenib resistance through aberrant PCD. Most tumor cells can partially evade PCD through epigenetic modifications. PCD in tumor cells involves several crucial escape mechanisms associated with sorafenib resistance, including apoptosis, ferroptosis, and autophagy pathways⁴².

3.1.1 Apoptosis

Apoptosis, the predominant form of cell death, is a critical mechanism triggered by chemotherapy and molecular targeting therapies⁴³. Apoptosis comprises endogenous and exogenous pathways. The endogenous apoptotic pathway results from increased mitochondrial permeability and the cytoplasmic release of pro-apoptotic molecules, such as cytochrome c. Caspase-9 responds to cytochrome c accumulation in the cytoplasm by forming apoptosomes and initiating caspase-mediated PCD. This process is tightly regulated by the B-cell lymphoma-2 (BCL-2) protein family, which includes pro-apoptotic members (BAX, BAK1, BID, BIM, PUMA, BAD, BIK, BMF, NOXA, and HRK) and anti-apoptotic members (BCL-2, BCL-XL, BCL-W, and MCL1). Anti-apoptotic proteins inhibit the release of cytochrome c from mitochondria, whereas pro-apoptotic proteins function by promoting this release^{44, 45}. Caspase-3, a downstream effector shared by both pathways, cleaves deoxyribonucleic acid to induce apoptosis⁴⁶. Since sorafenib primarily acts through the endogenous apoptosis pathway, we focus on how abnormalities in this pathway mediate sorafenib resistance (**Figure 2**).

3.1.1.1 Anti-apoptotic protein-induced resistance

Increased levels of anti-apoptotic proteins are partly attributed to epigenetic regulation of the KDM1A (lysine demethylase 1A)/ FKBP8 (FKBP prolyl isomerase 8)/ BCL2 (B-cell lymphoma-2) axis. The cytoplasmic localization and protein stability of KDM1A were promoted by acetylation at lysine-117 by the acetyl transferase KAT8. The stably expressed KDM1A demethylates FKBP8 in the cytoplasm, upregulating expression of the anti-apoptotic protein BCL-2⁴⁷. Zhou et al. also described a mechanism where the lncRNA TTN-AS1 inhibits miR-16-5p, thereby activating the phosphatase and tensin

homolog (PTEN)/protein kinase B (Akt) signaling pathway and upregulating BCL-2 protein expression. Overexpression of BCL-2 protein enhances HCC resistance to sorafenib ⁴⁸.

3.1.1.2 Decreased apoptotic protein-induced resistance

MiR-494, overexpressed in HCC, induces sorafenib resistance by suppressing the levels of apoptosis modulator proteins. Specifically, the reduction in p53 upregulated modulator of apoptosis (PUMA) protein expression results in decreased caspase activity, effectively inhibiting apoptosis ⁴⁹. Elevated circulating levels of miR-518d-5p have been associated with shorter sorafenib treatment duration and reduced OS. MiR-518d-5p mediated mitochondrial dysfunction, leading to the silencing of c-Jun and the obstruction of PUMA transcription. Consequently, the downregulation of PUMA significantly diminishes HCC sensitivity to sorafenib and inhibits the production of reactive oxygen species (ROS), further decreasing apoptosis in cancer cells ⁵⁰. The lncRNA NEAT1 is also highly expressed in HCC cells. High of NEAT1 levels can downregulate miR-335 expression thereby activating the downstream c-Met-Akt pathway and reducing the production of apoptotic proteins. This mechanism counteracts sorafenib-induced apoptosis and contributes to drug resistance ⁵¹. Dong et al. observed that miR-124-3p expression is downregulated in HCC tissues; this downregulation enhanced the dephosphorylation of AKT2 and silent information regulator sirtuin 1 (SIRT1 or sirtuin 1), resulting in decreased downstream forkhead box class O3a (FOXO3a) activity. Although FOXO3a generally plays a pro-apoptotic role via BIM promoter activation, its suppression reduces cellular sensitivity to sorafenib-induced apoptosis ⁵².

3.1.1.3 Inhibited caspase-3 cleavage-induced resistance

Shao et al. conducted a comparative analysis of differential miRNA expression between sorafenib-sensitive and sorafenib-resistant HCC cells, revealing a significant downregulation of miR-10b-3p in the resistant cell lines. This depletion of miR-10b-3p inhibits caspase-3 cleavage by upregulating cyclin E1, thereby reducing the capacity of sorafenib to induce apoptosis in HCC cells and ultimately leading to drug resistance ⁵³. The circRNA cDCBLD2, highly expressed in HCC, promotes HCC cell survival during sorafenib treatment. By competitively binding to miR-345-5p, cDCBLD2 enhances the stability of DNA topoisomerase II α (TOP2A) mRNA. Normally, miR-345-5p directly targets the TOP2A coding sequence to promote caspase-3 activation and induce apoptosis. However, in the presence of overexpressed cDCBLD2, this process is reversed, allowing the cells to acquire resistance

to sorafenib⁵⁴.

3.1.2 Ferroptosis

Ferroptosis represents a newly identified form of PCD that is induced by iron-dependent oxidative stress. Whereas apoptosis is triggered by caspase activity, ferroptosis is induced by ROS, which are byproducts of mitochondrial oxidative phosphorylation⁵⁵. ROS include superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($\bullet OH$), hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2)⁵⁶. Despite the higher metabolic rate of cancer cells leading to a greater ROS load and making them more susceptible to ferroptosis compared to normal cells, cancer cells employ epigenetic regulation to counteract the increases in ferroptosis caused by their high metabolism. This enhances their survival under oxidative stress and promotes resistance to sorafenib⁵⁷. Ferroptosis centers around three pathways: system xc⁻/glutathione peroxidase 4 (GPX4), lipid peroxidation, and iron metabolism. Epigenetic regulatory factors promote sorafenib resistance by modifying these processes to inhibit ferroptosis⁵⁸ (**Figure 3**).

3.1.2.1 System Xc⁻/GPX4 pathway

System Xc⁻ is a heterodimeric transporter composed of two subunits, solute carrier family 7 member 11 (SLC7A11) and solute carrier family 3 member 2 (SLC3A2). Its primary function is to mediate the exchange of extracellular cystine for intracellular glutamate. Upon uptake, cystine is reduced to cysteine, which serves as a key precursor for glutathione (GSH) synthesis. GPX4 inhibits ROS production by converting glutathione (GSH) to oxidized glutathione (GSSG) and reducing the cytotoxic lipid peroxide (L-OOH) to the corresponding alcohol (L-OH)⁵⁹. Inhibition of System Xc⁻ impairs cystine uptake, leading to GSH depletion, decreased GPX4 activity, and accumulation of lipid peroxides—ultimately triggering ferroptosis.

Sorafenib induces ferroptosis by inhibiting system xc⁻ activity⁶⁰. Consequently, some epigenetic regulators promote sorafenib resistance within HCC by restoring system xc⁻ activity. In HCC, the overexpression of lncRNA CASC11⁶¹ and lncRNA DUXAP8⁶² suppresses ferroptosis by upregulating SLC7A11 levels. Shi et al. uncovered an NSUN2/MALREF-mediated m5C methylation axis promoting lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) expression in HCC. MALAT1 suppresses ferroptosis by binding ELAVL1 to enhance its cytoplasmic translocation, thereby stabilizing SLC7A11. Importantly, the combination of MALAT1-IN1 (MALAT1 inhibitor) with sorafenib demonstrated enhanced antitumor efficacy in preclinical HCC models⁶³. Furthermore,

circTTC13 functions as a sponge for miR-513a-5p, indirectly elevating SLC7A11 expression to inhibit ferroptosis²⁴. Cytoplasmic lncRNA PVT1 competitively binds miR-195-5p to upregulate Pleomorphic Adenoma Gene 1 (PLAG1), subsequently increasing GPX4 expression. Pharmacological PLAG1 inhibition demonstrated synergistic effects with sorafenib across preclinical HCC models, though functional characterization of the PVT1/miR-195-5p/PLAG1 axis in ferroptosis modulation remains incomplete due to observed phenotypic heterogeneity⁶⁴.

3.1.2.2 The lipid peroxidation pathway

In ferroptosis, polyunsaturated fatty acid (PUFA) is activated into PUFA-CoA by Acyl-CoA synthetase long-chain family 4 (ACSL4) and subsequently esterified into phospholipid (PL)-PUFA complexes via LPCAT3. Under iron-dependent catalysis by lipoxygenases (LOXs) or ROS, PL-PUFA undergoes peroxidation to generate phospholipid hydroperoxides (PL-OOH). In the absence of GPX4-mediated detoxification, PL-OOH undergo pathological accumulation⁶⁵.

ACSL4 is an important regulator of fatty acid metabolism and a key determinant of cellular sensitivity to ferroptosis⁶⁶. ACSL4 and GPX4 respectively regulate ROS accumulation in positive and negative directions⁶⁷. ACSL4 inhibition leads to drug resistance in HCC. For example, Lu et al. observed a greater than tenfold increase in miR-23a-3p expression within sorafenib-resistant cells relative to the parental cell line. The upregulation of miR-23a-3p directly inhibits ferroptosis by suppressing ACSL4, blocking sorafenib sensitivity in HCC⁶⁸. Additionally, depletion of PUFA contributes to sorafenib resistance. Zhao et al. demonstrated a time-dependent decrease in lncRNA HNF4A-AS1 expression during prolonged sorafenib treatment, potentially mediated by HIF-1 α activation under hypoxic stress. HNF4A-AS1 destabilizes DECR1 mRNA via METTL3-mediated m6A methylation with YTHDF3 as the reader protein. However, HNF4A-AS1 deficiency leads to DECR1 overexpression, accelerating PUFA oxidation to deplete PUFA reservoirs and confer sorafenib resistance⁶⁹.

3.1.2.3 The iron metabolism pathway

Iron metabolism during ferroptosis comprises the following pathway: extracellular Fe³⁺ is transported into cells via the transferrin–transferrin receptor complex⁷⁰, then reduced to Fe²⁺ by the six-transmembrane epithelial antigen of prostate family member 3 (STEAP3) metal reductase in endosomes, and finally released into the cytosol through divalent metal transporter 1 (DMT1)⁷¹.

Excess Fe^{2+} is stored as ferritin (FTH1: Oxidizes Fe^{2+} to Fe^{3+} , promoting iron storage; FTL: Participates in the structural stability of ferritin and iron storage) or exported via membrane ferroportin 1 (FPN1)⁷². A fraction of intracellular Fe^{2+} participates in formation of the unstable labile iron pool (LIP), where highly oxidized Fe^{2+} generates highly toxic hydroxyl free radicals (ROS) through the Fenton reaction, effectively inducing cancer cell death⁷³. HCC cells develop drug resistance by inhibiting various steps of the process through intrinsic genetic alterations.

Sun et al. discovered that downregulation of miR-654-5p led to increased levels of the downstream protein heat shock protein family B member 1 (HSPB1), which inhibits iron uptake by suppressing transferrin receptor protein 1 (TFR1)⁷⁴. EZH2 enhances H3K27me3 in hepatocellular HCC, leading to reduced RNA polymerase II binding at the TFR2 promoter region, which ultimately results in downregulation of TFR2 expression⁷⁵. A significant decrease in the intracellular iron content reduces free radical production from the Fenton reaction, thereby inhibiting sorafenib-induced ferroptosis and enhancing drug resistance. Additionally, Gao et al. revealed that nuclear receptor coactivator 4 (NCOA4) binds ferritin for lysosomal degradation, elevating intracellular free iron. Elevated lncRNA URB1-AS1 suppresses NCOA4, thereby reducing free iron and ROS levels to inhibit sorafenib-induced ferroptosis.⁶⁰ Hao et al. identified sorafenib-induced upregulation of DUSP4 as an adaptive feedback response to MAPK pathway inhibition. Mechanistically, DUSP4-mediated phosphorylation of YTHDC1 at threonine residue T148 enhances its binding to SRSF3, thereby reducing nuclear retention of FTH1/FTL mRNA. This elevates FTH1/FTL translation efficiency, ultimately suppressing ferroptosis⁷⁶.

3.1.3 Autophagy

Autophagy, a self-degrading system usually accompanied by cell death (not a death mechanism), constitutes a lysosome-mediated physiological process for degrading and recycling damaged proteins and organelles. Autophagy is essential for maintaining cell homeostasis in unfavorable environments; dysregulated autophagy often poses substantial health risks with dichotomous effects (e.g., inhibiting tumor initiation while supporting tumor progression)^{77, 78}. Classical autophagy encompasses a multiphasic sequence comprising four pivotal stages: initiation, nucleation, maturation, and degradation. These stages involve the sequential activation and selective recruitment of autophagy-related proteins (ATGs). Upstream regulators of autophagy include mechanistic target of rapamycin

kinase 1 (mTORC1; inhibition) and adenosine monophosphate-activated protein kinase (AMPK; activation). Initiation involves activation of the ULK complex, which includes UNC-51-like kinase 1 (ULK1), ULK2, FIP200, ATG13, and ATG101. Nucleation comprises the response of autophagy-specific VPS34 complex I (including VPS34, beclin-1, ATG14, and VPS15) to the ULK complex, thereby catalyzing phosphatidylinositol-3-phosphate (PI3P) production on the autophagosome membrane. During autophagosome maturation, PI3P recruits the autophagy coupling machinery, in which the ATG5/ATG12/ATG16L1 complex guides ATG3 and ATG7 to conjugate LC3 to phosphatidylethanolamine (PE) on the membrane, forming LC3-II. ATG4 cleaves LC3 into its soluble form (LC3-I), enabling the link between LC3 and PE. Cancer cells can epigenetically modify any of these steps to achieve drug resistance ^{77, 78, 79} (**Figure 4**).

Li et al. found that sorafenib treatment led to miR-21 enrichment in the affected nucleus and promoted expression of the lncRNA SNHG1. SNHG1 activates the Akt/mTOR signaling pathway by promoting SLC3A2 transcription, thus inhibiting autophagy. This process decreases the capacity for autophagy to promote cell death, leading to sorafenib resistance ^{80, 81}. Additionally, miR-25 was highly expressed in HCC tissues, where it negatively regulated the expression of the downstream suppressor gene F-box and WD repeat domain-containing 7 (FBXW7), a component of the SKP1-cullin-1-F-box-protein (SCF) E3 ligase complex involved in autophagy-related ubiquitination. MiR-25 promotes autophagy and mediates sorafenib resistance by degrading FBXW7 mRNA and upregulating LC3-II protein levels ⁸². Hu et al. found that compared with neighboring tissues, HCC tissues showed increased miR-21-5p expression. miR-21-5p promoted the expression of ubiquitination-specific peptidase 24 (USP24); the interaction between USP24 and SIRT7 decreased the level of SIRT7 ubiquitination in HCC cells, while upregulating the LC3-II/I ratio and beclin-1 level. These modifications help to enhance protective autophagy in HCC cells, thus inducing sorafenib resistance ^{83, 84}. Chen et al. reported the combined use of hydroxychloroquine and sorafenib could overcome sorafenib resistance through inhibition of the Toll-like receptor 9 (TLR9)/SOD1/hsa-miR-30a-5p/beclin-1 resistance axis. Hsa-miR-30a-5p, a key miRNA targeting ATG5 and beclin-1, is significantly downregulated in drug-resistant cells; this alteration leads to significant autophagosome enrichment ⁸⁵. ATG4B plays a crucial role in autophagy and reportedly promotes HCC resistance ⁸⁶. Chen et al. found that the lncRNA CRNDE triggers protective autophagy by upregulating ATG4B in

HCC cells via sequestration of miR-543, whereas sorafenib therapy activates the CRNDE/ATG4B/autophagy pathway and reduces sorafenib sensitivity in HCC ⁸⁷. Lin et al. found that METTL3 was significantly downregulated in sorafenib-resistant cells; its key downstream target was identified as FOXO3. METTL3 downregulation inhibits m6A-dependent FOXO3 methylation and directly induces the transcription of ATG3, ATG5, ATG7, ATG12, ATG16L1, and other ATGs in HCC. Additionally, YTHDF1 is blocked from recognizing its m6A binding site, resulting in decreased FOXO3 mRNA stability; this process increases autophagic flux and induces sorafenib resistance ⁸⁸. Tao et al. found that the lncRNA BBOX1-AS1 was highly expressed in HCC. Furthermore, BBOX1-AS1 enhanced the stability of PHF8 mRNA by targeting miR-361-3p. PHF8 (i.e., KDM7B) is a histone demethylase that can trigger protective autophagy in HCC cells, leading to sorafenib resistance ⁵⁴.

3.2 Epigenetic modifications reprogram tumor metabolism

A defining feature of malignant tumors is unlimited proliferation, such that the body's normal metabolism cannot meet the corresponding growth requirements. To solve this problem and adapt to the harsh conditions created by drug treatments, cancer cells rewire their metabolic pathways to ensure survival, proliferation, and metastasis. Unlike normal cells, which primarily rely on glucose metabolism to meet their energy needs, most malignant tumors harness glucose metabolism along with a range of other substances, including lipids and cholesterol, to produce the energy necessary for their unchecked growth. To maximize the utilization of these additional substances, cancer cells undergo autonomous epigenetic modifications that increase the expression of proteins associated with metabolic function, greatly reducing their susceptibility to targeted drugs and facilitating resistance ^{89, 90} (Figure 5).

3.2.1 Carbohydrate metabolism

The liver has a high level of glucose metabolism, and aerobic glycolysis is essential for the rapid division of liver cancer cells. Zhang et al. found that glycolysis is usually more active in sorafenib-resistant HCC cells than in normal cells; this difference can be attributed to the downregulation of miR-374b expression in HCC cells under continuous sorafenib stimulation, which reduces the inhibitory effects on hnRNPA1. Upregulation of hnRNPA1 contributes to a shift in pyruvate kinase (PKM) gene splicing from PKM1 to PKM2. PKM2 is a key regulator of glycolysis and oxidative

phosphorylation; its enrichment in rapidly proliferating cells leads to sorafenib resistance⁹¹. Another study showed that sorafenib resistance was mediated by endoplasmic reticulum stress (ERS) and subsequent PKM2 accumulation. ERS activation significantly downregulated miR-188-5p expression and directly upregulated hnRNPA2B1 expression in HCC cells. hnRNPA2B1 promotes survival and suppresses apoptosis by upregulating PKM2⁹². Cardiotrophin-like cytokine 1 (CLCF1) is inversely regulated by upstream miR-30a-5p. Zhang et al. reported that sorafenib treatment significantly reduced the expression of miR-30a-5p, whereas overexpression of CLCF1 activated the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway and multiple glycolytic genes. This enhanced glycolytic process produces sorafenib resistance in HCC cells⁹³. High miR-494 levels negatively regulate glucose 6-phosphatase catalytic subunit (G6pc), resulting in the inhibition of glucose 6-phospho-glucose conversion; the accumulation of glucose 6-phosphate helps promote glycolytic metabolism and glycogen accumulation. Additionally, miR-494 blocks oxidative phosphorylation by activating hypoxia-inducing factor 1A (HIF-1A) pathway, which leads to lipid droplet storage. Enhancement of glycolysis process and lipid drop accumulation both create a favorable environment for HCC cell resistance to sorafenib-induced death, but the dominant role is glycolysis⁹⁴.

3.2.2 Lipid metabolism

Lipid metabolism reprogramming is a key feature of malignant tumors; the change of fatty acid metabolism in HCC cells is a distinguishing feature from normal liver cells. Increases in lipid uptake, storage, and lipogenesis contribute to rapid growth and drug resistance⁹⁵. The prevalence of metabolic-associated fatty liver disease (MAFLD) is increasing worldwide. In MAFLD patients, the liver is overwhelmed by the need to degrade fatty acids, resulting in toxic lipid accumulation and an increased risk of liver cancer⁹⁶. The lncRNA LINC01468, significantly upregulated in MAFLD and HCC, disrupts SH2 domain-containing inositol polyphosphate 5-phosphatase-2 (SHIP2) protein stability by modulating Cullin 4A (CUL4A)-mediated ubiquitination; this process activates the PI3K/AKT/mTOR signaling pathway, promoting de novo lipid biosynthesis and sorafenib resistance⁹⁷. Chen et al. observed significantly downregulated expression of lncRNA LINC01056 in the context of sorafenib resistance, and the underlying mechanism involved cancer cell transformation from glycolysis to fatty acid oxidation. LINC01056 knockout cells showed high levels of fatty acid oxidation (FAO) activity during sorafenib treatment. HCC tumor cells preferentially use FAO (rather than glycolysis) to increase

energy production (i.e., adenosine triphosphate [ATP] accumulation), yielding sorafenib resistance. Peroxisome proliferator-activated receptor α (PPAR α) is a ligand-activated transcription factor responsible for maintaining a balance between FAO and glycolysis. LINC01056-mediated sorafenib resistance is achieved by regulating the post-transcriptional activity of PPAR α ⁹⁸. From the above mechanisms, it is evident that the regulation of fatty acid oxidation (FAO), whether up- or down-regulated, contributes to sorafenib resistance. Indeed, the emergence of drug resistance in cancers can be linked to energy accumulation, and FAO serves as a significant source of energy. When FAO levels decline, this is frequently accompanied by an intensification of glycolysis or an accumulation of lipid droplets. The accumulation of these lipid droplets allows HCC to switch to FAO in a timely manner to supply energy when glycolysis becomes insufficient. The metabolism of HCC is a highly intricate physiological process, with various metabolic pathways often not functioning independently but rather complementing each other. This interdependence may explain why the bidirectional regulation of FAO promotes sorafenib resistance.

3.2.3 Amino acid metabolism

Altered amino acid metabolism is needed to support the malignant tumor phenotype and enhance anticancer drug resistance. Aspartic acid is essential for protein synthesis and nucleotide biosynthesis; however, cancer cells cannot convert asparagine to aspartic acid because they lack asparaginase activity ⁹⁹. Therefore, cancer cells extensively reprogram intracellular aspartic acid cycling to maintain sufficient levels of aspartic acid. Chen et al. found that the lncRNA LINC01234 binds the promoter of arginine succinate synthase 1 (ASS1) to inhibit its transcriptional activation, thereby suppressing the conversion of aspartic acid to urea in cancer cells. Additionally, the increase in cellular aspartic acid leads to activation of the mTORC1 pathway; increased mTORC1 activity similarly contributes to the development of sorafenib resistance in HCC ¹⁰⁰.

3.3. Epigenetic modifications facilitating the formation and maintenance of drug-resistant cells

Previous studies have demonstrated that stemness, which enables the formation and maintenance of drug-resistant cells, is a pivotal factor involved in drug resistance ¹⁰¹. Stemness primarily refers to the phenomenon where, after the majority of cancer cells are eliminated by drug treatment, a small subpopulation survives and undergoes renewal. These surviving cells exhibit acquired drug resistance, which they can sustain and transmit over an extended period, thereby impeding tumor eradication ¹⁰²

(Figure 6).

3.3.1 Cancer stem cell expansion

Cancer stem cells (CSCs), alternatively referred to as tumor-initiating cells (T-ICs), constitute the primary cellular group that imparts tumor stemness¹⁰³. These stationary, self-renewing cells reside within primary cancers, nestled in tumor niches where their extensive functional potential fuels cancer growth by re-establishing heterogeneity. Theoretically, a single CSC can rebuild an entire tumor; this near-immortality also provides robust treatment resistance¹⁰⁴.

MiR-3677-3p, upregulated in HBV+ HCC, drives sorafenib resistance by suppressing F-box only protein 31 (FBXO31) -a tumor-suppressive stem cell factor (SCF) ubiquitin ligase component. FBXO31 downregulation reduces forkhead box protein M1 (FOXM1) ubiquitination, stabilizing this oncoprotein; FOXM1 further activates OCT4 to enhance CSC expansion, thus sustaining therapeutic resistance^{105 106}. Chen et al. also observed high expression of DNMT3a and TET2 in CSCs, revealing close coordination between these two enzymes. The DNMT3a–TET2 axis promotes oncosphere formation and enhances the tumor-reconstructing capacity of CSCs, thereby increasing HCC tolerance to sorafenib¹⁰⁷. The upregulation of HDAC11 in HCC inhibits liver kinase B1 (LKB1) expression by triggering acetylation of histone H3 lysine 9 (H3K9ac) in the downstream LKB1 promoter region. Subsequent inhibition of the downstream AMPK signaling pathway indirectly enhances glycolysis and continuously activates CSCs, maintaining their stemness and sorafenib resistance¹⁰⁸. MiR-93 is also highly expressed in liver T-ICs, where it enhances self-renewal and tumorigenesis by downregulating myotubularin-associated protein 3 (MTMR3)¹⁰⁹. Qu et al. found that miR-361-3p upregulation promotes the expansion of liver T-ICs by negatively regulating SRY-box transcription factor 1 (SOX1)¹¹⁰. Furthermore, KDM5B (histone demethylase) is upregulated in sorafenib-resistant HCC and drives therapeutic resistance through activating PI3K/Akt pathway and CSC trait potentiation¹¹¹. Lai et al. demonstrated that elevated serum IL-6 levels were associated with significantly shorter overall survival in HCC patients. IL-6 was shown to upregulate DNMT3b and OCT4 expression. As a critical transcription factor, OCT4 promotes the conversion of HCC cells into stem-like cells. Mechanistically, IL-6 enhances OCT4 expression via a STAT3-dependent pathway involving DNMT3b, potentially facilitating sorafenib resistance¹¹². LARP4B, upregulated by METTL3-mediated, IGF2BP3-dependent m6A modification, promotes cancer stemness progression and impairs sorafenib efficacy by activating

the SPINK1-mediated EGFR pathway. Inhibition of LARP4B enhances the antitumor effect of sorafenib by disrupting the positive feedback loop of the LARP4B/SPINK1/p-AKT/C/EBP- β axis¹¹³. METTL3 stabilized and increased lncRNA KIF9-AS1 expression through an m6A-IGF2BP1-dependent mechanism. KIF9-AS1 enhances cancer stemness and sorafenib resistance in HCC by promoting Ubiquitin-specific peptidase 1 (USP1)-mediated deubiquitination of short stature homeobox (SHOX2)¹¹⁴.

3.3.2 Enhanced EMT

Epithelial–mesenchymal transition (EMT) is a process through which epithelial cells are transformed into mesenchymal cells. It is closely linked to the phenotypic heterogeneity of cancer cells and helps them rapidly adapt to various injuries, facilitating drug resistance¹¹⁵. Its key characteristics are loss of the epithelial marker E-cadherin and increased expression of the mesenchymal marker vimentin¹¹⁶. Notably, there exists a considerable overlap between the signaling pathways activated during EMT and those that propel cancer stem cells (CSCs), such as the Wnt, Hedgehog, and Notch signaling pathways. This overlap may offer partial insights into the heightened drug resistance¹¹⁷. Furthermore, some certain epigenetic regulatory factors contribute to sorafenib resistance by regulating the expression of EMT-associated proteins.

Sun et al. detected significantly diminished expression of the lncRNA LIMT (LINC01089) in HCC, which was associated with poor prognosis. The downregulation of LINC01089 contributes to increased expression of miR-665, which promotes sorafenib resistance by enhancing EMT¹¹⁸. Xu et al. reported that the lncRNA H19 reduced E-cadherin expression by promoting the expression of miR-675 and enhancing tumor cell migration and invasion properties, leading to sorafenib resistance¹¹⁹. HCC cells with upregulated LINC00540 exhibited intrinsic sorafenib resistance, an irregular and elongated EMT-like phenotype, and decreased E-cadherin expression. This resistance mechanism is attributed to LINC00540-mediated upregulation of aldo-keto reductase family 1 member C2 (AKR1C2) expression and promotion of EMT through competitive binding with miR-4677-3p¹²⁰. MiR-125b-5p also enhances EMT in HCC by targeting Ataxin-1; this interaction regulates sorafenib resistance¹²¹. Expression of the lncRNA DUXAP8 was significantly higher in HCC than in para-cancer normal tissues, potentially because of METTL3 binding to the m6A site on DUXAP8. Mechanistic analysis revealed that DUXAP8 activates the MAPK/ERK pathway via competitive binding to miR-584-5p,

thereby enhancing globule formation by HCC cells and promoting the expression of stem cell-related genes¹²². KIAA1429, a core m6A methyltransferase component, is upregulated in sorafenib-resistant liver cancer cells. Ye et al. reported that aberrant upregulation of KIAA1429 and the associated m6A methylation led to enhanced EMT, partially explaining the intrinsic origin of sorafenib resistance¹²³. CircMEMO1 functions as a molecular sponge for miR-106b-5p, enhancing TET1 expression and elevating Transcription Factor 21 (TCF21) mRNA 5hmC levels. This mechanism suppresses EMT and diminishes the stemness of HCC, thereby increasing the sensitivity of HCC cells to sorafenib. However, circMEMO1 expression is markedly downregulated in HCC tissues. The disruption of the circMEMO1/miR-106b-5p/TCF21 regulatory axis contributes to reduced sorafenib sensitivity in HCC¹²⁴.

3.4 Epigenetic modifications activates uncontrolled cell proliferation signaling pathway activity

Sorafenib primarily acts on serine-threonine kinases (e.g., Raf-1) and receptor tyrosine kinases such as VEGFR and platelet-derived growth factor receptor β (PDGFR- β). Complex signaling pathways within cancer cells respond to this targeted inhibition by suppressing angiogenesis and cell proliferation¹²⁵. Although sorafenib influences metastasis and proliferation through multiple targets, such as VEGFR, Raf, and PDGFR, some HCC tumors do not overexpress these targets.¹²⁶ An additional challenge is that epigenetic changes also mitigate sorafenib toxicity by selectively downregulating important targets in cell signaling pathways. Here, we explore how sorafenib resistance is induced via dysregulation of downstream cell proliferation signaling pathways through epigenetic modifications (**Figure 7**).

3.4.1 The PTEN/AKT/mTOR signaling pathway

The PI3K/AKT/mTOR signaling pathway is one of the most frequently dysregulated pathways in human cancers¹²⁷. PTEN is an important tumor suppressor that antagonizes the highly carcinogenic AKT/mTOR pathway¹²⁸. However, certain epigenetic regulators reduce PTEN expression or directly activate AKT, causing uncontrolled PTEN/AKT/mTOR signaling that desensitizes cancer cells to sorafenib¹²⁹. For example, miR-19a-3p is highly expressed in HCC; it promotes HCC metastasis and sorafenib resistance by inhibiting PTEN¹³⁰. CircITCH also participates in downstream regulation of

the PTEN/PI3K/Akt pathway by sponging miR-20b-5p, a negative regulator of PTEN. Continuous sorafenib treatment decreases circITCH expression in HCC cells and enhances downstream overexpression of miR-20b-5p, thereby reducing the tumor suppressor activity of PTEN ¹³¹.

3.4.2 The Ras/Raf/MEK/ERK signaling pathway

The Ras/Raf/MEK/ERK pathway is primarily known for promoting cell proliferation and survival, and its dysregulation is often associated with malignant tumor onset ¹³². Additionally, this pathway is involved in regulating apoptosis, cell cycle progression, cell migration, differentiation, metabolism, and other processes ¹³³. The significantly increased expression levels of Ras/Raf/MEK/ERK kinases in HCC play important roles in maintaining and promoting HCC differentiation ¹³⁴. Ras, a signal transduction activation switch, is reportedly mutated in 30% of HCC cases, suggesting that genetic alterations strongly contribute to HCC. Next, we discuss how the regulation of Ras/Raf/MEK/ERK pathway activation by epigenetic modifications induces sorafenib resistance.

Positive expression of HBV-encoded X protein (HBx) is a poor prognostic factor for HCC patients. HBx induces upregulation of the lncRNA TRERNA1, which targets miR-22-3p to activate the Ras/Raf/MEK/ERK signaling pathway, thereby promoting cell proliferation and sorafenib resistance ¹³⁵. Xu et al. found that circRNA-SORE, upregulated in sorafenib-resistant HCC cells, hindered the degradation of Y-box-binding protein 1 (YBX1) by E3 ubiquitin ligase processing factor 19 (PRP19) and prevented nuclear translocation of the carcinogenic protein YBX1. Thus, overexpression of downstream targets, including AKT, Raf-1, and ERK, mediates sorafenib resistance ¹³⁶. Liu et al. found that Src collagen homology 3 (Shc3) was significantly upregulated in HCC compared with normal liver tissue; this upregulation was caused by hypomethylation of its upstream promoter, which enhanced c-Jun binding. The resulting positive feedback loop promoted high Shc3 expression and c-Jun phosphorylation. Using major vault protein (MVP) as a bridge, this positive feedback loop enhanced Shc3-mediated MEK and ERK phosphorylation, creating a resistance pathway independent of c-Raf ¹³⁷. Additionally, Shc3 activates the β -catenin/T-cell factor (TCF) pathway by interacting with the cytoplasmic components of β -catenin, which reduces β -catenin degradation and increases the expression of MDR1 (multidrug resistance 1) in HCC, resulting in multidrug resistance ¹³⁸.

3.4.3 Alternative signaling pathway

The aforementioned downstream pathways are classical downstream receptor tyrosine kinase (RTK)

signaling pathways. Importantly, some HCC cells do not achieve resistance by direct inhibition of targeted drugs; instead, they activate alternative pathways that bypass the inhibitory effects of such drugs, promoting resistance. Sha et al. reported that sorafenib resistance can arise through activation of RTK replacement pathways to compensate for the loss of MAPK/ERK signaling pathways during sorafenib-mediated Raf inhibition. PLEKHG5, a Rho GTPase activator that responds to various extracellular and intracellular signaling pathways, is highly upregulated in sorafenib-resistant cells; HDAC2-mediated lysine deacetylation promotes its stability. PLEKHG5 overexpression promotes Rac1 and its downstream AKT/ NF- κ B phosphorylation, reducing sorafenib sensitivity¹³⁹.

3.5 Epigenetic modifications enable abnormal transport process

The potency of the anti-tumor effects of targeted drugs partly depends on the concentration of the drug accumulated within tumor cells. Additionally, the concentration of drugs within cancer cells hinges on the expression level of drug transporter proteins on the cell membrane. Malignant tumors can regulate the amount of transporter proteins through epigenetic mechanisms to decrease their internal drug concentration, thereby developing drug resistance. Another transport-related mechanism of drug resistance involves exosomes, which serve as crucial mediators for the transfer of drug resistance between resistant and sensitive cells, and are also regulated by epigenetic mechanisms. Here, we summarize the contributions of epigenetic modifications to transport-mediated sorafenib resistance in HCC (Figure 8).

3.5.1 Drug transporters

The elevated expression of ATP-binding cassette (ABC) transporters in HCC promotes drug efflux-mediated drug resistance¹⁴⁰. Some epigenetic regulators exacerbate sorafenib resistance by regulating ABC expression. For example, in the classical Wnt/ β -catenin signaling pathway, β -catenin translocates into the nucleus to regulate gene expression activity. Li et al. reported that miR-138-1-3p was significantly downregulated in sorafenib-resistant HCC, accompanied by an upregulation of its target, p21-activated kinase 5 (PAK5). PAK5 enhances the phosphorylation and nuclear translocation of β -catenin, promoting the transcriptional activity of the drug efflux protein ABCB1. Increased ABCB1 expression promotes drug resistance by exporting sorafenib. Importantly, miR-138-1-3p mimics have the potential to reverse this resistance¹⁴¹.

Another class of transporters has been identified; whereas ABC transporters are responsible for drug efflux, this new class of transporters is responsible for drug uptake into HCC cells ¹⁴². Reduced expression of these transporters leads to resistance through insufficient drug accumulation within HCC cells ¹⁴³. Chen et al. reported that the lncRNA NIFK-AS1 is upregulated by METTL3-dependent m6A methylation in HCC, resulting in the downregulation of the drug transporters OATP1B1 and OATP1B3, which are mainly responsible for sorafenib uptake. This reduction in OATP1B1 and OATP1B3 levels impairs sorafenib sensitivity in HCC cells, thereby contributing to sorafenib resistance at high NIFK-AS1 levels ¹⁴⁴. Hepatocyte nuclear factor 3 γ (HNF3C γ) serves as the core of a regulatory system that promotes LCSC differentiation and inhibits malignant transformation. Importantly, it enhances the sorafenib response through activation of the drug transporters OATP1B1 and OATP1B3. However, Zhou et al. revealed that METTL14-dependent m6A modification downregulates HNF3C- γ expression, directly leading to sorafenib resistance ¹⁴⁵.

3.5.2 Exosomes

Exosomes are cell-derived nanovesicles comprising bilateral lipid membranes that release their contents upon fusion with the cell membrane, facilitating intercellular communication and activating signaling pathways in target cells ¹⁴⁶. Additionally, exosome contents usually include proteins, DNA, and ncRNAs; drug-sensitive cells can develop resistance by absorbing the contents of exosomes from drug-resistant cells ¹⁴⁷. HCC cells generally produce more exosomes than normal cells which contribute to sorafenib resistance in HCC cells.

High expression of miR-4669 in circulating exosomes is a risk factor for poor HCC prognosis. A study revealed that miR-4669-overexpressing HCC cells exhibited gradual upregulation of SIRT1 and elevated levels of the lncRNA MVIH. Although no direct interaction between SIRT1 and MVIH was evident, their overexpression promoted microvascular infiltration in HCC tissues and enhanced sorafenib resistance ¹⁴⁸. Extracellular vesicles (EVs) derived from cancer-associated fibroblasts (CAFs) are rich in miR-1228, which inhibits stress-induced apoptosis; the communication property of EVs helps to transmit this inhibition. CAF-derived EVs deliver miR-1228-3p, which enhances sorafenib resistance by modulating the placenta-related protein 8 (PLAC8)/PI3K/AKT axis¹⁴⁹. The GOLPH3 oncoprotein is highly expressed in HCC, where it increases the BCL-2/Bax ratio and promotes sorafenib resistance by upregulating the exosomal level of miR-494-3p and enhancing PTEN

targeting¹⁵⁰. Huang et al. identified a significant enrichment of circRNA circUPF2 in exosomes derived from sorafenib-resistant HCC cells. Functioning as a molecular scaffold, circUPF2 recruits insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2), which stabilizes the downstream target SLC7A11 mRNA. This stabilization suppresses ferroptosis in HCC cells, ultimately conferring sorafenib resistance to previously sensitive HCC cells¹⁵¹. MiR-93 is markedly overexpressed in sorafenib-resistant HCC cells, where it drives drug resistance by directly targeting PTEN. Notably, miR-93 is selectively packaged into exosomes secreted by these resistant cells. These miR-93-laden exosomes act as critical vehicles for disseminating sorafenib resistance, reactivating the PI3K/AKT signaling pathway through their PTEN-targeting activity¹⁵².

4. Epigenetic modifications involved in lenvatinib resistance

More than a decade after the approval of sorafenib, a randomized phase III non-inferiority trial of lenvatinib demonstrated benefits in median and progression-free survival¹⁵³. In 2018, lenvatinib was approved by the FDA as a first-line targeted therapy for unresectable HCC¹⁵⁴. Lenvatinib has demonstrated non-inferiority to sorafenib in terms of overall survival (OS) and superiority across all secondary efficacy endpoints¹⁵⁵. However, despite its promising efficacy, the treatment of HCC (HCC) with lenvatinib is similarly impeded by the emergence of drug resistance. Given its relatively shorter clinical application history compared to sorafenib, research on the mechanisms underlying lenvatinib resistance is somewhat limited. In this chapter, we present an overview of the reported epigenetic mechanisms involved in lenvatinib resistance, with a particular emphasis on ncRNA regulation, RNA methylation, and histone post-translational modifications (**Figure 9**).

4.1 NcRNA regulation promotes lenvatinib resistance

4.1.1 NcRNAs enables tumors to escape programmed cell death

Yu et al. showed that lenvatinib promoted the expression of lncMT1JP, which increased the downstream expression of BCL-2 via competitive binding to miR-24-3p and decreased the cleavage of caspase-3, thereby enhancing resistance to the apoptosis-inducing effect of lenvatinib⁴⁸. Chronic lenvatinib exposure suppresses miR-128-3p expression in HCC cells, thereby driving hyperactivation of the c-Met/Akt/ERK signaling axis. Mechanistically, Akt activation inhibits phosphorylation of

GSK3 β , which subsequently blocks caspase-9 and caspase-3 proteolytic cleavage via GSK3 β inactivation, ultimately establishing an anti-apoptotic program that confers lenvatinib resistance¹⁵⁶. CircPIAS1 is upregulated in HCC and competitively sponges miR-455-3p to enhance NUPR1 expression, which transcriptionally activates FTH1. This cascade suppresses Fe²⁺-dependent Fenton reactions and confers lenvatinib resistance. Notably, combining ZZW-115 (a NUPR1 inhibitor) with lenvatinib synergistically suppresses tumor growth in circPIAS1-overexpressing murine models. Further investigation is warranted to delineate the precise mechanisms through which NUPR1 modulates ferroptosis pathways, which is critical for developing comprehensive therapeutic targeting strategies¹⁵⁷. The lncRNA HOTAIRM1, a conserved Hox gene cluster, is significantly upregulated in lenvatinib-resistant HCC; it activates the downstream autophagy-related factor beclin-1 and triggers autophagy to promote lenvatinib resistance through the suppression of miR-34a, a molecular sponge of HOTAIRM1¹⁵⁸.

4.1.2 NcRNAs enable the formation and maintenance of drug-resistant cells

Han et al. demonstrated that miR-183-5p overexpression in HCC induces MUC15 downregulation. MUC15 can restrict T-IC expansion by destabilizing c-MET and suppressing PI3K/AKT/SOX2 signaling. MUC15 degradation led to an increased proportion of T-ICs in HCC tissues, enhancing malignant proliferation and promoting lenvatinib resistance¹⁵⁹. Additionally, HCC cells and LCSCs display elevated expression of miR-3154, which promotes self-renewal in HCC by downregulating the mRNA and protein expression levels of hepatic nuclear factor 4 alpha (HNF4 α). This effect of miR-3154 results in a poor response to lenvatinib¹⁶⁰. Lnc-ZEB2-19 suppresses HCC stemness and metastasis by binding to Transformer 2 α (TRA2A) to induce RSPH14 mRNA degradation. Paradoxically, the expression of lnc-ZEB2-19 is commonly downregulated in HCC, which promotes lenvatinib resistance development¹⁶¹. Circ0007386 is significantly upregulated in HCC and acts as a molecular sponge for miR-507 to alleviate its suppression of CCNT2, thereby driving EMT and lenvatinib resistance¹⁶².

4.1.3 NcRNAs reprogram tumor metabolism

LINC01532 exhibits m6A-mediated upregulation in HCC and interacts with hnRNPK to facilitate G6PD pre-mRNA splicing. This post-transcriptional regulation enhances G6PD expression and pentose phosphate pathway (PPP) flux, resulting in accelerated NADPH biosynthesis. The

accumulated NADPH reinforces cellular antioxidant defenses, thereby attenuating lenvatinib-induced oxidative stress and apoptosis in HCC cells¹⁶³.

4.1.4 ncRNAs enable uncontrolled cell proliferation signaling pathway activity

CircCCNY recruits the E3 ubiquitin ligase SMURF1 to induce HSP60 ubiquitination and proteasomal degradation. This process liberates Raf kinase inhibitory protein (RKIP), thereby inactivating the MAPK survival signaling pathway through RKIP-mediated phosphatase recruitment, which consequently potentiates lenvatinib-induced tumor cell apoptosis. However, high-throughput sequencing analysis revealed a paradoxical downregulation of circCCNY in HCC, which likely impairs circCCNY's ability to suppress the MAPK pathway, thereby contributing to lenvatinib resistance¹⁶⁴. HCC with high basal Aurora-A expression levels is more prone to developing lenvatinib resistance. The underlying mechanism involves Aurora-A positively regulating hsa-circ-0058046 to competitively inhibit miR-424-5p and activate FGFR1 signaling. Combination therapy using the Aurora-A inhibitor MLN-8237 with lenvatinib synergistically reverses lenvatinib resistance. However, this study lacked human tissue samples to measure hsa-circ-0058046 expression in cancerous tissues and analyze its correlation with clinical features, particularly regarding lenvatinib resistance¹⁶⁵.

4.2 RNA methylation promotes lenvatinib resistance

METTL3 catalyzes m6A methylation of frizzled10 (FZD10) mRNA to modulate FZD10 expression. YTHDF2 is responsible for stabilizing the modified FZD10 mRNA. After upregulation, FZD10 promotes self-renewal of hepatic CSCs by activating β -catenin and YAP1; it also reduces HCC cell sensitivity to lenvatinib by activating the β -catenin/c-Jun/MEK/ERK axis. Furthermore, the promoter region of METTL3 contains c-Jun binding sites that enhance METTL3 expression, forming a stable positive feedback loop that contributes to persistent lenvatinib resistance¹⁶⁶. Additionally, mettl3-mediated m6A modification upregulates USP15 expression in HCC. USP15 stabilizes Lectin Galactoside-Binding Soluble 3 (LGALS3) by blocking its ubiquitin-proteasome system-mediated degradation, thereby activating the AKT/mTOR axis to promote cancer stemness, proliferation, and lenvatinib resistance. However, this study lacks clinical validation of the USP15/LGALS3/AKT/mTOR axis in lenvatinib-treated patient specimens, and the mechanistic details underlying USP15-driven AKT/mTOR activation remain unresolved¹⁶⁷. METTL1 and WD repeat-containing protein 4 (WDR4) were significantly upregulated in lenvatinib-resistant cells.

METTL1/WDR4 complex promoted the translation of EGFR pathway proteins through tRNA N7-methylguanosine (m7G) methylation modification, thereby conferring lenvatinib resistance¹⁶⁸. Wang et al. identified an amplification of the transcriptional coactivator 2 (CRTC2) gene located in the 1q21.3 region. CRTC2 interacts with PABP1 and recruits YTHDF2 to enhance the translation of specific m6A-mRNA via relocating mRNA from decay sites to polysomes. CRTC2-mediated enhanced translation of c-Jun confers resistance to lenvatinib¹⁶⁹.

N4-acetylcytidine (ac4C) is a conserved acetylation modification of mRNA. Although less studied than m6A, ac4C plays an important role in regulating gene expression stability¹⁷⁰. For example, ERS is a cancer cell defense mechanism induced by extracellular and intracellular stresses; this mechanism directly enhances the invasiveness and metastasis abilities of HCC cells by modifying the tumor microenvironment¹⁷¹. Pan et al. discovered that NAT10 is a lysine acetyltransferase that enhances ac4C modification of HSP90AA1 RNA and increases lenvatinib resistance in HCC cells during ERS.¹⁷²

4.3 Histone modification promotes lenvatinib resistance

Duan et al. reported that lncXIST was significantly upregulated in HCC and bound to the histone-modifying enzyme EZH2. This interaction led to increased H3K27me3 in the promoter region of nucleotide-binding oligomerization domain 2 (NOD2), thereby inhibiting NOD2 gene expression. Downregulation of the tumor suppressor NOD2 and subsequent activation of downstream ERK targets may partially explain the development of lenvatinib resistance^{173, 174}. Lu et al. discovered that HCC exhibit elevated lactylation levels, which promote lactylation at the K76 site of insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3), enhancing its binding to m6A-modified phosphate carboxykinase 2 (PCK2) and nuclear factor erythroid 2-related factor 2 (NRF2) mRNAs and boosting the antioxidant capacity of HCC cells. Upregulated PCK2 redirects carbon flux toward serine metabolism and one-carbon unit synthesis, maintaining high levels of S-adenosylmethionine (SAM) and serine. This metabolic shift reinforces m6A modification of PCK2 and NRF2 mRNAs, creating a positive feedback loop of IGF2BP3 lactylation-PCK2-SAM-m6A. This loop sustains elevated PCK2 and NRF2 expression, ultimately driving lenvatinib resistance¹⁷⁵.

4.4 Other lenvatinib mechanisms

CircMED27 exhibited marked upregulation in both serum and tumor tissues of HCC patients.

circMED27 functions as a molecular sponge for miR-655-3p, effectively relieving its inhibitory effect on the target gene USP28, thereby promoting USP28 protein expression. Subsequent functional analyses confirmed that this circMED27/miR-655-3p/USP28 regulatory axis critically contributes to the development of lenvatinib resistance in HCC¹¹. CircPIK3C3 is significantly downregulated in HCC, which relieves its negative regulation of miR-452-5p, leading to activation of the Wnt/ β -catenin signaling pathway and downregulation of the downstream tumor suppressor gene SOX15. This process has been demonstrated to be closely associated with lenvatinib resistance. However, the study did not establish a direct or more comprehensive link between SOX15 and lenvatinib resistance¹⁷⁶.

4.5 Shared epigenetic resistance mechanisms/targets of sorafenib and lenvatinib

The epigenetic mechanisms underlying sorafenib/lenvatinib resistance encompass shared regulatory processes, including DNA/RNA methylation, histone modifications, and non-coding RNA regulation and shared resistance outcomes involving dysregulated programmed cell death, metabolic reprogramming, enhanced cancer stem cell properties, and uncontrolled proliferative signaling pathways. Moreover, they exhibit shared core mechanisms in drug resistance evolution.

LncRNA Nuclear Paraspeckle Assembly Transcript 1 Variant 1 (NEAT1v1) switches HCC growth pattern from MEK/ERK-dependent to AKT-dependent via superoxide dismutase 2 (SOD2), conferring resistance to both sorafenib and lenvatinib¹⁷⁷. HCC cells with upregulated lncRNA AC026401.3 expression exhibit lenvatinib/sorafenib resistance. Mechanistically, AC026401.3 facilitates OCT1 recruitment to the E2F2 promoter, promoting its transcription. This transcriptional activation establishes an imbalance wherein tumor proliferation outpaces drug-induced apoptosis, ultimately driving resistance during prolonged exposure¹⁷⁸. Overexpressed YTHDF1 in CSCs drives CSC renewal and directly interacts with m6A-modified NOTCH1 transcripts, stabilizing their expression to promote lenvatinib/sorafenib resistance, exhibiting a dose-dependent resistance profile. Therapeutically, LNP-encapsulated YTHDF1-targeting siRNA synergistically suppressed tumor progression when combined with lenvatinib / sorafenib in murine models, revealing translational potential¹⁷⁹. Arechederra et al. identified an association between ADAMTSL5 hypermethylation and TKI resistance. Mechanistically, ADAMTSL5 enhanced resistance to multiple TKIs (e.g., sorafenib, lenvatinib) through RTK signaling activation, without altering receptor protein levels¹⁸⁰.

Additionally, sorafenib and lenvatinib resistance mechanisms share common molecular targets. For

instance, the LARP4B/IGF2BP3/SPINK1 axis mediates sorafenib resistance, while the IGF2BP3 lactylation-PCK2-SAM-m6A axis drives lenvatinib resistance, with IGF2BP3 serving as a shared target in both pathways.

5. Epigenetic drug resistance therapy and clinical translation

5.1 Targeting strategies for epigenetic drug resistance

Previous sections have outlined epigenetic mechanisms (e.g., DNA/RNA methylation, histone/non-coding RNA modifications) driving sorafenib/lenvatinib resistance in HCC. The central challenge lies in targeting these pathways to overcome resistance and guide clinical translation strategies. The epigenetic alterations in drug-resistant HCC cells offer novel avenues for early detection and modulation of drug resistance. The comprehensive review of epigenetic therapies provides valuable insights for developing strategies to reverse drug resistance and advancing their clinical translation.

5.1.1 Epigenetic drugs

Epigenetic therapies highlight the potential of epigenetic drugs to reverse HCC resistance. Current strategies focus on two approaches: the first approach involves combination therapies with other anticancer agents to block or reverse established drug resistance after its emergence¹⁸¹. The alternative strategy focuses on monitoring epigenetic changes during treatment and intervening with epigenetic drugs before the stabilization of resistant epigenetic states, which may delay or prevent resistance development - potentially enabling cancer to be managed as a chronic condition¹⁸². The two most extensively studied classes of epigenetic drugs currently are DNA methyltransferase inhibitors (DNMTis) and histone deacetylase inhibitors (HDACis)¹⁸³.

DNMTis, such as FDA-approved 5-azacytidine (5-aza) and decitabine (DAC), reverse malignant phenotypes by reactivating tumor suppressor genes via DNA hypomethylation. Other DNMTis that are not yet approved include SGI-110 (guadecitabine) and the recently developed selective DNMT1 inhibitor GSK3685032183^{184, 185}. To address the short half-life of 5-aza and DAC, zebularine, next-generation DNMTi, is an orally administered and more stable DNMTi that has been demonstrated preclinical efficacy¹⁸⁶. Emerging strategies combining DNMTis with sorafenib/lenvatinib demonstrate potential to reverse HCC resistance. For instance, nanaomycin A, a streptomyces-derived DNMT3b-selective quinone antibiotic, synergizes with sorafenib by suppressing IL-6-induced DNMT3b/OCT4

expression and restoring sorafenib sensitivity¹¹².

HDACis include vorinostat, belinostat, panobinostat, and romidepsin (FDA-approved), alongside investigational agents such as givinostat, resminostat, abexinostat, entinostat, mocetinostat, valproic acid, and butyrate^{185, 187}. Combining HDACis with sorafenib/lenvatinib shows promise in alleviating sorafenib/lenvatinib resistance. For instance, a phase I/II trial combining the HDAC inhibitor resminostat with sorafenib demonstrated safety and tolerability, achieving a 90% disease control rate in sorafenib-refractory HCC patients¹⁸⁸. The HDACi vorinostat reverses lenvatinib resistance by suppressing AKT activation, while co-treatment with lenvatinib and AKT/HDACi synergistically induces HCC cell apoptosis¹⁸⁹. Additionally, EZH2, as a key regulator of histone methylation, can be targeted by drugs that inhibit its activity to modulate epigenetic processes. Currently, the approved EZH2 inhibitors include tazemetostat and valemestostat (approved only in Japan), while other drugs in clinical trials include GSK126, CPI-1205, SHR2554 and PF-06821497¹⁹⁰. Studies have reported on the combined use of EZH2 inhibitors with targeted therapies to overcome drug resistance. Tazemetostat synergizes with sorafenib to enhance ferroptosis in sorafenib-resistant HepG2-SR cells⁷⁵.

Currently, epigenetic drugs remain limited to a few FDA-approved agents, with most candidates in preclinical or early clinical development. Future research should prioritize validating their therapeutic efficacy in HCC to unlock their significant potential. The synergistic combination of epigenetic drugs with sorafenib/lenvatinib poses a significant threat to resistant tumors. Despite substantial challenges in clinical translation, advances in research and technology are expected to accelerate their therapeutic implementation.

5.1.2 Targeting ncRNAs

ncRNAs are increasingly recognized as pivotal epigenetic regulators of cancer therapy resistance. Targeting these ncRNAs holds promise for reversing drug resistance and advancing clinical strategies. Current approaches primarily involve silencing oncogenic ncRNAs via small interfering RNA (siRNA) /short hairpin RNA (shRNA) -mediated knockdown. For example, lncRNA SNHG1 depletion restores sorafenib-induced apoptosis in HCC by suppressing the miR-21/SLC3A2/AKT axis⁸⁰. Similarly, circRNA-SORE silencing enhances sorafenib sensitivity through YBX1 downregulation¹³⁶. However, this paradigm does not universally apply, as resistance can also be mediated by downregulation of tumor-suppressive ncRNAs. Restoring their expression via mimics may resensitize resistant cells to

therapy. For instance, transfection of miR-128-3p mimics into HCC cells rescues lenvatinib's anti-proliferative efficacy in resistant HCC models.¹⁵⁶ The combination of specific inhibitors of ncRNAs with targeted drugs has also demonstrated promising efficacy against drug-resistant tumors. The combination of lncRNA MALAT1-IN1 (MALAT1 inhibitor) with sorafenib demonstrated enhanced antitumor efficacy in preclinical HCC models⁶³.

While some strategies have been validated in preclinical models, others await rigorous exploration. Integrating mature gene-editing technologies with clinical regimens may unlock novel solutions to combat resistance.

5.1.3 Targeting m6A modification

Strategies to reverse HCC resistance by regulating the m6A process have become a research hotspot in recent years. For instance, METTL3 enhances EGFR mRNA stability via m6A modification, promoting lenvatinib resistance. However, the METTL3 inhibitor STM2457 improved tumor response to lenvatinib in multiple murine HCC models¹⁹¹. Additionally, WD6305, as a potent and selective proteolysis-targeting chimera (PROTAC) degrader of the METTL3-METTL14 complex, also offers a novel approach for the treatment of drug-resistant tumors¹⁹². Notably, studies have reported that YTHDC1 ablation induces nuclear accumulation of FTH1/FTL transcripts, thereby partially counteracting sorafenib resistance⁷⁶.

Accumulating evidence indicates that natural products can reverse drug resistance by suppressing m6A modification. Liu et al. demonstrated that the natural compound radosiin effectively counteracts METTL8-mediated lenvatinib resistance¹⁹³. Zhao et al. reported that lobeline, a plant-derived natural product, reverses lenvatinib resistance in HCC through m6A modulation, potentially via UBE3B-related mechanisms¹⁹⁴. RNA methylation is a relatively newer form of epigenetic regulation, and in the future, more methylation inhibitors with targeted catalytic activity are expected to be developed.

5.1.4 Other epigenetic therapies

Certain therapeutic strategies do not directly target epigenetic regulators but instead act on upstream or downstream molecules of these regulatory molecules to indirectly reverse drug resistance, independent of epigenetic drugs or genetic interference. For example, the synergistic treatment with PUFA supplementation and sorafenib can overcome HNF4A-AS1/DEC1/PUFA axis-induced sorafenib resistance⁶⁹. The glycolysis inhibitor 2-DG suppresses IGF2BP3 accumulation and its lysine

lactylation, thereby reversing IGF2BP3 lactylation-mediated lenvatinib resistance.¹⁷⁵. Furthermore, some physiological substances have been shown to reverse drug resistance by modulating DNA methylation status. For instance, human menstrual blood-derived stem cells (MenSCs) enhance TET2 expression to reverse BCL2-interacting protein 3 (BNIP3)/ BCL2-interacting protein 3-like (BNIP3L) promoter hypermethylation in sorafenib-resistant HCC, thereby amplifying sorafenib-induced mitophagy and resistant-cell death¹⁹⁵.

The fundamental efficacy of these therapies stems from disrupting any component within the epigenetic drug resistance axis, thereby countering drug resistance. This implies that comprehensive exploration of targets in resistance mechanisms could expand therapeutic options for epigenetic treatments. However, compared to therapies directly targeting epigenetic regulatory molecules, research on their efficacy remains more limited.

5.2 Epigenetic therapies and types of drug resistance

The resistance to targeted therapy in HCC stems from either primary resistance or acquired resistance. Primary resistance typically involves inherent genetic alterations in tumors, manifesting as significant drug resistance during initial targeted treatment^{196, 197}. For instance, elevated intrinsic Aurora-A expression predisposes HCC to lenvatinib resistance¹⁶⁵. Moreover, genomic amplification of CRTC2 (1q21.3) promotes intrinsic lenvatinib resistance via co-condensate formation with the m6A reader YTHDF2, representing a novel innate resistance mechanism in HCC^{169, 198}. In contrast, acquired resistance develops through dynamic adaptive mechanisms during treatment, enabling certain tumor cells to survive therapy and evolve new resistant phenotypes. Clinically, this manifests as gradual loss of therapeutic efficacy after an initial response period¹⁹⁹. A representative example is the cDCBLD2/miR-345-5p/TOP2A resistance regulatory mechanism reported by Ruan et al. through in vitro establishment of sorafenib-resistant cell lines⁵⁴. Importantly, most of the drug resistance mechanisms summarized in this review fall into the category of acquired drug resistance. A hallmark feature of this resistance is its reversible nature, with drug sensitivity recoverable through genetic or pharmacological targeting of resistance-associated regulatory factors.

Fundamentally, primary resistance originates from tumor's inherent stable state, while acquired resistance represents a dynamic and reversible adaptive response. Generally, the prognosis of patients with primary resistance is worse than those with acquired resistance²⁰⁰. The significance of

distinguishing between two types of resistance for epigenetic therapy lies in selecting the appropriate treatment timing. The optimal intervention window for acquired drug resistance should target the early stages of resistance evolution. By monitoring dynamic changes in drug resistance regulatory factors and implementing epigenetic-targeted therapy during the incipient phase of resistance development, when tumors have not yet established a stable resistance ecosystem and residual sensitive cells remain responsive to the original treatment regimen, it becomes possible to effectively prevent further progression of drug resistance^{201, 202, 203}. However, overcoming intrinsic resistance necessitates early epigenetic/genetic interventions, provided that pretreatment detection of drug resistance-associated biomarkers could potentially prevent unnecessary delays in patient treatment caused by primary resistance. Regrettably, the prediction of potential drug resistance factors faces multiple obstacles in terms of cost, testing time, sensitivity, and specificity²⁰⁴.

5.3 Epigenetic therapy and HCC heterogeneity

HCC displays marked therapeutic heterogeneity across etiological subgroups. Comparative clinical evidence demonstrates lenvatinib's superior efficacy over sorafenib in HBV-associated HCC, while showing comparable outcomes in HCV-positive patients²⁰⁵. Intriguingly, sorafenib exhibits enhanced therapeutic responses in HCV-related HCC²⁰⁶. Differences in therapeutic responses due to etiological variations are also observed in epigenetic drug resistance regulation. HBV-associated HCC shows significant miR-193b downregulation driving sorafenib resistance through myeloid cell leukemia-1 (Mcl-1) overexpression²⁰⁷, whereas HCV-related HCC exhibits miR-193b upregulation and Mcl-1 downregulation, which enhances therapeutic sorafenib sensitivity²⁰⁸. Lai et al. reported that IL-6 promotes sorafenib resistance through the DNMT3b-OCT4-DNMT1 axis. Targeting DNMT3b enhances sorafenib sensitivity and improves its therapeutic efficacy against sorafenib-resistant HCC cells, particularly in HBV⁺ HCC¹¹² findings demonstrate that epigenetic drug resistance mechanisms and therapeutic strategies in HCC exhibit significant heterogeneity depending on etiological drivers. Characterizing these etiology-driven epigenetic divergences is critical for guiding precision therapeutic strategies.

Emerging evidence indicates that hepatitis B virus X protein (HBx) promotes HBV-HCC development and progression through interactions with DNMT3A, HDAC1, and ncRNAs^{209, 210}. Additionally, studies have reported HBx-mediated epigenetic mechanisms contributing to drug

resistance. For example, the aforementioned TRERNA1/miR-22-3p/NRAS axis sustains RAS/MAPK pathway activation, conferring sorafenib resistance in HCC cells. MiR-3677-3p suppresses the expression of FBXO31, thereby promoting sorafenib resistance in HBV-HCC¹⁰⁵. Whether these drug resistance mechanisms identified in HBV-HCC are similarly present in HCV-HCC, and whether significant differences exist between the two etiologies, warrants systematic investigation.

The escalating global burden of type 2 diabetes and obesity has established MAFLD as a predominant HCC etiology²¹¹. Clinical analyses indicate MAFLD-associated HCC patients typically present with older age at diagnosis, larger tumor dimensions, and more advanced disease stages. Notably, sorafenib demonstrates comparable therapeutic efficacy in MAFLD-related HCC to other etiological subtypes²¹², and this finding also applies to lenvatinib²¹³. Although Tomonari et al. reported enhanced responsiveness of lenvatinib in non-viral HCC compared to viral hepatitis-associated cases, these findings remain preliminary due to small cohort size and restriction to Child-Pugh class A patients. Nevertheless, this observation underscores the importance of etiological considerations in therapeutic decision-making²¹⁴. Consequently, elucidating epigenetic resistance mechanisms in MAFLD-related HCC remains imperative. Current mechanistic insights remain sparse, though pivotal findings emerge: Wang et al. demonstrated mechanistic linkage between LINC01468 upregulation and SHIP2 destabilization via CUL4A-dependent ubiquitination in MAFLD-HCC. This LINC01468/SHIP2 axis drives PI3K/AKT/mTOR pathway activation, promoting sorafenib resistance⁹⁷. Critical knowledge gaps persist, demanding systematic exploration of MAFLD-specific resistance networks and cross-etiological comparisons of their activation patterns to inform etiology-adapted therapeutic strategies.

Systematic comparative analysis of these etiological differences holds transformative potential for developing context-specific therapeutics, enabling rational therapeutic design to overcome clinical drug resistance through precision oncology frameworks in the future.

5.4 Epigenetic therapy and complexity of drug resistance

5.4.1 Bidirectional regulation of epigenetic molecules

The effects of epigenetic regulatory factors on downstream targets are not static. The same regulatory element can have completely opposite regulatory effects on different downstream molecules. This makes drug resistance in epigenetic regulation even more unpredictable and simultaneously highlights

the necessity of identifying cross-targets involved in multiple drug resistance mechanisms. YTHDF2 specifically binds to m6A-modified mRNAs through its YTH domain, typically mediating target mRNA degradation or translational repression. For instance, CRTC2 activity counteracts YTHDF2-mediated mRNA degradation, thereby promoting lenvatinib resistance¹⁶⁹. Beyond its degradation function, YTHDF2 paradoxically promotes drug resistance through mRNA stabilization mechanisms. YTHDF2 stabilizes m6A-modified FZD10 mRNA to promote lenvatinib resistance¹⁶⁶. METTL3- and METTL14-mediated m6A modification of SREBF2-AS1 promotes HCC progression and sorafenib resistance²¹⁵, while METTL14-dependent m6A methylation similarly induces sorafenib resistance through downregulation of HNF3 γ mRNA¹⁴⁵. Similar scenarios exist in histone modifications. For instance, H3K27me3 serves as a repressive mark, whereas H3K4me is found at the promoters of active genes³⁰.

5.4.2 Epigenetic therapy and drug resistance networks

Emerging studies have demonstrated intricate interactions among DNA/RNA methylation, histone modifications, and ncRNA regulation²¹⁶, constructing a sophisticated regulatory network underlying sorafenib/lenvatinib resistance. This network manifests as cooperative crosstalk between diverse epigenetic regulators, which collectively drive drug resistance through multi-layered molecular adaptations. For instance, m6A-modified SREBF2-AS1 orchestrates TET1 and FXR1 recruitment to activate SREBF2 transcription in sorafenib-resistant HCC²¹⁵. CircMEMO1 modulates sorafenib therapeutic sensitivity by regulating the promoter methylation status of TCF21¹²⁴. Similarly, lenvatinib resistance involves lncXIST-driven EZH2/H3K27me3 axis activation¹⁷⁸ and IGF2BP3 K76 lactylation-PCK2 m6A cooperation enhancing antioxidant defenses¹⁷⁵. MiR-21 promotes the methylation of PTEN by regulating the expression of the TET protein family, thereby enhancing HCC resistance²¹⁷.

The intricate drug resistance regulatory network poses a further crisis for epigenetic therapy, as monotherapy with a single epigenetic drug demonstrates poor efficacy in overcoming drug resistance that involves multiple regulatory mechanisms. Combination therapy with multiple epigenetic drugs demonstrate therapeutic promise: DNMTi-induced DNA demethylation fails to reactivate tumor suppressor genes due to persistent PRC2-mediated repression. As EZH2 catalyzes PRC2-dependent H3K27 trimethylation, combining EZH2i with DNMTis synergistically reactivates epigenetically

silenced therapeutic targets. Preclinical validation demonstrated DNMTi/EZH2i co-treatment (DAC+GSK126) enhances antitumor immunity and suppresses cancer stemness markers in HCC models, outperforming DNMTi monotherapy²¹⁸. While limited to preliminary cell line data, these findings underscore the necessity of multi-mechanism targeting to counteract epigenetic networks.

5.5 Personalized Epigenetic Therapy

The genetic basis for implementing personalized treatment is the heterogeneity of HCC. Essentially, tumor heterogeneity determines the different epigenetic drug resistance mechanisms among patients. Personalized epigenetic therapy embodies a precision oncology paradigm tailoring therapeutic regimens to individual epigenetic differences, aiming to optimize therapeutic outcomes while mitigating off-target toxicity. Central to this approach is the systematic identification of tumorigenic epigenetic drivers coupled with application of modality-specific epigenetic modifiers or genome-editing technologies²¹⁹. For instance, ncRNAs expression in drug-resistant tumors can be either upregulated or downregulated, depending on the specific ncRNAs' role in the resistance mechanism. Clinically, these differentially expressed miRNAs emerge as actionable biomarkers detectable during incipient resistance phases, enabling preemptive therapeutic adjustments²²⁰.

The clinical efficacy of epigenetic agents demonstrates interpatient variability attributable to tumor heterogeneity. This therapeutic challenge necessitates precision selection of targeted epigenetic modulators based on individual resistance mechanisms to achieve personalized therapeutic suppression of drug-resistant pathways. For instance, while histone modification-driven resistance mechanisms may share common epigenetic foundations, their distinct molecular mediators require tailored pharmacological interventions to optimize therapeutic outcomes. As evidenced by our prior investigations, histone demethylases KDM1A and KDM5B respectively drive drug resistance, wherein targeted pharmacological inhibition using respective antagonists effectively reverses these resistance mechanisms. For example, the combination of a KDM5B inhibitor and lenvatinib can restore p15 levels and enhance the sensitivity of resistant cells to lenvatinib²²¹. KDM5B-targeting drugs include CPI-455; KDM5-C49; and GSK-J1²²². Huang et al. reported that the activity of KDM1A is essential for maintaining the stemness of cancer stem cells. Therefore, KDM1A inhibitors restore the sensitivity of sorafenib-resistant HCC cells to sorafenib in vivo by inhibiting the Wnt/ β -catenin signaling pathway²²³. The KDM1A inhibitor currently comprises TCP, ORY-1001, GSK-2879552, IMG-7289,

INCB059872, CC-90011, and ORY-2001197²²⁴. Additionally, the combination of HDAC2 inhibitor CAY10683 and sorafenib significantly slowed down the growth of drug-resistant tumors¹³⁹. Therefore, it is evident that while these drug resistance mechanisms are all mediated by histone modifications, the distinct regulatory factors involved lead to variations in the drugs employed. This forms the epigenetic basis for personalized treatment.

Precision oncology frameworks ultimately aim to circumvent therapeutic vulnerabilities arising from tumor diversity through systematic molecular profiling of resistance mechanisms. Accelerating the empirical validation of HCC-associated epigenetic networks and computational modeling of tumor heterogeneity will enable clinically actionable precision therapeutic paradigms.

5.6 Epigenetic therapy and nanomedicine

Nanomedicine developed based on tumor characteristics and mechanisms is ushering in a new era of cancer therapy. Nanomedicine refers to drug delivery systems designed and fabricated using nanotechnology, typically ranging in size from 10-200 nm. By encapsulating drug molecules in nanocarriers (such as liposomes, polymeric nanoparticles, micelles, dendrimers, and inorganic nanoparticles) and modifying these carriers to enable precise drug release at tumor sites, nanomedicine can significantly improve drug solubility, stability, pharmacokinetic properties, and targeting capability. This leads to enhanced therapeutic efficacy while reducing side effects^{225, 226, 227}. Combination therapy derived from nanomedicine represents a promising strategy for treating drug-resistant HCC. For instance, Zhou et al. designed a mirNA-loading PLGA-PLL (polylactic acid-glycolic acetic copolymer grafted hyper-branched polylysine) carrier and synthesized a novel polymer nanoparticle (Ab-miR-NPs) by modifying it with GPC3 antibody hgc33 and loading it with miR let-7b-5p. Ab-miR-NPs increased the sensitivity of sorafenib resistant HCC by interfering with the expression of IGF1R and inhibiting the activity of Ras/Raf and PI3K/Akt signaling pathways associated with cell resistance²²⁸

In summary, nanomaterials demonstrate vast application potential in the field of dual-drug delivery. By integrating smart responsiveness and targeted modification strategies, nanomedicine is expected to provide more effective and safer therapeutic solutions for drug-resistant HCC treatment.

Summary and Prospects

Overall, the current therapeutic landscape for HCC remains inadequate, with drug resistance

representing a critical challenge. Extensive research has highlighted the pivotal role of epigenetic modifications in conferring resistance to both sorafenib and lenvatinib. In this comprehensive review, we have systematically reviewed these mechanisms from the abnormal regulation of ncRNA regulation, DNA methylation, RNA methylation, and histone modification. These epigenetic modifications affect the physiological behaviors including PCD inhibition, metabolic reprogramming, the formation and maintenance of drug-resistant cells, cell proliferation signaling pathway dysregulation and abnormal HCC transport process, ultimately leading to resistance to sorafenib and lenvatinib. Our review also explores the therapeutic potential and research directions of epigenetic interventions from multiple perspectives, including strategies targeting epigenetic drug resistance mechanisms, the influence of resistance subtypes and tumor heterogeneity on treatment efficacy. The proactive advancement of personalized treatment represents a critical future clinical direction, despite being a highly demanding approach requiring substantial technological and financial resources. We further discuss the emerging promise of nanomedicine in overcoming therapeutic resistance, an innovative strategy poised to benefit patients with advanced-stage malignancies. However, current research on sorafenib/lenvatinib drug resistance mechanisms remains incomplete, and epigenetic therapy represents an emerging field. Based on these limitations, we propose critical unresolved questions and outline promising research directions in this area.

(1) Mechanistic studies may involve potential conflicts and biases in experimental design or data analysis. Firstly, the use of small clinical sample sizes in some mechanistic studies, such as the study by Yu et al., which included only 20 cases¹¹⁴. Secondly, the reliance on induced drug-resistant hepatocellular carcinoma cell lines as resistance models rather than clinical drug-resistant tumor models; the dominance of preclinical studies raises concerns about translational validity, as mouse models cannot fully replicate human conditions. Thirdly, confirmation bias may occur in data interpretation, with some studies selectively reporting positive correlations between drug resistance mechanisms and resistant phenotypes. For instance, Li et al. reported that lncRNA PVT1 promotes sorafenib resistance by positively regulating GPX4⁶⁴, which contradicts the earlier finding by He et al. that PVT1 directly interacts with miR-214-3p to inhibit GPX4²²⁹. Therefore, further investigation is warranted to determine whether lncRNA PVT1 exerts a negative regulatory effect on drug resistance.

(2) Although preliminary studies have identified shared drug resistance mechanisms and

therapeutic targets between lenvatinib and sorafenib, significant unexplored aspects remain in this field, warranting further mechanistic and translational investigations. Targeting these shared therapeutic resistance mechanisms or common molecular targets may enable dual sensitization of HCC to both sorafenib and lenvatinib. However, this hypothesis requires rigorous validation through further investigation. Successful clinical translation of such therapeutic strategies could provide substantial survival benefits for patients with advanced-stage HCC.

(3) Epigenetic complexity arises from multilayered dynamic regulation and tumor heterogeneity. Drug resistance mechanisms form intricate regulatory networks through interconnected interactions, exacerbating therapeutic challenges. Future research on epigenetic drug resistance should extend beyond mechanistic studies and epigenetic drug development to address challenges posed by complex regulatory networks and tumor heterogeneity. Combination therapies must evolve beyond pairing targeted agents with resistance-reversing drugs; they should also pay attention to investigate multi-epigenetic drug combinations to maximally counteract resistance mechanisms. It is noteworthy that nanomedicine represents a promising alternative strategy to overcome these barriers.

(4) Epigenetic therapies show promise but face fragmented mechanistic understanding and early-stage development, with most agents still experimental. Expanding approved drugs and validating their HCC efficacy is critical. Approved epigenetic therapies show variable efficacy due to tumor heterogeneity and resistance mechanisms. Advancing understanding of resistance pathways and patient-specific responses could address these limitations. Combination strategies pairing epigenetic drugs with targeted therapies show substantial potential to overcome resistance, though multicenter trials are needed.

In a word, the translation of existing research findings into practical and usable clinical drugs is a central focus of future research endeavors. With continued discovery of drug resistance mechanisms, we anticipate an expansion of comprehensive clinical treatment options in HCC, which could significantly benefit patients with mid-stage and advanced HCC.

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Author contributions

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Data availability

No datasets were generated or analysed during the current study.

Declarations

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71:209-49.
2. Wang W, Wei C. Advances in the early diagnosis of hepatocellular carcinoma. *Genes Dis.*

2020;7:308-19.

3. Kim DY, Han KH. Epidemiology and surveillance of hepatocellular carcinoma. *Liver Cancer*. 2012;1:2-14.
4. Sugawara Y, Hibi T. Liver transplantation for patients with hepatocellular carcinoma: its current status and advances. *Biosci Trends*. 2022;16:207-11.
5. Wang K, Wang C, Jiang H, Zhang Y, Lin W, Mo J, et al. Combination of ablation and immunotherapy for hepatocellular carcinoma: where we are and where to go. *Front Immunol*. 2021;12:792781.
6. Chang Y, Jeong SW, Jang JY, Kim YJ. Recent updates of transarterial chemoembolization in hepatocellular carcinoma. *Int J Mol Sci*. 2020;21:8165.
7. Tümen D, Heumann P, Gülow K, Demirci CN, Cosma LS, Müller M, et al. Pathogenesis and current treatment strategies of hepatocellular carcinoma. *Biomedicines*. 2022;10:3202.
8. Girardi DM, Sousa LP, Miranda TA, Haum FNC, Pereira GCB, Pereira AAL. Systemic therapy for advanced hepatocellular carcinoma: current stand and perspectives. *Cancers (Basel)*. 2023;15:1680.
9. Llovet JM, Pinyol R, Kelley RK, El-Khoueiry A, Reeves HL, Wang XW, et al. Molecular pathogenesis and systemic therapies for hepatocellular carcinoma. *Nat Cancer*. 2022;3:386-401.
10. Li L, Yu S, Chen J, Quan M, Gao Y, Li Y. miR-15a and miR-20b sensitize hepatocellular carcinoma cells to sorafenib through repressing CDC37L1 and consequent PPIA downregulation. *Cell Death Discov*. 2022;8:297.
11. Zhang P, Sun H, Wen P, Wang Y, Cui Y, Wu J. circRNA circMED27 acts as a prognostic factor and mediator to promote lenvatinib resistance of hepatocellular carcinoma. *Mol Ther Nucleic Acids*. 2022;27:293-303.
12. Oura K, Morishita A, Hamaya S, Fujita K, Masaki T. The roles of epigenetic regulation and the tumor microenvironment in the mechanism of resistance to systemic therapy in hepatocellular carcinoma. *Int J Mol Sci*. 2023;24.
13. Su X, Li Y, Ren Y, Cao M, Yang G, Luo J, et al. A new strategy for overcoming drug resistance in liver cancer: epigenetic regulation. *Biomed Pharmacother*. 2024;176:116902.
14. Nebbioso A, Tambaro FP, Dell'Aversana C, Altucci L. Cancer epigenetics: moving forward. *PLoS*

Genet. 2018;14:e1007362.

15. Grady WM, Yu M, Markowitz SD. Epigenetic alterations in the gastrointestinal tract: current and emerging use for biomarkers of cancer. *Gastroenterology*. 2021;160:690-709.
16. Xie W, Sun H, Li X, Lin F, Wang Z, Wang X. Ovarian cancer: epigenetics, drug resistance, and progression. *Cancer Cell Int*. 2021;21:434.
17. Barbieri I, Kouzarides T. Role of RNA modifications in cancer. *Nat Rev Cancer*. 2020;20:303-22.
18. Zhuang H, Yu B, Tao D, Xu X, Xu Y, Wang J, et al. The role of m6A methylation in therapy resistance in cancer. *Mol Cancer*. 2023;22:91.
19. Li Q, Li Z, Fan Z, Yang Y, Lu C. Involvement of non-coding RNAs in the pathogenesis of myocardial ischemia/reperfusion injury. *Int J Mol Med*. 2021;47:1-1.
20. Borley J, Brown R. Epigenetic mechanisms and therapeutic targets of chemotherapy resistance in epithelial ovarian cancer. *Ann Med*. 2015;47:359-69.
21. Yan H, Bu P. Non-coding RNA in cancer. *Essays Biochem*. 2021;65:625-39.
22. Shah M, Sarkar D. HCC-related lncRNAs: roles and mechanisms. *Int J Mol Sci*. 2024;25.
23. Patop IL, Wüst S, Kadener S. Past, present, and future of circRNAs. *EMBO J*. 2019;38:e100836.
24. Zhang Y, Yao R, Li M, Fang C, Feng K, Chen X, et al. CircTTC13 promotes sorafenib resistance in hepatocellular carcinoma through the inhibition of ferroptosis by targeting the miR-513a-5p/SLC7A11 axis. *Mol Cancer*. 2025;24:32.
25. Wang Y, Ma X, Zhang T, Wang L, Liu Y, Zhou G, et al. Research progress of DNA methylation in the screening of cervical cancer and precancerous lesions. *Interdiscip Med*. 2025;3:e20240043.
26. Toh TB, Lim JJ, Chow EK. Epigenetics in cancer stem cells. *Mol Cancer*. 2017;16:29.
27. Meng H, Cao Y, Qin J, Song X, Zhang Q, Shi Y, et al. DNA methylation, its mediators and genome integrity. *Int J Biol Sci*. 2015;11:604-17.
28. Wajapeyee N, Gupta R. Epigenetic alterations and mechanisms that drive resistance to targeted cancer therapies. *Cancer Res*. 2021;81:5589-95.
29. Romero-Garcia S, Prado-Garcia H, Carlos-Reyes A. Role of DNA methylation in the resistance to therapy in solid tumors. *Front Oncol*. 2020;10:1152.
30. Audia JE, Campbell RM. Histone modifications and cancer. *Cold Spring Harb Perspect Biol*. 2016;8:a019521.

31. Borley J, Brown R. Epigenetic mechanisms and therapeutic targets of chemotherapy resistance in epithelial ovarian cancer. *Ann Med*. 2015;47:359-69.
32. Sun L, Zhang H, Gao P. Metabolic reprogramming and epigenetic modifications on the path to cancer. *Protein Cell*. 2022;13:877-919.
33. Xie S, Chen W, Chen K, Chang Y, Yang F, Lin A, et al. Emerging roles of RNA methylation in gastrointestinal cancers. *Cancer Cell Int*. 2020;20:585.
34. Yang B, Wang JQ, Tan Y, Yuan R, Chen ZS, Zou C. RNA methylation and cancer treatment. *Pharmacol Res*. 2021;174:105937.
35. Kong H, Sun J, Zhang W, Zhang H, Li H. Long intergenic non-protein coding RNA 1273 confers sorafenib resistance in hepatocellular carcinoma via regulation of methyltransferase 3. *Bioengineered*. 2022;13:3108-21.
36. Li J, Xie H, Ying Y, Chen H, Yan H, He L, et al. YTHDF2 mediates the mRNA degradation of the tumor suppressors to induce AKT phosphorylation in N6-methyladenosine-dependent way in prostate cancer. *Mol Cancer*. 2020;19:152.
37. Kudo M, Ueshima K, Ikeda M, Torimura T, Tanabe N, Aikata H, et al. Randomised, multicentre prospective trial of transarterial chemoembolisation (TACE) plus sorafenib as compared with TACE alone in patients with hepatocellular carcinoma: TACTICS trial. *Gut*. 2020;69:1492-501.
38. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359:378-90.
39. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, 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;10:25-34.
40. Bruix J, da Fonseca LG, Reig M. Insights into the success and failure of systemic therapy for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol*. 2019;16:617-30.
41. Godlewski M, Kobylińska A. Programmed cell death - strategy for maintenance cellular organisms homeostasis. *Postepy Hig Med Dosw (Online)*. 2016;70:1229-44.
42. Meng Q, Ding B, Ma P, Lin J. Interrelation between programmed cell death and immunogenic cell death: take antitumor nanodrug as an example. *Small Methods*. 2023;7:e2201406.
43. Renehan AG, Booth C, Potten CS. What is apoptosis, and why is it important? *BMJ*.

- 2001;322:1536-8.
44. Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. *Nat Rev Clin Oncol*. 2020;17:395-417.
 45. Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. *Cell Mol Immunol*. 2021;18:1106-21.
 46. Wong RS. Apoptosis in cancer: from pathogenesis to treatment. *J Exp Clin Cancer Res*. 2011;30:87.
 47. Lv S, Zhao X, Zhang E, Yan Y, Ma X, Li N, et al. Lysine demethylase KDM1A promotes cell growth via FKBP8-BCL2 axis in hepatocellular carcinoma. *J Biol Chem*. 2022;298:102374.
 48. Yu T, Yu J, Lu L, Zhang Y, Zhou Y, Zhou Y, et al. MT1JP-mediated miR-24-3p/BCL2L2 axis promotes Lenvatinib resistance in hepatocellular carcinoma cells by inhibiting apoptosis. *Cell Oncol (Dordr)*. 2021;44:821-34.
 49. Pollutri D, Patrizi C, Marinelli S, Giovannini C, Trombetta 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;9:4.
 50. Fernández-Tussy P, Rodríguez-Agudo R, Fernández-Ramos D, Barbier-Torres L, Zubiete-Franco I, Davalillo SL, et al. Anti-miR-518d-5p overcomes liver tumor cell death resistance through mitochondrial activity. *Cell Death Dis*. 2021;12:555.
 51. Chen S, Xia X. Long noncoding RNA NEAT1 suppresses sorafenib sensitivity of hepatocellular carcinoma cells via regulating miR-335-c-Met. *J Cell Physiol*. 2019;234:14999-5009.
 52. Dong ZB, Wu HM, He YC, Huang ZT, Weng YH, Li H, et al. MiRNA-124-3p.1 sensitizes hepatocellular carcinoma cells to sorafenib by regulating FOXO3a by targeting AKT2 and SIRT1. *Cell Death Dis*. 2022;13:35.
 53. Shao YY, Chen PS, Lin LI, Lee BS, Ling A, Cheng AL, et al. Low miR-10b-3p associated with sorafenib resistance in hepatocellular carcinoma. *Br J Cancer*. 2022;126:1806-14.
 54. Ruan Y, Chen T, Zheng L, Cai J, Zhao H, Wang Y, et al. cDCBLD2 mediates sorafenib resistance in hepatocellular carcinoma by sponging miR-345-5p binding to the TOP2A coding sequence. *Int J Biol Sci*. 2023;19:4608-26.
 55. Bai X, Kang J, Wei S, Wang Y, Liu Y, Yuan B, et al. A pH responsive nanocomposite for

- combination sonodynamic-immunotherapy with ferroptosis and calcium ion overload via SLC7A11/ACSL4/LPCAT3 pathway. *Exploration*. 2025;5:20240002.
56. Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mechanisms and health implications. *Cell Res*. 2021;31:107-25.
 57. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol*. 2021;22:266-82.
 58. Cong T, Luo Y, Fu Y, Liu Y, Li Y, Li X. New perspectives on ferroptosis and its role in hepatocellular carcinoma. *Chin Med J (Engl)*. 2022;135:2157-66.
 59. Li J, Cao F, Yin HL, Huang ZJ, Lin ZT, Mao N, et al. Ferroptosis: past, present and future. *Cell Death Dis*. 2020;11:88.
 60. Gao Y, Tong M, Wong TL, Ng KY, Xie YN, Wang Z, et al. Long noncoding RNA URB1-antisense RNA 1 (AS1) suppresses sorafenib-induced ferroptosis in hepatocellular carcinoma by driving ferritin phase separation. *ACS Nano*. 2023;17:22240-58.
 61. Chen F, Wang L. Long noncoding RNA CASC11 suppresses sorafenib-triggered ferroptosis via stabilizing SLC7A11 mRNA in hepatocellular carcinoma cells. *Discov Oncol*. 2023;14:145.
 62. Shi Z, Li Z, Jin B, Ye W, Wang L, Zhang S, et al. Loss of LncRNA DUXAP8 synergistically enhanced sorafenib induced ferroptosis in hepatocellular carcinoma via SLC7A11 de-palmitoylation. *Clin Transl Med*. 2023;13:e1300.
 63. Shi CJ, Pang FX, Lei YH, Deng LQ, Pan FZ, Liang ZQ, et al. 5-methylcytosine methylation of MALAT1 promotes resistance to sorafenib in hepatocellular carcinoma through ELAVL1/SLC7A11-mediated ferroptosis. *Drug Resist Updat*. 2025;78:101181.
 64. Li J, Li Y, Wang D, Liao R, Wu Z. PLAG1 interacts with GPX4 to conquer vulnerability to sorafenib induced ferroptosis through a PVT1/miR-195-5p axis-dependent manner in hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2024;43:143.
 65. Chen X, Kang R, Kroemer G, Tang D. Organelle-specific regulation of ferroptosis. *Cell Death Differ*. 2021;28:2843-56.
 66. Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol*. 2017;13:91-8.
 67. Ding K, Liu C, Li L, Yang M, Jiang N, Luo S, et al. Acyl-CoA synthase ACSL4: an essential target

- in ferroptosis and fatty acid metabolism. *Chin Med J (Engl)*. 2023;136:2521-37.
68. Lu Y, Chan YT, Tan HY, Zhang C, Guo W, Xu Y, 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;41:3.
 69. Zhao Y, Han S, Zeng Z, Zheng H, Li Y, Wang F, et al. Decreased lncRNA HNF4A-AS1 facilitates resistance to sorafenib-induced ferroptosis of hepatocellular carcinoma by reprogramming lipid metabolism. *Theranostics*. 2024;14:7088-110.
 70. Cheng Y, Zak O, Aisen P, Harrison SC, Walz T. Structure of the human transferrin receptor-transferrin complex. *Cell*. 2004;116:565-76.
 71. Li Y, Du Y, Zhou Y, Chen Q, Luo Z, Ren Y, et al. Iron and copper: critical executioners of ferroptosis, cuproptosis and other forms of cell death. *Cell Commun Signal*. 2023;21:327.
 72. Zanninelli G, Loréal O, Brissot P, Konijn AM, Slotki IN, Hider RC, et al. The labile iron pool of hepatocytes in chronic and acute iron overload and chelator-induced iron deprivation. *J Hepatol*. 2002;36:39-46.
 73. Camarena V, Huff TC, Wang G. Epigenomic regulation by labile iron. *Free Radic Biol Med*. 2021;170:44-9.
 74. Sun J, Liu Q, Jiang Y, Cai Z, Liu H, Zuo H. Engineered small extracellular vesicles loaded with miR-654-5p promote ferroptosis by targeting HSPB1 to alleviate sorafenib resistance in hepatocellular carcinoma. *Cell Death Discov*. 2023;9:362.
 75. Lai Y, Han X, Xie B, Xu Y, Yang Z, Wang D, et al. EZH2 suppresses ferroptosis in hepatocellular carcinoma and reduces sorafenib sensitivity through epigenetic regulation of TFR2. *Cancer Sci*. 2024;115:2220-34.
 76. Hao SH, Ma XD, Xu L, Xie JD, Feng ZH, Chen JW, et al. Dual specific phosphatase 4 suppresses ferroptosis and enhances sorafenib resistance in hepatocellular carcinoma. *Drug Resist Updat*. 2024;73:101052.
 77. Debnath J, Gammoh N, Ryan KM. Autophagy and autophagy-related pathways in cancer. *Nat Rev Mol Cell Biol*. 2023;24:560-75.
 78. Qian C, Zhang Y, Chen X, Zhu C, Dong X, Chen W, et al. Nanomedicine-encouraged cellular autophagy promoters favor liver fibrosis progression reversal. *View*. 2024;5:20240003.

79. Klionsky DJ, Petroni G, Amaravadi RK, Baehrecke EH, Ballabio A, Boya P, et al. Autophagy in major human diseases. *EMBO J.* 2021;40:e108863.
80. Li W, Dong X, He C, Tan G, Li Z, Zhai B, et al. LncRNA SNHG1 contributes to sorafenib resistance by activating the Akt pathway and is positively regulated by miR-21 in hepatocellular carcinoma cells. *J Exp Clin Cancer Res.* 2019;38:183.
81. Zhai B, Hu F, Jiang X, Xu J, Zhao D, Liu B, et al. Inhibition of Akt reverses the acquired resistance to sorafenib by switching protective autophagy to autophagic cell death in hepatocellular carcinoma. *Mol Cancer Ther.* 2014;13:1589-98.
82. Feng X, Zou B, Nan T, Zheng X, Zheng L, Lan J, et al. MiR-25 enhances autophagy and promotes sorafenib resistance of hepatocellular carcinoma via targeting FBXW7. *Int J Med Sci.* 2022;19:257-66.
83. Hu Z, Zhao Y, Mang Y, Zhu J, Yu L, Li L, et al. MiR-21-5p promotes sorafenib resistance and hepatocellular carcinoma progression by regulating SIRT7 ubiquitination through USP24. *Life Sci.* 2023;325:121773.
84. Ding M, Jiang CY, Zhang Y, Zhao J, Han BM, Xia SJ. SIRT7 depletion inhibits cell proliferation and androgen-induced autophagy by suppressing the AR signaling in prostate cancer. *J Exp Clin Cancer Res.* 2020;39:28.
85. Chen MY, Yadav VK, Chu YC, Ong JR, Huang TY, Lee KF, et al. Hydroxychloroquine (HCQ) modulates autophagy and oxidative DNA damage stress in hepatocellular carcinoma to overcome sorafenib resistance via TLR9/SOD1/hsa-miR-30a-5p/Beclin-1 axis. *Cancers (Basel).* 2021;13:3227.
86. Zhang N, Wu Y, Lyu X, Li B, Yan X, Xiong H, et al. HSF1 upregulates ATG4B expression and enhances epirubicin-induced protective autophagy in hepatocellular carcinoma cells. *Cancer Lett.* 2017;409:81-90.
87. Chen L, Sun L, Dai X, Li T, Yan X, Zhang Y, et al. LncRNA CRNDE promotes ATG4B-mediated autophagy and alleviates the sensitivity of sorafenib in hepatocellular carcinoma cells. *Front Cell Dev Biol.* 2021;9:687524.
88. Lin Z, Niu Y, Wan A, Chen D, Liang H, Chen X, et al. RNA m(6)A methylation regulates sorafenib resistance in liver cancer through FOXO3-mediated autophagy. *EMBO J.* 2020;39:e103181.

89. Cao Y. Adipocyte and lipid metabolism in cancer drug resistance. *J Clin Invest.* 2019;129:3006-17.
90. You M, Xie Z, Zhang N, Zhang Y, Xiao D, Liu S, et al. Signaling pathways in cancer metabolism: mechanisms and therapeutic targets. *Signal Transduct Target Ther.* 2023;8:196.
91. 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;9:765-78.
92. Zhou B, Lu D, Wang A, Cui J, Zhang L, Li J, et al. Endoplasmic reticulum stress promotes sorafenib resistance via miR-188-5p/hnRNPA2B1-mediated upregulation of PKM2 in hepatocellular carcinoma. *Mol Ther Nucleic Acids.* 2021;26:1051-65.
93. Zhang Z, Tan X, Luo J, Yao H, Si Z, Tong JS. The miR-30a-5p/CLCF1 axis regulates sorafenib resistance and aerobic glycolysis in hepatocellular carcinoma. *Cell Death Dis.* 2020;11:902.
94. Bergamini C, Leoni I, Rizzardi N, Melli M, Galvani G, Coda CA, et al. MiR-494 induces metabolic changes through G6pc targeting and modulates sorafenib response in hepatocellular carcinoma. *J Exp Clin Cancer Res.* 2023;42:145.
95. Cheng C, Geng F, Cheng X, Guo D. Lipid metabolism reprogramming and its potential targets in cancer. *Cancer Commun (Lond).* 2018;38:27.
96. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med.* 2018;24:908-22.
97. Wang H, Wang Y, Lai S, Zhao L, Liu W, Liu S, et al. LINC01468 drives NAFLD-HCC progression through CUL4A-linked degradation of SHIP2. *Cell Death Discov.* 2022;8:449.
98. Chan YT, Wu J, Lu Y, Li Q, Feng Z, Xu L, et al. Loss of lncRNA LINC01056 leads to sorafenib resistance in HCC. *Mol Cancer.* 2024;23:74.
99. Sullivan LB, Luengo A, Danai LV, Bush LN, Diehl FF, Hosios AM, et al. Aspartate is an endogenous metabolic limitation for tumour growth. *Nat Cell Biol.* 2018;20:782-8.
100. Chen M, Zhang C, Liu W, Du X, Liu X, Xing B. Long noncoding RNA LINC01234 promotes hepatocellular carcinoma progression through orchestrating aspartate metabolic reprogramming. *Mol Ther.* 2022;30:2354-69.
101. Ebrahimi N, Afshinpour M, Fakhr SS, Kalkhoran PG, Shadman-Manesh V, Adelian S, et al.

- Cancer stem cells in colorectal cancer: signaling pathways involved in stemness and therapy resistance. *Crit Rev Oncol Hematol*. 2023;182:103920.
102. Skvortsova I. Cancer stem cells: what do we know about them? *Cells*. 2021;10(6):1523.
 103. Liu A, Zhu J, Wu G, Cao L, Tan Z, Zhang S, et al. Antagonizing miR-455-3p inhibits chemoresistance and aggressiveness in esophageal squamous cell carcinoma. *Mol Cancer*. 2017;16:106.
 104. Najafi M, Farhood B, Mortezaee K. Cancer stem cells (CSCs) in cancer progression and therapy. *J Cell Physiol*. 2019;234:8381-95.
 105. He H, Zhou J, Cheng F, Li H, Quan Y. MiR-3677-3p promotes development and sorafenib resistance of hepatitis B-related hepatocellular carcinoma by inhibiting FOXM1 ubiquitination. *Hum Cell*. 2023;36:1773-89.
 106. Sher G, Masoodi T, Patil K, Akhtar S, Kuttikrishnan S, Ahmad A, et al. Dysregulated FOXM1 signaling in the regulation of cancer stem cells. *Semin Cancer Biol*. 2022;86:107-21.
 107. Cheng T, Zhou C, Bian S, Soback K, Liu Y. Coordinated activation of DNMT3a and TET2 in cancer stem cell-like cells initiates and sustains drug resistance in hepatocellular carcinoma. *Cancer Cell Int*. 2024;24:110.
 108. Bi L, Ren Y, Feng M, Meng P, Wang Q, Chen W, et al. HDAC11 regulates glycolysis through the LKB1/AMPK signaling pathway to maintain hepatocellular carcinoma stemness. *Cancer Res*. 2021;81:2015-28.
 109. Li J, Zhong X, Wang X, Xu F, Yang J, Lu J, et al. miR-93 regulates liver tumor initiating cells expansion and predicts chemotherapeutic response of patients. *Arch Biochem Biophys*. 2021;703:108871.
 110. Qu S, Zhang X, Wu Y, Li H, Zhai J, Wu D. miR-361-3p regulates liver tumor-initiating cells expansion and chemo-resistance. *J Cancer*. 2021;12:1483-92.
 111. Liu J, Nie C. KDM5B regulates the PTEN/PI3K/Akt pathway to increase sorafenib-resistance in hepatocellular carcinoma. *Anticancer Drugs*. 2022;33:840-9.
 112. Lai SC, Su YT, Chi CC, Kuo YC, Lee KF, Wu YC, et al. DNMT3b/OCT4 expression confers sorafenib resistance and poor prognosis of hepatocellular carcinoma through IL-6/STAT3 regulation. *J Exp Clin Cancer Res*. 2019;38:474.

113. Wang C, Dong R, Yang F, Zheng L, Liu Y, Yan Y, et al. LARP4B promotes hepatocellular carcinoma progression and impairs sorafenib efficacy by activating SPINK1-mediated EGFR pathway. *Cell Death Discov.* 2024;10:208.
114. Yu Y, Lu XH, Mu JS, Meng JY, Sun JS, Chen HX, et al. N6-methyladenosine-modified long non-coding RNA KIF9-AS1 promotes stemness and sorafenib resistance in hepatocellular carcinoma by upregulating SHOX2 expression. *World J Gastroenterol.* 2024;30:5174-90.
115. Manfioletti G, Fedele M. Epithelial-mesenchymal transition (EMT) 2021. *Int J Mol Sci.* 2022;23:5848.
116. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol.* 2014;15:178-96.
117. Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. *Curr Opin Cell Biol.* 2005;17:548-58.
118. Sun J, Zheng X, Wang B, Cai Y, Zheng L, Hu L, et al. LncRNA LIMT (LINC01089) contributes to sorafenib chemoresistance via regulation of miR-665 and epithelial to mesenchymal transition in hepatocellular carcinoma cells. *Acta Biochim Biophys Sin (Shanghai).* 2022;54:261-70.
119. Xu Y, Liu Y, Li Z, Li H, Li X, Yan L, et al. Long non-coding RNA H19 is involved in sorafenib resistance in hepatocellular carcinoma by upregulating miR-675. *Oncol Rep.* 2020;44:165-73.
120. Xu K, Wang X, Hu S, Tang J, Liu S, Chen H, et al. LINC00540 promotes sorafenib resistance and functions as a ceRNA for miR-4677-3p to regulate AKR1C2 in hepatocellular carcinoma. *Heliyon.* 2024;10:e27322.
121. Hirao A, Sato Y, Tanaka H, Nishida K, Tomonari T, Hirata M, et al. MiR-125b-5p is involved in sorafenib resistance through ataxin-1-mediated epithelial-mesenchymal transition in hepatocellular carcinoma. *Cancers (Basel).* 2021;13:4917.
122. Liu Z, Lu J, Fang H, Sheng J, Cui M, Yang Y, et al. m6A modification-mediated DUXAP8 regulation of malignant phenotype and chemotherapy resistance of hepatocellular carcinoma through miR-584-5p/MAPK1/ERK pathway axis. *Front Cell Dev Biol.* 2021;9:783385.
123. Kuang Y, Cheng Y, Wang J, Li H, Cao X, Wang Y. KIAA1429 mediates epithelial mesenchymal transition in sorafenib-resistant hepatocellular carcinoma through m6A methylation modification. *Cancer Med.* 2023;12:7222-33.

124. Dong ZR, Ke AW, Li T, Cai JB, Yang YF, Zhou W, et al. CircMEMO1 modulates the promoter methylation and expression of TCF21 to regulate hepatocellular carcinoma progression and sorafenib treatment sensitivity. *Mol Cancer*. 2021;20:75.
125. Zheng A, Chevalier N, Calderoni M, Dubuis G, Dormond O, Ziros PG, et al. CRISPR/Cas9 genome-wide screening identifies KEAP1 as a sorafenib, lenvatinib, and regorafenib sensitivity gene in hepatocellular carcinoma. *Oncotarget*. 2019;10:7058-70.
126. Kong FH, Ye QF, Miao XY, Liu X, Huang SQ, Xiong L, et al. Current status of sorafenib nanoparticle delivery systems in the treatment of hepatocellular carcinoma. *Theranostics*. 2021;11:5464-90.
127. Janku F, Yap TA, Meric-Bernstam F. Targeting the PI3K pathway in cancer: are we making headway? *Nat Rev Clin Oncol*. 2018;15:273-91.
128. Christine A, Park MK, Song SJ, Song MS. The equilibrium of tumor suppression: DUBs as active regulators of PTEN. *Exp Mol Med*. 2022;54:1814-21.
129. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. *Front Oncol*. 2014;4:64.
130. Jiang XM, Yu XN, Liu TT, Zhu HR, Shi X, Bilegsaikhan 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.
131. Li X, Yin X, Bao H, Liu C. Circular RNA ITCH increases sorafenib-sensitivity in hepatocellular carcinoma via sequestering miR-20b-5p and modulating the downstream PTEN-PI3K/Akt pathway. *Mol Cell Probes*. 2023;67:101877.
132. Wu PK, Becker A, Park JI. Growth inhibitory signaling of the Raf/MEK/ERK pathway. *Int J Mol Sci*. 2020;21:5436.
133. Li L, Zhao GD, Shi Z, Qi LL, Zhou LY, Fu ZX. The Ras/Raf/MEK/ERK signaling pathway and its role in the occurrence and development of HCC. *Oncol Lett*. 2016;12:3045-50.
134. Hou J, Lin L, Zhou W, Wang Z, Ding G, Dong Q, et al. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell*. 2011;19:232-43.
135. Song W, Zheng C, Liu M, Xu Y, Qian Y, Zhang Z, et al. TRERNA1 upregulation mediated by

- HBx promotes sorafenib resistance and cell proliferation in HCC via targeting NRAS by sponging miR-22-3p. *Mol Ther.* 2021;29:2601-16.
136. Xu J, Ji L, Liang Y, Wan Z, Zheng W, Song X, et al. CircRNA-SORE mediates sorafenib resistance in hepatocellular carcinoma by stabilizing YBX1. *Signal Transduct Target Ther.* 2020;5:298.
 137. Liu Y, Zhang X, Yang B, Zhuang H, Guo H, Wei W, et al. Demethylation-induced overexpression of Shc3 drives c-Raf-independent activation of MEK/ERK in HCC. *Cancer Res.* 2018;78:2219-32.
 138. Liu Y, Zhuang H, Cao F, Li J, Guo Y, Zhang J, et al. Shc3 promotes hepatocellular carcinoma stemness and drug resistance by interacting with β -catenin to inhibit its ubiquitin degradation pathway. *Cell Death Dis.* 2021;12:278.
 139. Sha Y, Pan M, Chen Y, Qiao L, Zhou H, Liu D, et al. PLEKHG5 is stabilized by HDAC2-related deacetylation and confers sorafenib resistance in hepatocellular carcinoma. *Cell Death Discov.* 2023;9:176.
 140. Fung SW, Cheung PF, Yip CW, Ng LW, Cheung TT, Chong CC, et al. The ATP-binding cassette transporter ABCF1 is a hepatic oncofetal protein that promotes chemoresistance, EMT and cancer stemness in hepatocellular carcinoma. *Cancer Lett.* 2019;457:98-109.
 141. Li TT, Mou J, Pan YJ, Huo FC, Du WQ, Liang J, et al. MicroRNA-138-1-3p sensitizes sorafenib to hepatocellular carcinoma by targeting PAK5 mediated β -catenin/ABCB1 signaling pathway. *J Biomed Sci.* 2021;28:56.
 142. Ciută AD, Nosol K, Kowal J, Mukherjee S, Ramírez AS, Stieger B, et al. Structure of human drug transporters OATP1B1 and OATP1B3. *Nat Commun.* 2023;14:5774.
 143. Anabtawi N, Drabison T, Hu S, Sparreboom A, Talebi Z. The role of OATP1B1 and OATP1B3 transporter polymorphisms in drug disposition and response to anticancer drugs: a review of the recent literature. *Expert Opin Drug Metab Toxicol.* 2022;18:459-68.
 144. Zhang S, Xu J, Chen Q, Zhang F, Wang H, Guo H. lncRNA MT1JP-overexpression abolishes the silencing of PTEN by miR-32 in hepatocellular carcinoma. *Oncol Lett.* 2021;22:604.
 145. Zhou T, Li S, Xiang D, Liu J, Sun W, Cui X, et al. m6A RNA methylation-mediated HNF3 γ reduction renders hepatocellular carcinoma dedifferentiation and sorafenib resistance. *Signal*

- Transduct Target Ther. 2020;5:296.
146. Liu J, Ren L, Li S, Li W, Zheng X, Yang Y, et al. The biology, function, and applications of exosomes in cancer. *Acta Pharm Sin B*. 2021;11:2783-97.
 147. Dai J, Su Y, Zhong S, Cong L, Liu B, Yang J, et al. Exosomes: key players in cancer and potential therapeutic strategy. *Signal Transduct Target Ther*. 2020;5:145.
 148. Nakano T, Chen CL, Chen IH, Tseng HP, Chiang KC, Lai CY, et al. Overexpression of miR-4669 enhances tumor aggressiveness and generates an immunosuppressive tumor microenvironment in hepatocellular carcinoma: its clinical value as a predictive biomarker. *Int J Mol Sci*. 2023;24:7908.
 149. Zhang Y, Pan Q, Shao Z. Extracellular vesicles derived from cancer-associated fibroblasts carry tumor-promotive microRNA-1228-3p to enhance the resistance of hepatocellular carcinoma cells to sorafenib. *Hum Cell*. 2023;36:296-311.
 150. Gao Y, Yin Z, Qi Y, Peng H, Ma W, Wang R, et al. Golgi phosphoprotein 3 promotes angiogenesis and sorafenib resistance in hepatocellular carcinoma via upregulating exosomal miR-494-3p. *Cancer Cell Int*. 2022;22:35.
 151. Dong FL, Xu ZZ, Wang YQ, Li T, Wang X, Li J. Exosome-derived circUPF2 enhances resistance to targeted therapy by redeploying ferroptosis sensitivity in hepatocellular carcinoma. *J Nanobiotechnology*. 2024;22:298.
 152. Bao Y, Xu S, Zhou J, Zhao C, Dai S, Zhang Y, et al. Exosomal miR-93 derived from hepatocellular carcinoma cell promotes the sorafenib resistance of hepatocellular carcinoma through PTEN/PI3K/Akt pathway. *J Biochem Mol Toxicol*. 2024;38:e23666.
 153. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet*. 2018;391:1163-73.
 154. Nair A, Reece K, Donoghue MB, Yuan WV, Rodriguez L, Keegan P, et al. FDA supplemental approval summary: lenvatinib for the treatment of unresectable hepatocellular carcinoma. *Oncologist*. 2021;26:e484-e491.
 155. Zhao Y, Zhang YN, Wang KT, Chen L. Lenvatinib for hepatocellular carcinoma: from preclinical mechanisms to anti-cancer therapy. *Biochim Biophys Acta Rev Cancer*. 2020;1874:188391.
 156. Xu X, Jiang W, Han P, Zhang J, Tong L, Sun X. MicroRNA-128-3p mediates lenvatinib resistance

- of hepatocellular carcinoma cells by downregulating c-Met. *J Hepatocell Carcinoma*. 2022;9:113-26.
157. Zhang XY, Li SS, Gu YR, Xiao LX, Ma XY, Chen XR, et al. CircPIAS1 promotes hepatocellular carcinoma progression by inhibiting ferroptosis via the miR-455-3p/NUPR1/FTH1 axis. *Mol Cancer*. 2024;23:113.
158. Gu D, Tong M, Wang J, Zhang B, Liu J, Song G, et al. Overexpression of the lncRNA HOTAIRM1 promotes lenvatinib resistance by downregulating miR-34a and activating autophagy in hepatocellular carcinoma. *Discov Oncol*. 2023;14:66.
159. Han T, Zheng H, Zhang J, Yang P, Li H, Cheng Z, et al. Downregulation of MUC15 by miR-183-5p.1 promotes liver tumor-initiating cells properties and tumorigenesis via regulating c-MET/PI3K/AKT/SOX2 axis. *Cell Death Dis*. 2022;13:200.
160. Wei Y, Wei L, Han T, Ding S. miR-3154 promotes hepatocellular carcinoma progression via suppressing HNF4 α . *Carcinogenesis*. 2022;43:1002-14.
161. Cao M, Ren Y, Li Y, Deng J, Su X, Tang Y, et al. Lnc-ZEB2-19 inhibits the progression and lenvatinib resistance of hepatocellular carcinoma by attenuating the NF- κ B signaling pathway through the TRA2A/RSPH14 axis. *Int J Biol Sci*. 2023;19:3678-93.
162. Feng Y, Liang L, Jia W, Wang J, Xu C, Zhu D, et al. Circ_0007386 promotes the progression of hepatocellular carcinoma through the miR-507/ CCNT2 axis. *J Hepatocell Carcinoma*. 2024;11:1095-112.
163. Hu Y, Chen M, Sun S, Zhang C, Xin Z, Sun X, et al. Long non-coding RNA LINC01532 sustains redox homeostasis and accelerates lenvatinib resistance in hepatocellular carcinoma. *J Adv Res*. 2025.
164. Yang L, Tan W, Wang M, Wei Y, Xie Z, Wang Q, et al. circCCNY enhances lenvatinib sensitivity and suppresses immune evasion in hepatocellular carcinoma by serving as a scaffold for SMURF1 mediated HSP60 degradation. *Cancer Lett*. 2025;612:217470.
165. Abudoureyimu M, Sun N, Chen W, Lin X, Pan F, Wang R. Aurora-A promotes lenvatinib resistance experimentally through hsa-circ-0058046/miR-424-5p/FGFR1 axis in hepatocellular carcinoma. *Int J Immunopathol Pharmacol*. 2025;39:3946320251316692.

166. Wang J, Yu H, Dong W, Zhang C, Hu M, Ma W, et al. N6-methyladenosine-mediated up-regulation of FZD10 regulates liver cancer stem cells' properties and lenvatinib resistance through WNT/ β -catenin and Hippo signaling pathways. *Gastroenterology*. 2023;164:990-1005.
167. Fang R, Jia Z, Xin Y, Zhao K, Qin W, Lu H, et al. N6-methyladenosine-modification of USP15 regulates chemotherapy resistance by inhibiting LGALS3 ubiquitin-mediated degradation via AKT/mTOR signaling activation pathway in hepatocellular carcinoma. *Cell Death Discov*. 2025;11:3.
168. Huang M, Long J, Yao Z, Zhao Y, Zhao Y, Liao J, et al. METTL1-mediated m7G tRNA modification promotes lenvatinib resistance in hepatocellular carcinoma. *Cancer Res*. 2023;83:89-102.
169. Wang M, Zou F, Wang S, Yang Y, Xia C, Chen L, et al. CRTC2 forms co-condensates with YTHDF2 that enhance translational efficiency of m6A-modified mRNAs to drive hepatocarcinogenesis and lenvatinib resistance. *Cancer Res*. 2025.
170. Yang W, Li HY, Wu YF, Mi RJ, Liu WZ, Shen X, et al. ac4C acetylation of RUNX2 catalyzed by NAT10 spurs osteogenesis of BMSCs and prevents ovariectomy-induced bone loss. *Mol Ther Nucleic Acids*. 2021;26:135-47.
171. Xu D, Liu Z, Liang MX, Fei YJ, Zhang W, Wu Y, et al. Endoplasmic reticulum stress targeted therapy for breast cancer. *Cell Commun Signal*. 2022;20:174.
172. Pan Z, Bao Y, Hu M, Zhu Y, Tan C, Fan L, et al. Role of NAT10-mediated ac4C-modified HSP90AA1 RNA acetylation in ER stress-mediated metastasis and lenvatinib resistance in hepatocellular carcinoma. *Cell Death Discov*. 2023;9:56.
173. Duan A, Li H, Yu W, Zhang Y, Yin L. Long noncoding RNA XIST promotes resistance to lenvatinib in hepatocellular carcinoma cells via epigenetic inhibition of NOD2. *J Oncol*. 2022;2022:4537343.
174. Wang J, Wang GG. No easy way out for EZH2: its pleiotropic, noncanonical effects on gene regulation and cellular function. *Int J Mol Sci*. 2020;21:9501.
175. Lu Y, Zhu J, Zhang Y, Li W, Xiong Y, Fan Y, et al. Lactylation-driven IGF2BP3-mediated serine metabolism reprogramming and RNA m6A-modification promotes lenvatinib resistance in HCC. *Adv Sci (Weinh)*. 2024;11:e2401399.

176. Yuan F, Tang Y, Liang H, Cao M, Ren Y, Li Y, et al. CircPIK3C3 inhibits hepatocellular carcinoma progression and lenvatinib resistance by suppressing the Wnt/ β -catenin pathway via the miR-452-5p/SOX15 axis. *Genomics*. 2025;117:110999.
177. Tsuchiya H, Shinonaga R, Sakaguchi H, Kitagawa Y, Yoshida K. NEAT1-SOD2 axis confers sorafenib and lenvatinib resistance by activating AKT in liver cancer cell lines. *Curr Issues Mol Biol*. 2023;45:1073-85.
178. Wang Y, Tan K, Hu W, Hou Y, Yang G. LncRNA AC026401.3 interacts with OCT1 to intensify sorafenib and lenvatinib resistance by activating E2F2 signaling in hepatocellular carcinoma. *Exp Cell Res*. 2022;420:113335.
179. Zhang X, Su T, Wu Y, Cai Y, Wang L, Liang C, et al. N6-methyladenosine reader YTHDF1 promotes stemness and therapeutic resistance in hepatocellular carcinoma by enhancing NOTCH1 expression. *Cancer Res*. 2024;84:827-40.
180. Arechederra M, Bazai SK, Abdouni A, Sequera C, Mead TJ, Richelme S, et al. ADAMTSL5 is an epigenetically activated gene underlying tumorigenesis and drug resistance in hepatocellular carcinoma. *J Hepatol*. 2021;74:893-906.
181. Morel D, Jeffery D, Aspeslagh S, Almouzni G, Postel-Vinay S. Combining epigenetic drugs with other therapies for solid tumours - past lessons and future promise. *Nat Rev Clin Oncol*. 2020;17:91-107.
182. Hata AN, Niederst MJ, Archibald HL, Gomez-Caraballo M, Siddiqui FM, Mulvey HE, et al. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nat Med*. 2016;22:262-9.
183. Gomez S, Tabernacki T, Kobyra J, Roberts P, Chiappinelli KB. Combining epigenetic and immune therapy to overcome cancer resistance. *Semin Cancer Biol*. 2020;65:99-113.
184. Pappalardi MB, Keenan K, Cockerill M, Kellner WA, Stowell A, Sherk C, et al. Discovery of a first-in-class reversible DNMT1-selective inhibitor with improved tolerability and efficacy in acute myeloid leukemia. *Nat Cancer*. 2021;2:1002-17.
185. Suraweera A, O'Byrne KJ, Richard DJ. Epigenetic drugs in cancer therapy. *Cancer Metastasis Rev*. 2025;44:37-61.
186. Fernández-Barrena MG, Arechederra M, Colyn L, Berasain C, Avila MA. Epigenetics in

- hepatocellular carcinoma development and therapy: the tip of the iceberg. *JHEP Rep.* 2020;2:100167.
187. New M, Olzscha H, La Thangue NB. HDAC inhibitor-based therapies: can we interpret the code? *Mol Oncol.* 2012;6:637-56.
 188. Bitzer M, Horger M, Giannini EG, Ganten TM, Wörns MA, Siveke JT, et al. Resminostat plus sorafenib as second-line therapy of advanced hepatocellular carcinoma - The SHELTER study. *J Hepatol.* 2016;65:280-8.
 189. Yan S, Chen L, Zhuang H, Yang H, Yang Y, Zhang N, et al. HDAC inhibition sensitize hepatocellular carcinoma to lenvatinib via suppressing AKT activation. *Int J Biol Sci.* 2024;20:3046-60.
 190. Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. *J Hematol Oncol.* 2020;13:104.
 191. Wang L, Yang Q, Zhou Q, Fang F, Lei K, Liu Z, et al. METTL3-m(6)A-EGFR-axis drives lenvatinib resistance in hepatocellular carcinoma. *Cancer Lett.* 2023;559:216122.
 192. Du W, Huang Y, Chen X, Deng Y, Sun Y, Yang H, et al. Discovery of a PROTAC degrader for METTL3-METTL14 complex. *Cell Chem Biol.* 2024;31:177-83.e11.
 193. Liu Y, Chen M, Wang XX, Gao Y, Han X, Wang S, et al. Targeting METTL8 with Rabdosiin overcomes lenvatinib resistance in hepatocellular carcinoma. *Exp Cell Res.* 2025;444:114389.
 194. Zhao L, Ma H, Jiang Y, Li Y, Qiao L, Chen Y, et al. Identification of an m6A natural inhibitor, Lobeline, that reverses lenvatinib resistance in hepatocellular tumors. *J Nat Prod.* 2024;87:1983-93.
 195. Zhou S, Liu Y, Zhang Q, Xu H, Fang Y, Chen X, et al. Human menstrual blood-derived stem cells reverse sorafenib resistance in hepatocellular carcinoma cells through the hyperactivation of mitophagy. *Stem Cell Res Ther.* 2023;14:58.
 196. De Mattia E, Cecchin E, Guardascione M, Foltran L, Di Raimo T, Angelini F, et al. Pharmacogenetics of the systemic treatment in advanced hepatocellular carcinoma. *World J Gastroenterol.* 2019;25:3870-96.
 197. Marin JJ, Romero MR, Briz O. Molecular bases of liver cancer refractoriness to pharmacological treatment. *Curr Med Chem.* 2010;17:709-40.
 198. Zhang R, Dai J, Yao F, Zhou S, Huang W, Xu J, et al. Hypomethylation-enhanced CRTC2

- expression drives malignant phenotypes and primary resistance to immunotherapy in hepatocellular carcinoma. *iScience*. 2024;27:109821.
199. Marusyk A, Janiszewska M, Polyak K. Intratumor heterogeneity: the Rosetta Stone of therapy resistance. *Cancer Cell*. 2020;37:471-84.
 200. Zhang Y, Lei J, Ma H, Zuo S. Impact of type of lenvatinib resistance on prognosis and second-line regimen in patients with virus-associated HCC. *J Hepatocell Carcinoma*. 2024;11:1507-17.
 201. Gifford G, Paul J, Vasey PA, Kaye SB, Brown R. The acquisition of hMLH1 methylation in plasma DNA after chemotherapy predicts poor survival for ovarian cancer patients. *Clin Cancer Res*. 2004;10:4420-6.
 202. Zhang SM, Cai WL, Liu X, Thakral D, Luo J, Chan LH, et al. KDM5B promotes immune evasion by recruiting SETDB1 to silence retroelements. *Nature*. 2021;598:682-7.
 203. Vera O, Rodriguez-Antolin C, de Castro J, Karreth FA, Sellers TA, Ibanez de Caceres I. An epigenomic approach to identifying differential overlapping and cis-acting lncRNAs in cisplatin-resistant cancer cells. *Epigenetics*. 2018;13:251-63.
 204. Lei J, Dai H, Zhang Y, Ou G, Peng Liang Z, Lu Y, et al. Prognostic impact of primary versus secondary resistance to sorafenib in patients with HCC. *Ther Adv Med Oncol*. 2025;17:17588359241299678.
 205. Casadei Gardini A, Puzzoni M, Montagnani F, Marisi G, Tamburini E, Cucchetti A, et al. Profile of lenvatinib in the treatment of hepatocellular carcinoma: design, development, potential place in therapy and network meta-analysis of hepatitis B and hepatitis C in all Phase III trials. *Onco Targets Ther*. 2019;12:2981-8.
 206. De Matteis S, Ghatti M, Gramantieri L, Marisi G, Casadei-Gardini A. Sorafenib in the treatment of virus-related HCC: differences between HCV and HBV. *Onco Targets Ther*. 2021;14:4305-8.
 207. Mao K, Zhang J, He C, Xu K, Liu J, Sun J, et al. Restoration of miR-193b sensitizes Hepatitis B virus-associated hepatocellular carcinoma to sorafenib. *Cancer Lett*. 2014;352:245-52.
 208. Braconi C, Valeri N, Gasparini P, Huang N, Taccioli C, Nuovo G, et al. Hepatitis C virus proteins modulate microRNA expression and chemosensitivity in malignant hepatocytes. *Clin Cancer Res*. 2010;16:957-66.
 209. Zheng DL, Zhang L, Cheng N, Xu X, Deng Q, Teng XM, et al. Epigenetic modification induced

- by hepatitis B virus X protein via interaction with de novo DNA methyltransferase DNMT3A. *J Hepatol.* 2009;50:377-87.
210. Zhang B, Han S, Feng B, Chu X, Chen L, Wang R. Hepatitis B virus X protein-mediated non-coding RNA aberrations in the development of human hepatocellular carcinoma. *Exp Mol Med.* 2017;49:e293.
 211. Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol.* 2021;18:223-38.
 212. Howell J, Samani A, Mannan B, Hajiev S, Motedayen Aval L, Abdelmalak R, et al. Impact of NAFLD on clinical outcomes in hepatocellular carcinoma treated with sorafenib: an international cohort study. *Ther Adv Gastroenterol.* 2022;15:17562848221100106.
 213. Singal AG, Nagar SP, Hitchens A, Davis KL, Iyer S. Real-world effectiveness of lenvatinib in hepatocellular carcinoma patients with nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol.* 2023;21:830-1.e1.
 214. Tomonari T, Sato Y, Tanaka H, Mitsuhashi T, Hirao A, Tanaka T, et al. Therapeutic efficacy of lenvatinib in nonviral unresectable hepatocellular carcinoma. *JGH Open.* 2021;5:1275-83.
 215. Wu X, Zeng M, Wei Y, Lu R, Huang Z, Huang L, et al. METTL3 and METTL14-mediated N(6)-methyladenosine modification of SREBF2-AS1 facilitates hepatocellular carcinoma progression and sorafenib resistance through DNA demethylation of SREBF2. *Sci Rep.* 2024;14:6155.
 216. Arif KMT, Elliott EK, Haupt LM, Griffiths LR. Regulatory mechanisms of epigenetic miRNA relationships in human cancer and potential as therapeutic targets. *Cancers (Basel).* 2020;12.
 217. Cao LQ, Yang XW, Chen YB, Zhang DW, Jiang XF, Xue P. Exosomal miR-21 regulates the TETs/PTENp1/PTEN pathway to promote hepatocellular carcinoma growth. *Mol Cancer.* 2019;18:148.
 218. Zhang L, Li HT, Shereda R, Lu Q, Weisenberger DJ, O'Connell C, et al. DNMT and EZH2 inhibitors synergize to activate therapeutic targets in hepatocellular carcinoma. *Cancer Lett.* 2022;548:215899.
 219. Majchrzak-Celińska A, Baer-Dubowska W. Pharmacoepigenetics: an element of personalized therapy? *Expert Opin Drug Metab Toxicol.* 2017;13:387-98.
 220. Sethi S, Ali S, Sethi S, Sarkar FH. MicroRNAs in personalized cancer therapy. *Clin Genet.*

2014;86:68-73.

221. Tang C, Tang C, Zhu X, Wang S, Yang Y, Miao Y, et al. Loss of AXIN1 regulates response to lenvatinib through a WNT/KDM5B/p15 signalling axis in hepatocellular carcinoma. *Br J Pharmacol.* 2025;182:1394-409.
222. Fu YD, Huang MJ, Guo JW, You YZ, Liu HM, Huang LH, et al. Targeting histone demethylase KDM5B for cancer treatment. *Eur J Med Chem.* 2020;208:112760.
223. Huang M, Chen C, Geng J, Han D, Wang T, Xie T, et al. Targeting KDM1A attenuates Wnt/ β -catenin signaling pathway to eliminate sorafenib-resistant stem-like cells in hepatocellular carcinoma. *Cancer Lett.* 2017;398:12-21.
224. Fang Y, Liao G, Yu B. LSD1/KDM1A inhibitors in clinical trials: advances and prospects. *J Hematol Oncol.* 2019;12:129.
225. Cao Z, Liu J, Yang X. Deformable nanocarriers for enhanced drug delivery and cancer therapy. *Exploration.* 2024;4:20230037.
226. Elemike EE, Onunkwo IC, Ughumiakpor O, Alawuru F, Mukoro A, Ishom P, et al. Bio-nanomaterials: Promising anticancer properties and treatment strategies. *Nano Transmed.* 2025;4:100076.
227. Lei C. Nanotechnology: Paving the way for advanced healthcare and beyond. *Nano Transmed.* 2023;2:100023.
228. Zhou S, Ma Y, Liu X, Yu P, Huang N, Song L, et al. Targeted delivery of glypican 3 (GPC3) antibody-modified microRNA (miR let-7b-5p) polymer nanoparticles to sorafenib-resistant hepatocellular carcinoma cells. *J Biomed Nanotechnol.* 2021;17:677-90.
229. He GN, Bao NR, Wang S, Xi M, Zhang TH, Chen FS. Ketamine induces ferroptosis of liver cancer cells by targeting lncRNA PVT1/miR-214-3p/GPX4. *Drug Des Devel Ther.* 2021;15:3965-78.

Figure 1. Schematic diagram of epigenetic regulation mechanisms and drug resistance outcomes. The mechanisms of epigenetic regulation contributing to drug resistance include: DNA/RNA methylation, histone modifications, and non-coding RNA regulation; while the consequential manifestations of drug resistance encompass: escaped programmed cell death, tumor metabolic reprogramming, formation and maintenance of drug-resistant cells, uncontrolled cell proliferation signaling pathways and abnormal transport processes. *Image created by Figdraw.*

Figure 2. Epigenetic modifications enhance sorafenib resistance by inhibiting apoptosis, involving the following regulators: a. KDM1A and lncRNA TTN-AS1 promote sorafenib resistance by upregulating anti-apoptotic proteins. b. miR-494, lncRNA NEAT1, miR-124-3p, and miR-518d-5p promote resistance by suppressing pro-apoptotic proteins. c. miR-10b-3p, circRNAs, and cDCBLD2 promote resistance by directly inhibiting caspase-3 cleavage. *Image created by Figdraw.*

Figure 3. Epigenetic modifications contribute to sorafenib resistance by suppressing ferroptosis, involving the following regulators: a. lncRNA CASC11, lncRNA DUXAP860, lncRNA MALAT1, circTTC13, and lncRNA PVT1 inhibit ferroptosis by targeting the System Xc⁻/GPX4 pathway. b. miR-23a-3p and lncRNA HNF4A-AS1 suppress ferroptosis by targeting the lipid peroxidation pathway. c. miR-654-5p, lncRNA URB1-AS1, EZH2 and YTHDC1 suppress ferroptosis by targeting the iron metabolism pathway with reduced intracellular free Fe²⁺ levels. *Image created by Figdraw.*

Figure 4. Epigenetic modification promotes sorafenib resistance by regulating autophagy. Some types of autophagy have prodeath effects and cause resistance when inhibited, whereas other types of autophagy have protective effects and cause resistance when enhanced. Classical autophagy comprises four key steps: initiation, nucleation, maturation, and degradation; epigenetic modification regulates autophagosome formation by affecting one or more of these processes. Epigenetic regulatory factors involved in the above processes include ncRNAs (e.g., miR-21, miR-25, miR-21-5p, hsa-miR30a-5p, lncRNA CRNDE, lncRNA BBOX1-AS1), RNA methylation modification enzymes (e.g., METTL3, YTHDF1), histone demethylase (PHF8). *Image created by Figdraw.*

Figure 5. Epigenetic modifications promote sorafenib resistance through metabolic reprogramming, involving the following regulators: a. miR-374b, miR-188-5p, miR-30a-5p and miR-494 promote resistance by regulating

glycolysis. b. lncRNA LINC01468 and lncRNA LINC01056 promote resistance by regulating lipid metabolism. c. lncRNA LINC01234 promotes resistance by regulating aspartate metabolism. *Image created by Figdraw.*

Figure 6. Epigenetic modifications promote sorafenib resistance by facilitating the formation and maintenance of drug-resistant cells, involving the following regulators: a. miR-3677-3p, miR-93, miR-361-3p, DNMT3a, DNMT3b, TET2, KDM5B, HDAC11, and METTL3 drive resistance by stimulating CSC expansion. b. LINC01089, lncRNA H19, miR-125b-5p, LINC00540, circMEMO1, lncRNA DUXAP8, KIAA1429, and METTL3 drive resistance through EMT activation. *Image created by Figdraw.*

Figure 7. Epigenetic modifications promote sorafenib resistance by activating uncontrolled cell proliferation signaling pathways, involving the following regulators: a. miR-19a-3p and circITCH drive resistance by modulating the PI3K/AKT/mTOR signaling pathway. b. miR-22-3p, circRNA-SORE, and hypomethylation of the Shc3 upstream promoter drive resistance by modulating the Ras/Raf/MEK/ERK signaling pathway. c. HDAC2 drives resistance by upregulating PLEKHG5 expression and activating the Rac1/AKT/NF- κ B pathway. *Image created by Figdraw.*

Figure 8. Epigenetic modification promotes sorafenib resistance by regulating HCC transport process, involving the following regulators: a. miR-138-1-3p promotes resistance by upregulating the drug efflux pump ABCB1. b. METTL3 and METTL14 promote resistance by downregulating drug uptake transporters OATP1B1 and OATP1B3. c. miR-4669, miR-1228, miR-494-3p, miR-93, and circUPF2 promote resistance via exosome-mediated transport. *Image created by Figdraw.*

Figure 9. Epigenetic modifications play crucial roles in lenvatinib resistance. The reported epigenetic mechanisms of lenvatinib resistance involve ncRNA regulation, RNA methylation/acetylation, and histone modification. NcRNAs promotes lenvatinib resistance through regulating PCD (e.g., lncRNAAC026401.3, lncMT1JP, lncRNA HOTAIRM1, miR-128-3p and circPIAS1), CSCs expansion (e.g., miR-183-5p, miR-3154, LCC-ZEB2-19 and circ0007386), metabolism reprogramming (e.g., LINC01532), and uncontrolled cell proliferation signaling pathway activity (e.g., CircCCNY and hsa-circ-0058046); key regulators of RNA methylation/acetylation include METTL3, METTL1, YTHDF2, and NAT10; histone modifications involve IGF2BP3-mediated lactylation and EZH2-dependent NOD2 methylation. *Image created by Figdraw.*

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Abbreviations

Abbreviation	Full name
HCC	hepatocellular carcinoma
VEGF	vascular endothelial growth factor
ncRNA	non-coding RNA
SAM	s-adenosylmethionine
DNMT	DNA methyltransferase
TET	ten-eleven translocation
HAT	histone acetyltransferase
HDAC	histone deacetylase
HMT	histone methyltransferase
HDM	histone demethylase
METTL	methyltransferase-like 3
YTHDF	YT521-B homology domain-containing family proteins
PDGF	platelet-derived growth factor
PCD	programmed cell death
BCL-2	B-cell lymphoma-2
PTEN	phosphatase and tensin homolog
SIRT1	silent information regulator sirtuin 1
FOXO3a	forkhead box class O3a
ROS	reactive oxygen species
GPX4	glutathione peroxidase 4
SLC7A11	solute carrier family 7 member 11
SLC3A2	solute carrier family 3 member 2
GSH	glutathione
GSSG	glutathione disulfide
ACSL4	acyl-CoA synthetase long-chain family 4
HSPB1	heat shock protein family B member 1
TFR1	transferrin receptor protein 1
NCOA4	nuclear receptor coactivator 4
SOD1	superoxide dismutase 1
CCS	copper chaperone for superoxide dismutase

ATG	autophagy-related protein
mTORC	mechanistic target of rapamycin kinase
AMPK	adenosine monophosphate-activated protein kinase
ULK	UNC-51-like kinase
PI3P	phosphatidylinositol-3-phosphate
FBXW7	F-box and WD repeat domain-containing 7
SCF	SKP1-cullin-1-F-box-protein
USP24	ubiquitination-specific peptidase 24
EMT	epithelial-mesenchymal transition
PKM	pyruvate kinase M
PI3K	phosphoinositide 3-kinase
ERS	endoplasmic reticulum stress
HIF-1A	hypoxia-inducing factor 1A
MAFLD	metabolic associated fatty liver disease
FAO	fatty acid oxidation
ASS1	arginine succinate synthase 1
CSC	cancer stem cell
T-IC	tumor-initiating cell
OCT	octamer-binding transcription factor
SOX1	SRY-box transcription factor 1
AKR1C2	aldo-keto reductase family 1 member C2
HBx	HBV-encoded X protein
Shc3	src collagen homology 3
MVP	major vault protein
RTK	receptor tyrosine kinase
ABC	ATP-binding cassette
EV	extracellular vesicle
CAF	cancer-associated fibroblast
GSK-3 β	glycogen synthase kinase-3 β
IGF2BP	insulin-like growth factor 2 mRNA-binding protein

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