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Review Article

Potential therapies for HCC involving targeting the ferroptosis pathway

Denghui Li[1*], Mengjie Zhang[1*], Ju Liu[2], Zhifang Li[2], Bing Ni[1]

[1]Department of Pathophysiology, College of High Altitude Military Medicine, Third Military Medical University, Chongqing 400038, China; [2]Department of Foreign Languages, College of Basic Medical Sciences, Third Military Medical University, Chongqing 400038, China. [*]Equal contributors.

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Abstract: Liver cancer ranks as the third leading cause of cancer-related mortality worldwide, predominantly in the form of hepatocellular carcinoma (HCC). Conventional detection and treatment approaches have proven inadequate for addressing the elevated incidence and mortality rates associated with HCC. However, a significant body of research suggests that combating HCC through the induction of ferroptosis is possible. Ferroptosis is a regulated cell death process characterized by elevated levels of reactive oxygen species (ROS) and lipid peroxide accumulation, both of which are dependent on iron levels. In recent years, there has been an increasing focus on investigating ferroptosis, revealing its potential as an inhibitory mechanism against various diseases, including tumors. Therefore, ferroptosis induction holds great promise for treating multiple types of cancers, including HCC. This article provides a review of the key mechanisms involved in ferroptosis and explores the potential application of multiple targets and pathways associated with ferroptosis in HCC treatment to improve therapeutic outcomes.

Keywords: Hepatocellular carcinoma, ferroptosis, therapy, positive regulation, negative regulation

Introduction

The global incidence of liver cancer reached over 900,000 cases in 2020, representing 4.7% of all newly diagnosed cancers worldwide; however, liver cancer accounted for a significant proportion (8.3%) of total cancer-related mortalities [1]. Furthermore, liver cancer was ranked as the third leading cause of cancer-related deaths globally [2]. The most common liver cancer form is HCC, accounting for up to 90% of cases. Numerous studies have demonstrated that various liver disease backgrounds, recurrence rates, and tumor heterogeneity are key factors associated with the treatment and prognosis of HCC patients [3]. Risk factors for developing HCC include obesity, fatty liver, cirrhosis, aflatoxin exposure, alcohol consumption, and diabetes [4].

From an epidemiological perspective, frequent consumption of a high-iron diet has been associated with an increased risk of HCC [5], suggesting that targeting iron-induced cell death could be a potential strategy for combating liver cancer. The investigation of iron-induced cell death in tumors is gaining momentum, supported by a plethora of evidence indicating the susceptibility of various tumor types, such as HCC and renal cell carcinoma [6]. It not only impacts the initiation and progression of cancer but also influences neurodegenerative diseases [7] and cardiovascular disorders [8] and is associated with various pathological physiological processes. For instance, liver cells undergo morphological alterations or ischemia-reperfusion injury following ART treatment [9]. Furthermore, it regulates the progression of various diseases, including hemolytic disorders [10], autoimmune diseases [11], and acute myeloid leukemia [12]. This review will briefly introduce ferroptosis-related pathophysiology and will focus mainly on the potential targets and pathways involved in the negative and positive regulation of ferroptosis for the treatment of HCC.

Ferroptosis-related physiology and pathophysiology

Comprehensive ferroptosis and fundamental characteristics

The concept of ferroptosis was initially proposed by Dixon et al., who postulated that it is a

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recently discovered iron-dependent regulated cell death pathway [13]. Stockwell et al. also identified erastin, a novel compound discovered through experimental screening that selectively induces cell death in cells with RAS mutations. This mechanism is distinct from traditional apoptosis [14]. Subsequently, a replication of the experiment was conducted by his team, wherein they screened for an additional compound called RAS synthetic lethality 3 (RSL3), which also elicits a comparable form of cell death [15]. Erastin or RSL3 (now defined as a class I and II ferroptosis inducer) inhibits the antioxidant system by increasing intracellular iron accumulation. Multiple experiments have shown that the consumption of lipid peroxidation inhibitors, iron chelators (e.g., deferoxamine), and polyunsaturated fatty acids (PUFAs) can inhibit this type of cell death, while the provision of exogenous iron (e.g., ferric citrate) promotes it [15]. Iron, a redox-active metal, can participate in the Fenton reaction, thereby facilitating the generation of excessive ROS and augmenting oxidative damage [16]. The Fenton reaction is a nonenzymatic chain reaction involving the oxidation of organic substrates by Fe^{2+} and peroxide (H_2O_2), resulting in the generation of lipid hydroperoxides (PLOOH) [17].

The morphological changes observed in ferroptosis are similar to those observed in pathological processes such as apoptosis, necrosis, and autophagy, although ferroptosis is distinct from pathological processes [18]. Morphologically, it is characterized by cell membrane rupture and vesiculation, a reduction in mitochondrial volume, decreased or no cristae, membrane wrinkling and shrinkage, outer membrane fragmentation, and increased membrane potential. Nevertheless, the nuclear structure remains intact without condensation or the formation of apoptotic bodies [19]. Moreover, no evidence of vesicle encapsulation or disruption of plasma membrane integrity resembling that of autophagic cells was observed in ferroptotic cells [20].

Iron metabolism in ferroptosis

The predominant forms of iron in an unstable iron pool are Fe^{2+} and glutathione-complexed species [21, 22]. Elevated iron levels have previously been identified as a predisposing factor for various cancers, including HCC [23]. In the physiological state, cellular iron is predominantly internalized into the cell via endocytosis facilitated by transferrin receptor 1 (TFR1), which specifically binds and uptakes iron complexed with transferrin [24]. Repressing TFR1 expression can effectively impede this process and mitigate ferroptosis [25]. Subsequently, it can be eliminated via exosomes and extracellular vesicles that contain ferritin [26]. As the primary driving factor of this process, prominin 2 exerts a significant influence on ferroptosis [27], and the downregulation of ferritin expression results in an increase in the labile iron pool, increasing the susceptibility of cells to ferroptosis [28]. Ceruloplasmin (CP) regulates iron levels to inhibit ferroptosis in HCC cells [29] (**Figure 1**).

Detection of ferroptosis

Iron abundance serves as a crucial indicator for detecting ferroptosis, and FRET iron probe-1 (FIP-1) can function as a fluorescent agent in labile iron during this process [30]. In addition, the level of lipid peroxidation can be quantified using inductively coupled plasma-mass spectrometry (ICP-MS) with Prussian blue staining [13]. The utilization of peroxidized phospholipids significantly enhances this characteristic, thereby making LC-MS quantitative monitoring of peroxidized phosphatidylcholine a valuable indicator for detecting ferroptosis [31]. Along with Liperfluo, BODIPY-C11, a fluorescent probe that undergoes a color change from red to green in iron-dead cells, is a commonly employed technique for quantifying lipid peroxidation in the context of ferroptosis [32]. The assessment of nicotinamide adenine dinucleotide phosphate (NADPH) activity can serve as an indicator of ferroptosis, reflecting the enzymatic function of glutathione peroxidase 4 (GPX4) [33]. Glutathione reductase (GR) facilitates the regeneration of reduced glutathione (GSH) through the reduction of oxidized glutathione (GSSG), utilizing NADPH as an electron donor (**Figure 1**). Consequently, the abundance of NADPH can also serve as a potential indicator of iron depletion [34].

Ferroptosis mechanisms and regulatory factors

Ferroptosis is characterized by the upregulation of ROS and the accumulation of lipid peroxides resulting from the depletion of GSH and inactivation of GPX4 [35]. GSH functions as a substrate for GPX4, safeguarding cells against

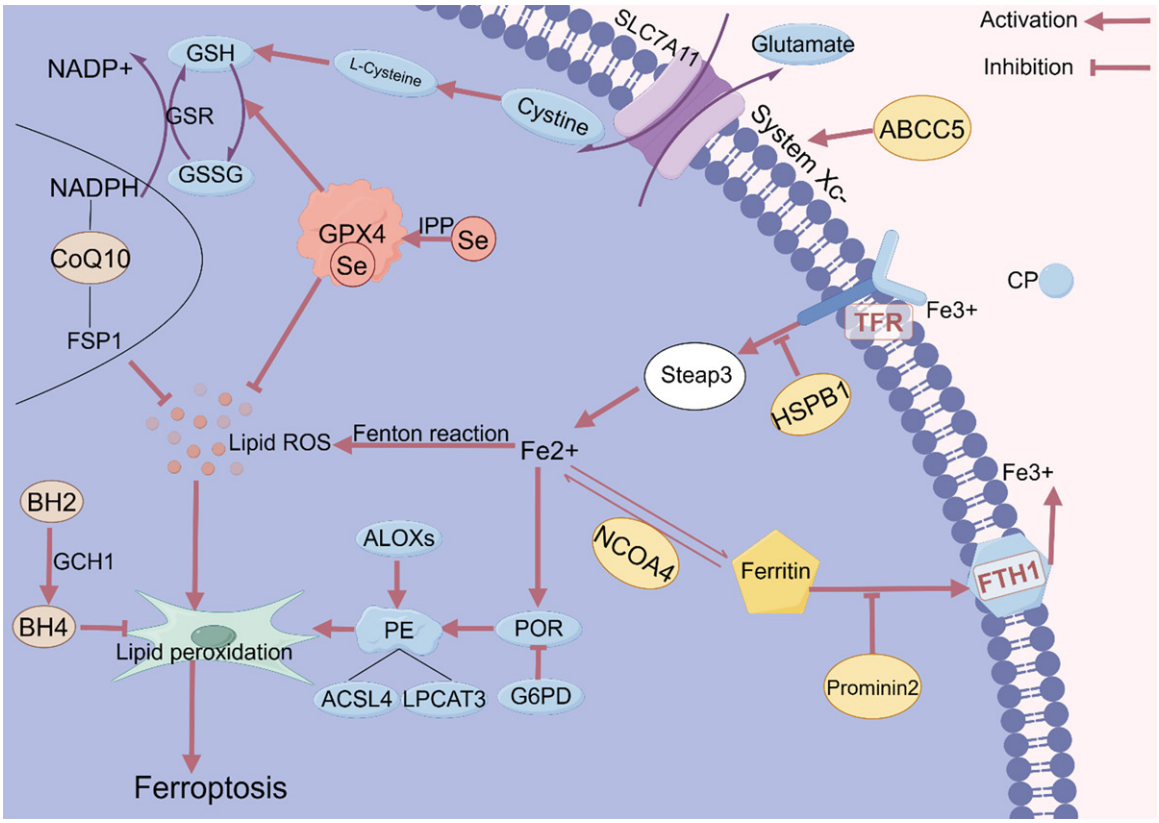

Figure 1. Molecular mechanisms of ferroptosis. The primary mechanism underlying ferroptosis is the induction of iron-induced lipid peroxidation. System Xc- mediates cystine uptake and the synthesis of GSH, while GPX4 plays a pivotal role in initiating lipid peroxidation. Phosphatidylethanolamine activates lipid peroxidation via the action of oxygenases such as ALOX and POR, accompanied by the catalysis of ACSL4 and LPCAT3. Extracellular iron enters the cell mainly through the TFR, the free iron in the cell is stored in ferritin, and through the mutual conversion of NCOA4 with Fe^{2+}, iron drives FTH1 to transport iron to the extracellular space through ferritinophagy. In addition, the CoQ10 and BH4 systems influence ferroptosis via the lipid peroxidation pathway. Abbreviations: System Xc-, Cystine-glutamate antiporter system; ALOXs, lipoxygenases; BH4, tetrahydrobiopterin; BH2, dihydrobiopterin; FTH1, ferritin heavy chain 1; HSPB1, heat shock protein beta-1; ABCC5, ATP-binding cassette transporter C5; ACSL4, acyl-CoA synthetase long chain family member 4; CoQ10, coenzyme Q10; G6PD, glucose-6-phosphate dehydrogenase; PE, phosphatidylethanolamine; GPX4, glutathione peroxidase 4; GSH, glutathione; GSR, glutathione-disulfide reductase; GSSG, oxidized glutathione; LPCAT3, lysophosphatidylcholine acyltransferase 3; NCOA4, nuclear receptor coactivator 4; POR, cytochrome p450 oxidoreductase; IPP, isopentenyl pyrophosphate; CP, ceruloplasmin; NCOA4, nuclear receptor coactivator 4; POR, cytochrome p450 oxidoreductase; SLC7A11, solute carrier family 7 member 11; ACLS4, acyl-CoA synthetase long chain family member 4; STEAP3, STEAP3 metalloreductase; TFR, transferrin receptor; GCH1, GTP cyclohydrolase I.

damage caused by lipid peroxidation. Disruption of GPX4 activity or inhibition of GSH synthesis can trigger ferroptosis [36]. In recent years, an increasing number of studies have elucidated the targets and pathways involved in ferroptosis. Ferroptosis inducers (FINs) can be classified into three categories. Category 1 includes erastin and DPI2, which inhibit the Xc- system and reduce GSH levels to suppress the antioxidant system. Category 2 comprises DPI7, DPI10, RSL3, etc., which directly inhibit GPX4, leading to peroxidation. Category 3 consists of drugs such as sorafenib and artemisinin derivatives that induce iron-dependent cell death. Ferroptosis inhibitors can be categorized into five classes: iron chelators, protein synthesis inhibitors, ROS inhibitors, antioxidants, and transaminase inhibitors [37].

The core events in the process of ferroptosis involve lipid peroxidation and iron accumulation, which are two pivotal factors triggering oxidative damage during this process [38]. Understanding the fundamental molecular mechanism underlying ferroptosis is crucial for elucidating its defensive mechanisms [39].

It is widely acknowledged that the substrate responsible for lipid peroxidation primarily comprises polyunsaturated fatty acids incorporated into membrane phospholipids (PL-PUFAs) [40]. Lipid peroxidation in PL-PUFAs can occur through two pathways. The first pathway involves nonenzymatic lipid autoxidation, where Fe^{2+} catalyzes the Fenton reaction, leading to the production of a significant amount of ROS. Subsequently, ROS trigger lipid peroxidation in PL-PUFAs [17]. In addition, membrane PUFAs are the primary targets of ROS attack [41]. The second pathway is the enzymatic-catalyzed reaction. Lipoxygenase (LOX) and cytochrome P450 oxidoreductase (POR) initiate lipid peroxidation [42], after which acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) participate in the biosynthesis and remodeling of phosphatidylethanolamine (PE), a pivotal phospholipid that triggers ferroptosis [43]. Upregulation of ACSL4 also leads to an increase in PUFAs [43], and downregulation of ACSL4 and LPCAT3 can attenuate intracellular lipid peroxidation. For instance, pioglitazone, a pharmacological agent, exerts inhibitory effects on ACSL4 to impede the conversion of free fatty acids into fatty acyl-CoA [44]. In addition, glucose-6-phosphate dehydrogenase (G6PD) modulates POR metabolism to inhibit ferroptosis [45] (**Figure 1**).

Three systems for the elimination of lipid peroxides have been identified, namely, the GSH system, the coenzyme Q10 (CoQ10) system, and the tetrahydrobiopterin (BH4) system [46] (**Figure 1**). The damage caused by the aforementioned systems can potentially result in ferroptosis. Among these systems, the GSH system was reported earliest and most extensively. The key components of the GSH system include: (1) The cystine-glutamate antiporter system (system Xc-), which is a widely distributed amino acid countertransport protein located in the phospholipid bilayer. It belongs to the heterodimeric amino acid transporter family and consists of heterodimers formed by disulfide bonds linking solute carrier family 7 member 11 (SLC7A11) and solute carrier family 3 member 2 (SLC3A2). This system is responsible for cellular cystine uptake while releasing glutamate [43]. By reducing the input of cysteine and inhibiting GSH production, SLC7A11 indirectly suppresses the activity of GPX4 [47]. This crucial mechanism enables ATP-binding cassette transporter C5 (ABCC5) to increase GSH levels by stabilizing the protein SLC7A11, thereby inhibiting ferroptosis [48]. (2) Cystine, the absorbed form of syngas in cells, is exchanged with glutamate through the Xc- system at a 1:1 ratio. GSH serves as a substrate for the synthesis of GSH, which is the reduced product of cysteine within cells [49], and inhibition of cystine uptake via the Xc- system can induce ferroptosis [50]. (3) GSH, under the action of glutathione peroxidase (GPX), reduces ROS and possesses antioxidant properties [51]. The Hippo-YAP/TAZ pathway plays a crucial role in maintaining the homeostasis of GSH in the body by activating transcription factor 4 (ATF4) in a TEAD-dependent manner, enhancing the expression of SLC7A11, and promoting sorafenib resistance in HCC cells [52]. Ribonucleotide reductase subunit M2 (RRM2) also affects ferroptosis in HCC cells by stimulating the synthesis of intracellular GSH [53]. (4) GPX4, as a selenoprotein, converts GSH into glutathione (GSSG) and utilizes GSH to transform toxic lipid peroxides into nontoxic lipid alcohols. Additionally, it can inhibit the activity of LOX [54]; therefore, inhibition of GPX4 activity can lead to the accumulation of lipid peroxides. It has also been reported that activation of GPX4 inhibits inflammation caused by lipid peroxidation and attenuates the arachidonic acid (AA) and nuclear factor κB (NF-κB) signaling pathways, thereby mitigating ROS-mediated ferroptosis [55]. In conclusion, it can be inferred that the system Xc-/cysteine/GSH/GPX4 axis plays a pivotal role in modulating lipid peroxidation and eliciting ferroptosis.

The main component of the CoQ10 antioxidant system is ferroptosis suppressor protein 1 (FSP1), which was discovered by Bersuker et al. in hundreds of cancer cell lines [56]. Subsequently, FSP1 was shown to inhibit lipid peroxidation and prevent ferroptosis by facilitating ubiquinone (CoQ10) regeneration [57]. Doll et al. first discovered that FSP1-CoQ10-NAD[P]H, a distinct and parallel pathway, can inhibit phospholipid peroxidation and ferroptosis in conjunction with GPX4 [56]. The newly reported chemical inducer FIN56 appears to induce ferroptosis by targeting coenzyme Q10 [58].

BH4 is an endogenous antioxidant that protects cells from ferroptosis independently of GPX4 [59]. GCH1 (GTP cyclohydrolase I) acts as

the rate-limiting enzyme of BH4, forming the GCH1/tetrahydrobiopterin metabolic pathway. This pathway may involve the generation of ROS and the inhibition of ferroptosis. The underlying mechanism is attributed to the antioxidant effect of BH4/BH2 (dihydrobiopterin) [60].

The other regulatory factors of ferroptosis primarily include the mevalonate (MVA) pathway, transsulfuration pathway, and HSF1-HSPB1 system [61] (**Figure 1**). The MVA pathway regulates the maturation of selenocysteine-specific tRNA via isopentenyl pyrophosphate (IPP), thereby affecting the efficient translation and synthesis of GPX4 and ultimately governing ferroptosis [62]. Moreover, the production of COQ10 is also a significant outcome of the MVA pathway [63]. The transsulfuration process involves the enzymatic conversion of methionine and glucose into homocysteine followed by its subsequent transformation of cysteine, which plays a crucial role in cellular defense against ferroptosis [64]. This groundbreaking work conducted by Harry Eagle during the 1950s and 1960s is highly important [65]. Heat shock proteins (HSPs) are a group of heat stress proteins that exhibit molecular chaperone activity [66], and heat shock factor (HSF) serves as the primary transcription factor for synthesizing HSPs [67]. Sun et al. conducted experimental research investigating the inhibitory effect of HSF1 knockdown on the protein expression of heat shock protein beta-1 (HSPB1) induced by erastin, which acts as a negative regulator of ferroptosis and lipid peroxides through its phosphorylation [68]. This erastin-induced ferroptosis was observed with increased expression of HSF1-HSPB1, whereas overexpression of HSPB1 had the opposite effect [69].

The negative regulatory pathways of ferroptosis in HCC treatment

In 2022, three studies proposed a potential therapeutic strategy for the treatment of various diseases through the negative regulation of ferroptosis. Yang et al. demonstrated that salidroside decreased the viability of HT22 cells, attenuated the accumulation of oxidative products, and significantly reduced lipid peroxidation and ROS accumulation within cells, subsequently leading to the upregulation of GPX4 and SLC7A11 expression. This effect was achieved by inhibiting ferroptosis via activation of the Nrf2/HO1 signaling pathway [70]. These findings have significant implications for therapeutic approaches for Alzheimer's disease. Yang et al. found that in a d-galactosamine/lipopolysaccharide (D-GalN/LPS) model, Maresin1 (MaR1) can inhibit ROS expression and increase GSH levels in mouse tissues, preventing acute liver injury (ALI) caused by ferroptosis through activation of Nrf2/HO-1/GPX4 expression [71]. This study underscores the significant therapeutic implications of MaR1 for ALI via the modulation of ferroptotic processes. Yuan et al. reported that oxygen-glucose deprivation/reoxygenation (OGD/R) in an in vitro stroke model can decrease the expression of GPX4 and SLC7A11. However, treatment with kaempferol after neuronal exposure increased the protein levels of GPX4 and SLC7A11, confirming its ability to activate the Nrf2/SLC7A11/GPX4 signaling pathway [72]. This finding demonstrates the vital significance of ferroptosis in the treatment of ischemia/reperfusion-related cell death and its major role in stroke therapy. These findings also suggest that ferroptosis can be negatively regulated to kill HCC cells through different mechanisms (**Table 1**).

Lipid peroxidation pathway in HCC

Xie et al. screened a flavonoid compound, baicalin, from a natural product library as a potential ferroptotic inhibitor by reducing iron accumulation and inhibiting lipid peroxidation [73]. Alpha-tocopherol (vitamin E) can also regulate lipid peroxidation to maintain the homeostasis of GPX4, thereby exerting inhibitory effects on ferroptosis. Foods abundant in vitamin E, such as brown rice, can be utilized to ameliorate lipid peroxidation induced by GPX4 imbalance and reduce cellular toxicity [74]. GPX4 has been proven to cause ferroptosis through lipid peroxidation. Moreover, vitamin E, a fat-soluble antioxidant, plays a crucial role in safeguarding cell membranes against oxidative damage. Consequently, supplementation with vitamin E has potential for mitigating GPX4-mediated ferroptosis [33]. As early as 70 years ago, the natural antioxidant vitamin E was found to be associated with elements such as selenium, cyste and lipid peroxidation [75]. Recent studies have further substantiated the role of selenium in preventing ferroptosis [76].

Table 1. Regulators of ferroptosis

Factor/Pathway	Effects	Mechanism	Ref
NFE2L2 (Nrf2)	Negative	Suppresses ferroptosis	[80]
Rb	Negative	Inhibits sorafenib-induced ferroptosis	[108]
MiR-214/circ0097009	Negative	Enhances Erastin-induced ferroptosis	[98]
CircIL4R	Negative	Inhibits ferroptosis	[102]
CISD2	Negative	Inhibits ferroptosis	[95]
CISD1	Positive	Regulates mitochondrial iron uptake	[96]
P53	Positive	Inhibits SLC7A11 expression and cystine uptake	[119]
IFN-γ	Positive	Inhibits system Xc- activity	[133]
TGF-β1	Positive	Inhibits xCT expression	[131]
Sorafenib	Positive	Suppresses the activity of system Xc-	[109, 110]
FC	Positive	Induction of ferroptosis by ferritin phagocytosis	[144]

In 2020, Miotto et al. investigated the mechanism by which ferrostatin-1 functions as an antioxidant to protect against ferroptosis by inhibiting lipid peroxidation [77]. Fer-1 is localized to the endoplasmic reticulum (ER) membrane, suggesting that its primary site of action may also be on the ER membrane [78]. A study conducted by Abrams et al. revealed that 1,2-dioxolane (FINO2), a cyclic peroxide screened among organic peroxides, can induce ferroptosis [79]. In a research article published in February this year, Professors Stockwell, Wei Min, and Woerp investigated the subcellular localization of FINO2, and lipophilic ferroptosis-induced experiments further revealed that lipid peroxidation predominantly occurs within the endoplasmic reticulum (ER), followed by the cellular membrane [80].

Nrf2 core pathway in HCC

Nrf2 is a pivotal transcription factor implicated in diverse biological processes, including iron metabolism. It can counteract lipid peroxidation and safeguard cells against oxidative damage [80] and serves as a key negative regulatory factor in ferroptosis. P62, a signaling protein found in various precancerous liver diseases and HCC, is highly expressed and can induce NRF2 activation [81]. The p62-Keap1-Nrf2 antioxidant signaling pathway has been demonstrated by Sun et al. to play a pivotal role in safeguarding HCC cells against ferroptosis through the upregulation of p62 activation to prevent Keap1-mediated degradation of Nrf2, thereby augmenting the anticancer efficacy of sorafenib and other therapeutic agents. These findings underscore the critical importance of the p62-Keap1-NRF2 pathway in protecting HCC cells from iron-induced cytotoxicity [82]. The intracellular accumulation of Nrf2 further triggers the activation of heme oxygenase-1 (HO-1), quinone oxidoreductase 1 (NQO1), ferritin heavy chain 1 (FTH1), and other iron-related factors, thereby inhibiting cellular iron overload and peroxidation and thus mediating HCC cell resistance to ferroptosis [82]. The precise role of HO-1 in iron death remains elusive, as conflicting reports suggest that excessive activation of HO-1 may release ferrous ions and contribute to ferroptosis [83], while others have reported that inhibiting HO-1 can exacerbate iron death [84]. Therefore, further investigation is required to elucidate the involvement of HO-1 in iron-induced death. In summary, the p62-Keap1-Nrf2 signaling pathway likely modulates ferroptosis and HCC development by regulating iron and ROS metabolism. Notably, Sun et al. (2020) demonstrated the potential for ROS accumulation via Keap1 deubiquitination and Nrf2 ubiquitination [85]. Furthermore, upon sorafenib treatment in HCC cells, the downstream metallothionein 1G (MT-1G) gene of the p62-Keap1-Nrf2 pathway exhibited increased expression levels, potentially attributed to transcriptional regulation by NRF2. Notably, Nrf2 can upregulate MT-1G expression via the cysteine-glycine pathway; conversely, inhibiting MT-1G can enhance GSH consumption and lipid peroxidation while promoting sorafenib-induced ferroptosis [86]. These findings suggest that MT-1G plays a negative regulatory role in ferroptosis in HCC cells. Sigma-1 receptors (S1Rs) activate antioxidant response elements and inhibit ROS production through NRF2 [87]. S1Rs are highly expressed in liver cells, providing protection against sorafenib-induced fer-

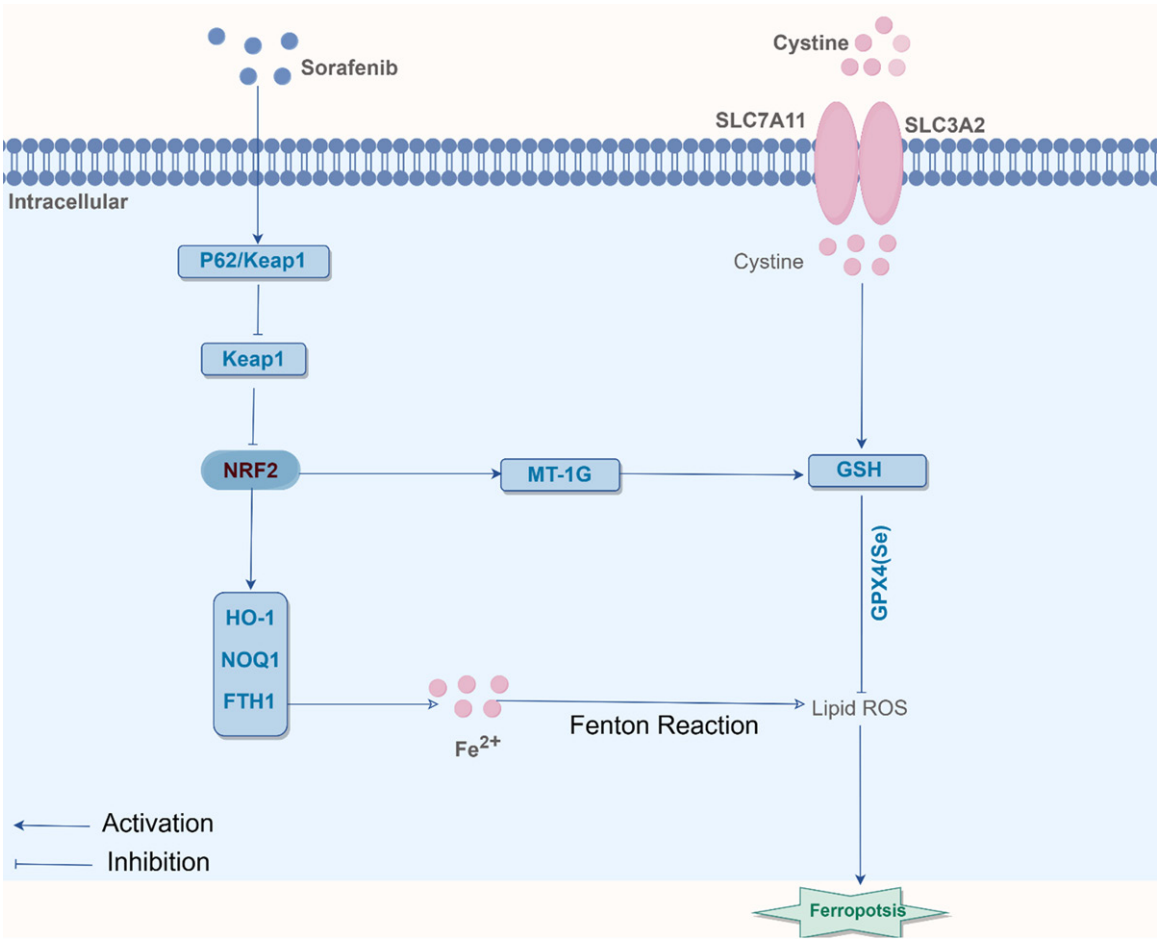

Figure 2. Schematic model of how the Nrf2 core pathway regulates ferroptosis. The Nrf2 pathway is regulated by p62 and keap1, leading to the modulation of ferroptosis through the activation of FTH, HO-1, NQO1, MT-1G, and SLC7A11. Abbreviations: P62, tumor protein P62; Nrf2, nuclear factor erythroid 2-related factor 2; MT-1G, metallothionein 1G; HO-1, heme oxygenase-1; NQO1, quinone oxidoreductase 1.

roptosis in HCC cells. Conversely, the inhibition of S1Rs promotes ferroptosis in HCC cells [88]. Sulfhydryl oxidase-1 functions as a disulfide catalyst targeting the epidermal growth factor receptor and promotes its ligand degradation in lysosomes. Suppressing NRF2 activation attenuates the antioxidant capacity of HCC cells. Overexpression of this enzyme enhances sorafenib-induced ferroptosis both in vitro and in vivo [89]. GSH transferase zeta 1 (GSTZ1) is an enzyme involved in the metabolism of phenylalanine/tyrosine [90]. It is downregulated in sorafenib-resistant HCC cell lines, and its depletion enhances the activation of the NRF2 pathway and increases GPX4 levels, thereby inhibiting ferroptosis [90, 91]. Ren et al. discovered that disulfiram/copper (DSF/Cu) disrupts mitochondrial homeostasis, resulting in lipid peroxidation and triggering ferroptosis, which has specific cytotoxic effects on HCC cells. Suppression of NRF2 expression via RNA interference enhances lipid peroxidation and validates its involvement in activating the p62-Keap1-Nrf2 pathway. Notably, inhibition of NRF2 also impacts the sensitivity of HCC cells to sorafenib [92]. Yang et al. reported that ginkgolide B (GB), the primary constituent of Ginkgo biloba leaf extract, is involved in apoptosis and is potentially regulated via the Nrf2 signaling pathway [93]. Sun et al. discovered that trigonelline, a compound, effectively inhibits the activity of Nrf2. In addition, Sid found that by targeting Nrf2,5-aminoimidazole-4-carboxy-1-beta-D-ribofuranoside (AICAR) can regulate the antioxidant capacity of HCC cells [94]. These findings underscore the pivotal role of NRF2-related pathways in inducing ferroptosis in HCC (**Figure 2**).

The CDGSH pathway in HCC

The CDGSH protein family, characterized by an iron-sulfur cluster domain, comprises a group of mitochondrial outer membrane proteins. Recent studies conducted by Li et al. confirmed that CISD2, a member of the iron-sulfur domain, is more highly expressed in HCC cells than in normal cells and that CISD2 leads to an increase in iron ion and ROS levels, thereby promoting ferroptosis in drug-resistant cells [95]. Combination therapy with sorafenib and CISD2 inhibition has also emerged as a treatment option for HCC. Another family member, CISD1, negatively regulates ferroptosis in cells through its role in mediating mitochondrial iron uptake [96] and preventing mitochondrial lipid peroxidation [97].

Noncoding RNAs in HCC

The occurrence and development of HCC are influenced by various noncoding RNAs, including microRNAs (miRs), circular RNAs (circRNAs) and long noncoding RNAs (lncRNAs) [98]. The overexpression of microRNAs in tumor cells exposed to erastin was found to increase ROS levels, increase iron ion concentrations, and reduce GSH levels, ultimately resulting in ferroptosis [99]. Furthermore, it was observed that overexpression of miR-148a in HCC suppresses cell proliferation, while its downregulation may enhance iron uptake mediated by transferrin receptor (TRF1) binding [100]. These findings offer new insights into targeted HCC treatment through the regulation of ferroptosis.

There are also studies on the role and mechanism of lncRNAs and circRNAs in ferroptosis. Qi et al. reported that the lncRNA GA binding protein transcription factor B1-antisense RNA1 (GABPB1-AS1) can be upregulated in HepG2 cells by erastin, which in turn reduces GABPB1 protein expression and further reduces the level of peroxiredoxin-5 (PRDX5) peroxidase, resulting in decreased antioxidant capacity in cells [91]. Therefore, the lncRNA GABPB1-AS1 can significantly improve the prognosis of patients with HCC, suggesting that the mechanism by which erastin induces ferroptosis in HepG2 hepatoma cells involves lncRNA regulation.

It has been reported that a novel circular RNA, cIARS (hsa_circ_0008367), facilitates ferroptosis by suppressing autophagy through the modulation of the RNA-binding protein ALKBH5 [101]. In HCC tissues, the circinterleukin-4 receptor (CircIL4R) is highly expressed [102], and CircIL4R can inhibit the miR-541-3p/Gpx4 pathway by targeting ATF4, thereby upregulating Gpx4 expression and inducing ferroptosis in liver cancer cells [103]. Additionally, Xu et al. recently discovered that circIL4R can act as a sponge for miR-541-3p, thereby influencing GPX4 expression and suppressing tumor growth [93]. These findings collectively reveal a unique circIL4R/miR-541p/GPX4 axis and provide new insights for the treatment of HCC.

Other pathways in HCC

ATP-binding cassette (ABC) transporters are crucial membrane proteins involved in the transportation of substances. Through GO and KEGG enrichment analyses, Zhang et al. discovered that ABC transporter subfamily B member 6 (ABCB6) potentially regulates ferroptosis in HCC cells by modulating intracellular iron levels [104]. Experimental evidence has also demonstrated that overexpression of ABCB6 promotes the proliferation of Huh7 cells [105]. In 2020, Tang et al. constructed diagnostic and prognostic models to showcase the crucial role of genes such as ABCB6 in providing personalized treatment for liver cancer patients [106].

The retinoblastoma (Rb) protein can regulate the transcription of diverse genes [107]. Louander et al. employed RNA interference to attenuate Rb expression in HCC cells upon exposure to sorafenib, resulting in a two- to three-fold increase in the cell death rate [108], suggesting that HCC cells exhibiting decreased RB protein expression exhibit increased susceptibility to ferroptosis.

Positive regulatory pathways of ferroptosis for HCC treatment

Pathways involved in sorafenib-induced ferroptosis

Sorafenib inhibits GSH synthesis by specifically targeting and suppressing the activity of system Xc-, thereby reducing cellular cysteine uptake. Consequently, this mechanism leads to a decrease in GPX4 enzyme activity due to the accumulation of ROS, ultimately inducing lipid peroxidation [109, 110]. Sorafenib inhibits the Xc- system through three pathways: (1) By

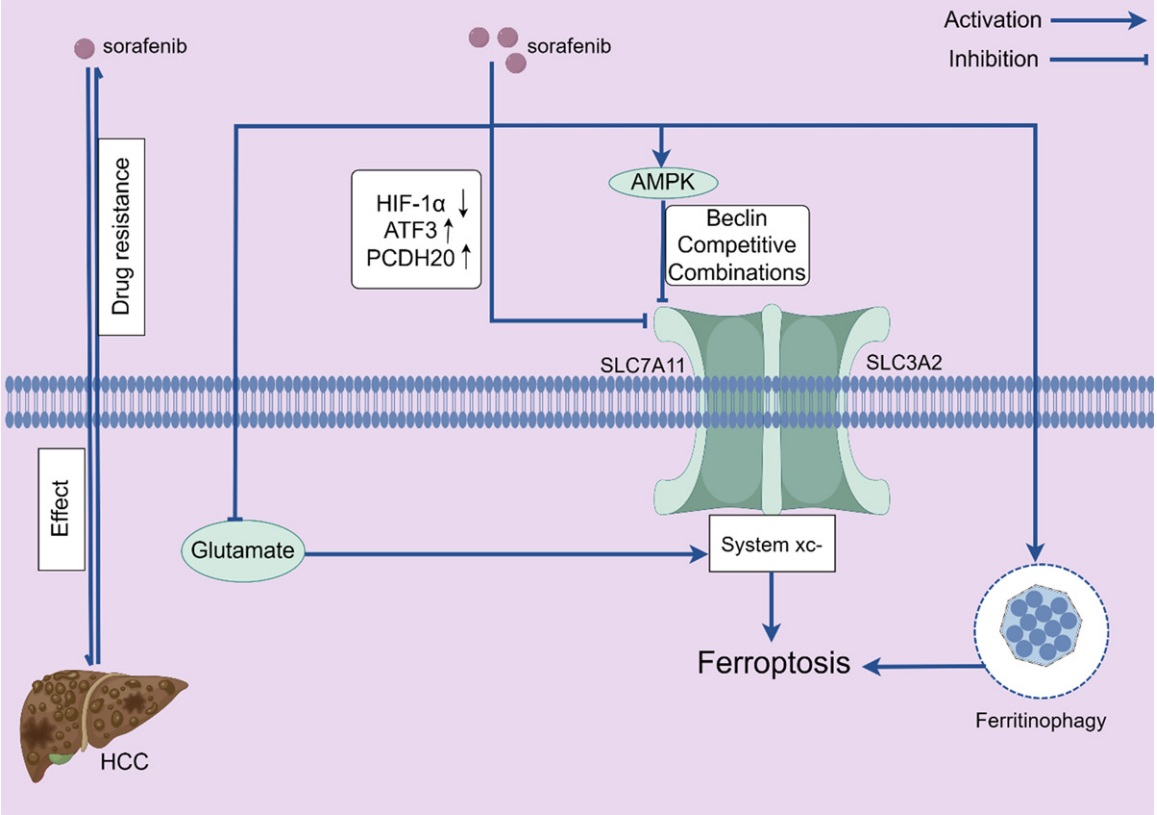

Figure 3. The pathways affected by sorafenib induce ferroptosis and confer resistance in HCC. The figure shows that sorafenib can induce ferroptosis in HCC cells, which establishes a dynamic equilibrium of drug resistance to sorafenib. Abbreviations: HIF-1α, hypoxia-inducible factor 1α; ATF3, activating transcription factor 3; PCDH20, protocadherin-20.

downregulating hypoxia-inducible factor 1α (HIF-1α) protein levels, subsequently suppressing SLC7A11 transcription, and inhibiting system Xc- [111]; by upregulating activating transcription factor 3 (ATF3), which inhibits SLC7A11 transcription to suppress system Xc- [112]; and by regulating protocadherin-20 (PCDH20), which inhibits NRF2 and subsequently suppresses SLC7A11 transcription to inhibit system Xc- [113]. (2) Sorafenib activates the AMPK signaling pathway to enhance binding affinity with SLC7A11 proteins, thereby suppressing the activity of system Xc- [114]. (3) Sorafenib can also inhibit this system through the modulation of glutamine levels [115]. Notably, sorafenib activates the key autophagy factor Beclin-1 to trigger ferritinophagy, leading to ferroptosis [116]. Sorafenib can induce ferroptosis in HCC cells; conversely, HCC cells can develop resistance to sorafenib, yet ferroptosis counteracts this resistance mechanism [117] (**Figure 3**).

P53 and SLC7A11 pathways in HCC

Jiang et al. discovered that the combination of the P53 gene and ROS induced a remarkable death rate exceeding 90% in H1299 cells, whereas the sole use of ROS did not result in any cell death [118]. This finding suggested that the antioxidant capacity of these cells was inhibited by the p53 gene in certain aspects. Recent research conducted by Zhang et al. revealed that phosphorylation of Ser46 in the zinc finger domain 498 of the p53 protein, along with a mutation at Ser47, leads to suppression of its transcriptional activity. Furthermore, mutated p53 induces ferroptosis in HCC cells and promotes the development and occurrence of HCC [119]. Ou et al. revealed that p53 upregulates the expression of spermidine/spermine N1-acetyltransferase 1 (SAT1), thereby increasing the levels of arachidonate 15-lipoxygenase (ALOX15) through the transcriptional inhibition of SLC7A11. This conse-

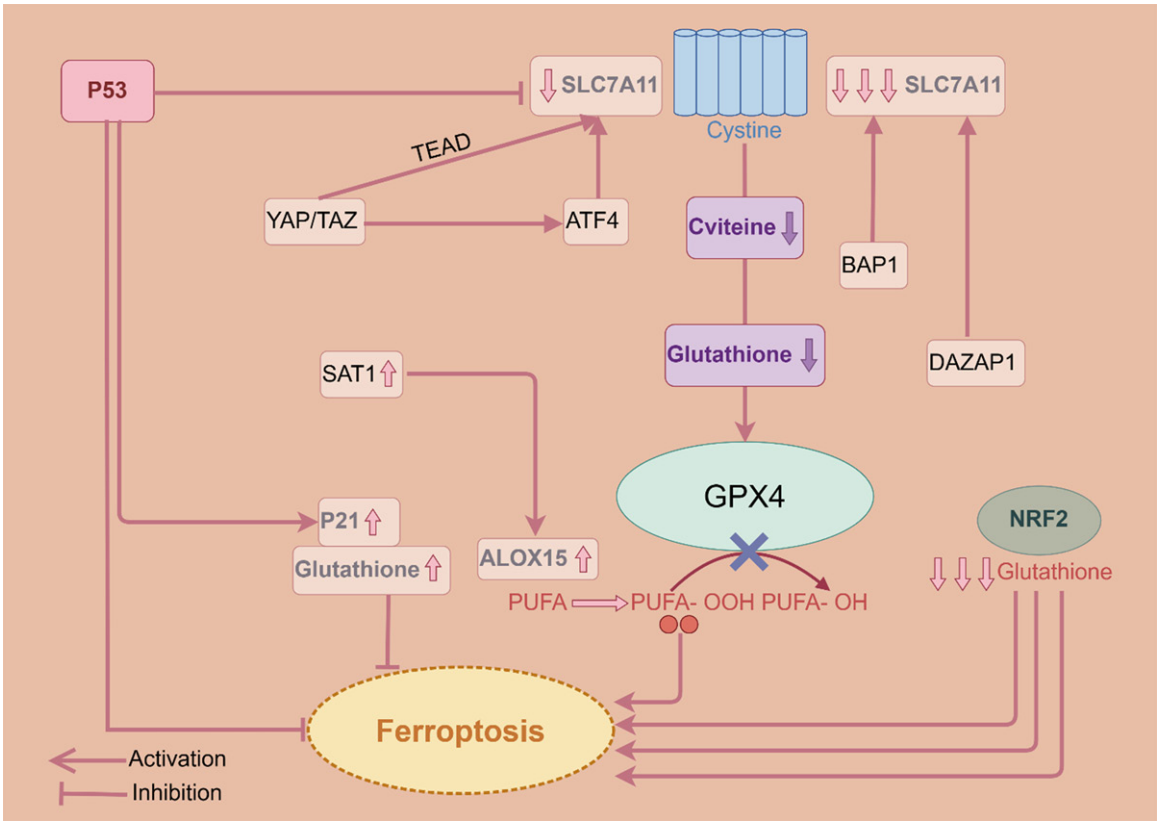

Figure 4. Role of SLC7A11 in ferroptosis. SLC7A11 is targeted by numerous molecules and proteins in HCC cells. DAZAP1 regulates ferroptosis through binding to the 3' region of SLC7A11, while BAP1 inhibits SLC7A11 to suppress cystine uptake. The promoter region of p53 binds to and activates SLC7A11, with additional factors also capable of modulating SLC7A11. Abbreviations: BAP1, BRCA1-associated protein 1; ATF4, activating transcription factor 4; DAZAP1, deleted in azoospermia-associated protein 1; SAT1, spermidine/spermine N1-acetyltransferase 1; ALOX15, arachidonate 15-lipoxygenase.

quently modulates Xc- system activity and facilitates the accumulation of lipid peroxidation products, ultimately inducing ferroptosis [120]. SLC7A11 is a direct target of p53, and its expression is suppressed by p53 through binding to p53 response elements in its promoter region [121]. This regulatory pathway of p53-mediated inhibition was experimentally confirmed in glioblastoma cells by Wang et al. [122]. SAT1 has been shown to bind to the promoter site of SLC7A11 and modulate its transcription and expression, resulting in enhanced production of ROS and lipid peroxidation when SAT1 is overexpressed [123]. In summary, SAT1 acts as a downstream effector of p53 that triggers ferroptosis via ROS generation. STAT6 acts as a negative regulator of ferroptosis by competitively binding with CREB-binding protein (CBP) at p53 [124]. Tarangelo et al. demonstrated that p21 can also facilitate the establishment of the p21-p53 axis, thereby delaying ferroptosis induced by cysteine deprivation in human fibrosarcoma HT-1080 cells [125]. Zhu et al. also reported the crucial role of the ALOX12-mediated ferroptotic pathway in facilitating the anticancer effects of p53 [126]. Furthermore, p53 interacts with solute carrier family 25 member 28 (SLC25A28) to enhance mitochondrial iron accumulation [127] (**Figure 4**).

Similarly, SLC7A11 also serves as a pivotal target in the regulation of various other proteins. The RNA-binding protein deleted in azoospermia-associated protein 1 (DAZAP1) can bind to the 3' noncoding region of SLC7A11, thereby impeding its expression and governing GPX4, thus facilitating HCC progression [128]. Similarly, the tumor suppressor BRCA1-associated protein 1 (BAP1) represses cystine uptake by suppressing SLC7A11 expression, inducing lipid peroxidation and triggering ferroptosis

[129]. Other tumor suppressor genes, such as beclin 1 (BECN1), can also exert negative regulatory effects [114]. Notably, inhibiting lactate uptake can also yield similar outcomes [130] (**Figure 4**).

Immune pathway in HCC

Transforming growth factor beta receptor I (TGF-β1) regulates cell growth and development and participates in cell signal transduction. Moreover, TGF-β1 indirectly inhibits the Xc- system through a reduction in XCT protein expression, leading to enhanced ROS levels [131]. This ultimately mediates lipid peroxidation and increases the susceptibility of HCC cells to GPX4 inhibitors [131]. Consequently, a recent clinical trial (NCT01246986) demonstrated that the TGF-β1 kinase inhibitor galunisertib had a longer survival and better safety profile in HCC patients [132]. Interferon-gamma (IFN-γ), derived from $CD8^+$ T cells of the immune system, can downregulate the expression levels of SLC7A11 and SLC3A2, thereby inhibiting the activity of system Xc- and sensitizing HCC cells to ferroptosis. This mechanism is associated with the STAT pathway and holds potential as an immunotherapeutic approach for treating HCC [133].

Increased expression of ACSL4 may occur through various molecular or protein pathways [134]. The YAP pathway, for instance, can induce ferroptosis by upregulating ACSL4 [135]. Conversely, inhibition of ACSL4 sensitizes cells to ferroptosis [136]. For example, SRC, a nonreceptor protein tyrosine kinase, acts as an inhibitor of ferroptosis by activating STAT3 to suppress ACSL4. Moreover, integrin α6β4 can mediate the aforementioned mechanism of SRC inhibition to suppress ferroptosis [136].

Mitochondrial pathway in HCC

Wang et al. demonstrated that the knockout of mitochondrial ferritin (FtMt) resulted in increased lipid peroxidation and disrupted glutathione cerebral ischemia/reperfusion (I/R) [137], which are classical features of ferroptosis, confirming the crucial role of FtMt in cellular protection against ferroptosis and I/R-induced brain injury. Some studies have shown that removal of mitochondria does not prevent ferroptosis, suggesting that mitochondria may not be involved in this process [78]. However, morphological changes associated with ferroptosis, such as mitochondrial fragmentation and crista enlargement, are closely related to changes in mitochondria [13]. Moreover, certain inhibitors targeting ferroptosis have been found to act on mitochondria [138]. Gao et al. discovered that inhibition of the mitochondrial TCA cycle leads to ferroptosis suppression and highlighted the pivotal role of mitochondria in cysteine deprivation-induced ferroptosis [139]. Nevertheless, the involvement of mitochondria in ferroptosis remains a subject of ongoing debate. Huang et al. demonstrated that the NUPR1 inhibitor ZZW-115 induces ferroptosis in HCC cells by disrupting mitochondrial homeostasis and function, resulting in the accumulation of ROS [140]. Yagoda et al. identified voltage-dependent anion channels (VDACs), which are responsible for ion and metabolite transport in mitochondria, as one of the targets of erastin. Knocking out the DAC2 or VDAC3 genes through RNA interference conferred Erastin tolerance, suggesting that VDAC proteins may trigger nonapoptotic cell death via specific pathways [141]. Subsequent experiments confirmed that Erastin-induced VDAC dysfunction leads to ferroptosis activation.

Autophagy-related pathways in HCC

Autophagy can degrade ferritin, reduce iron storage capacity, increase the intracellular iron concentration, and promote ferroptosis [142]. Nuclear receptor coactivator 4 (NCOA4) acts as a selective receptor mediating ferritin autophagy by facilitating its transport to the lysosome for degradation. By modulating the flux of NCOA4-mediated ferritin transport, it plays a crucial role in maintaining cellular homeostasis and regulating cellular sensitivity to ferroptosis [143]. This autophagic process involves the ATG5-ATG7-NCOA4 pathway [116]. The novel ferroptotic inducer Formosanin C (FC) enhances the expression of NCOA4 via iron autophagy to facilitate ferroptosis. The involvement of NCOA4 in ferritinophagy suggests the therapeutic potential of FC for treating ferroptosis-resistant HCC patients [144].

Ferroptosis in HCC therapy

The treatment options for HCC can be divided into surgical and nonsurgical approaches. However, due to the absence of early diagnostic symptoms and markers, most HCC patients

are diagnosed at an advanced stage [145]. Surgical treatment is pursued for less than 30% of these patients [146]. Nonsurgical targeted therapy is commonly employed for HCC [147], while interventional therapy that obstructs the tumor's blood supply is frequently utilized for unresectable cases [148]. Additionally, chemotherapy [149], traditional Chinese medicine [150], radiation therapy [151], and nanoparticles also exhibit promising prospects in HCC treatment [152].

Cisplatin can directly bind to GSH, forming a complex that deactivates GSH and induces ferroptosis [153]. The combination of cisplatin with erastin leads to the synthesis of diphenylethylene dichloride, which consumes intracellular glutathione and induces ferroptosis [154]. Additionally, other drugs, such as sulfasalazine and buthionine sulfoximine, indirectly deplete glutathione and induce ferroptosis [155]. Bai et al. reported that haloperidol promoted sorafenib-induced ferroptosis, even at relatively low doses of both drugs described above [156]. This finding suggested that haloperidol may enhance the efficacy of sorafenib in HCC patients. In addition, it can also reduce the dose of sorafenib applied, a finding that provides a new strategy for combination therapy in HCC.

Polyphyllin, a traditional Chinese medicine, can exert its anti-HCC effects by inducing ferroptosis, which has profound significance for our understanding of the mechanism underlying the antitumor activity of traditional Chinese medicine and improving the treatment of HCC and other tumors. Ooko et al. experimentally established a correlation between the expression of artemisinin and mRNAs associated with ferroptosis [157]. It seems debatable whether artemisinin and its derivatives directly induce ferroptosis. However, the aforementioned findings suggest the potential therapeutic application of artemisinin or its derivatives in ferroptosis.

Recently, reports have suggested that radiotherapy induces the generation of reactive oxygen species (ROS) through ionizing radiation (IR), which subsequently triggers the upregulation of SLC7A11 and suppresses ferroptosis [158]. In a recent study by Yin et al., it was discovered that cluster element (CLTRN) acts as a radiosensitive locus, potentially enhancing HCC cell radiosensitivity by modulating ferroptosis via the glutathione metabolism pathway. This process may be regulated by the NRF1/RAN (oncogene family)/DLD (dihydroceramide desaturase) protein complex [159]. These findings suggest that CLTRN is a promising target for radiotherapy in HCC treatment. It has also been reported that carbon ion (CI) irradiation can induce endoplasmic reticulum (ER) stress and activate the unfolded protein response (UPR) in HCC cells, triggering the PKR-like ER kinase (PERK) pathway. This pathway regulates p53 expression on the one hand and promotes autophagy through ATF4 expression on the other hand, ultimately leading to ferroptosis mediated by mitochondria [160]. Therefore, targeting PERK with drugs combined with CI radiotherapy holds significant clinical significance.

When low-density lipoprotein (LDL)-docosahexaenoic acid (DHA) nanoparticles are used to treat rats and human liver cancer cells, lipid peroxidation, GSH depletion, GPX4 inactivation, and cell death occur sequentially, which was later confirmed as ferroptosis [161]. This finding suggests a novel molecular mechanism underlying the effectiveness of LDL-DHA against HCC. Chen et al. also demonstrated the potential of nanobubbles in combination with oxygen-enhanced sonodynamic therapy (SDT) for the treatment of HCC through ferroptosis [162], a mechanism that has garnered significant attention. Another study developed sorafenib-loaded manganese-doped mesoporous silica nanoparticles that exhibit a dual mechanism for intracellular GSH depletion. The degradation of MMSNs leads to the consumption of intracellular GSH, while sorafenib inhibits system Xc- and suppresses GSH synthesis [152]. This nanomedicine possesses dual potential for GSH depletion and holds significant promise for inducing ferroptosis in HCC cells, thereby offering profound opportunities for combating HCC through the utilization of nanomaterials to induce ferroptosis.

Conclusion and perspectives

In this review, we present the epidemiology and etiology of HCC and the physiology and pathophysiology of ferroptosis, along with its mechanisms and regulatory factors. Additionally, we explored therapeutic approaches for HCC through the modulation of ferroptosis via multiple targets and pathways. We have highlight-

ed advancements in liver cancer therapy using emerging technologies or compounds while providing insights into novel molecular entities and drugs for future HCC treatment. The integration of ferroptosis therapy with complementary therapies such as immunotherapy, nanotechnology and traditional Chinese medicine presents promising prospects for application. Although precise reports on numerous molecular mechanisms are still lacking, we strongly suggest that ferroptosis holds promise for the treatment of HCC patients.

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Disclosure of conflict of interest

None.

Address correspondence to: Zhifang Li, Department of Foreign Languages, College of Basic Medical Sciences, Third Military Medical University, Chongqing 400038, China. Tel: +86-23-68752188; E-mail: happykarenli2003@163.com; Bing Ni, Department of Pathophysiology, College of High Altitude Military Medicine, Third Military Medical University, Chongqing 400038, China. Tel: +86-23-68752116; E-mail: nibing@tmmu.edu.cn

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