Ferroptosis in cancer (Review)

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Abstract. Ferroptosis is a type of programmed cell death depending on iron and reactive oxygen species. This unique cell death process has attracted a great deal of attention in the field of cancer research over the past decade. Research on the association of ferroptosis signal pathways and cancer development indicated that targeting ferroptosis has great potential for cancer therapy. In the present study, the latest research progress of ferroptosis was reviewed, focusing on the relationship between ferroptosis and the development of cancer, in order to further promote the clinical application of ferroptosis in cancer.

Contents

1. Introduction

Ferroptosis, first identified and reported by Dixon *et al* in 2012 (1), is a cell death process depending on iron and reactive oxygen species (ROS) that differs from apoptosis, necrosis and autophagy. It is characterized by a redox imbalance, elevated level of ROS, rupture of the outer mitochondrial membrane, dense contents and the decrease or loss of cristae of mitochondria (2). Previous studies have shown that ferroptosis may be induced in both humans and animals under a variety of physiological conditions and pathological stresses. A number of cancer-related signaling pathways have been identified to be associated with ferroptosis, and some cancer cells even relied on ferroptosis defenses for survival. In recent years, the discovery of new ferroptosis biomarkers, such as nicotinamide adenine dinucleotide phosphate (NADPH), hyperoxidized PRDX3, 7-DHC, has contributed to a clearer understanding of ferroptosis and the application of ferroptosis in the prevention and treatment of related diseases. Particularly, ferroptosis has been revealed to be a congenital tumor suppressor mechanism that mediates the anticancer activity of multiple tumor suppressors (3-5). Based on such important findings, how to regulate cellular ferroptosis to intervene in the occurrence and development of cancer has become a current research focus which may provide new therapeutic clues to combat this disease.

2. Ferroptosis mechanism

Iron metabolism. Iron is one of the essential trace minerals of the body (6). It plays an essential role in the oxidation-reduction catalysis and bioenergetics of the cells, and is also involved in the formation of toxic oxygen radicals. A previous study demonstrated that a high consumption of iron raises the risk of several cancer forms, including hepatocellular carcinoma and breast cancer (7). Compared with normal cells, cancer cells require more iron for proliferation. Accordingly, iron uptake is expedited, and their intracellular concentration is raised in rapidly proliferating cancer cells (8). Although it has not yet been fully understood as regard to how iron metabolism plays a role in the process of ferroptosis, it is well known to be critical for ferroptosis. According to Dixon *et al* (1), extracellular iron supplementation rendered cells more vulnerable to ferroptosis inducers, and iron complexes prevented the development of ferroptosis in cells both *in vivo* and *in vitro*. Hou *et al* (9) reported a rise of intracellular active iron concentration during the activation of ferroptosis in cells. In 2017, Li *et al* (10) found that both extra heme and non-heme iron could cause ferroptosis in cells. Thus, triggering iron-dependent ferroptosis by modulation of diverse active iron forms could provide a novel cancer therapeutic strategy.

Ferroptosis is a type of cell death that is caused by an increase in the labile iron pool in cells (11). The main mechanism of iron uptake in cells is through the binding of Fe^{3+} to

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transferrin (TF) and its recognition by the TF receptor (TFRC) to enter the cell. Subsequently, Fe^{3+} is separated from TF in the nuclear endosome, reduced to Fe^{2+} by six-transmembrane epithelial antigen of prostate 3, and then released into the cytoplasm via solute carrier family 39 member 8 (SLC39A8), SLC39A14 (zinc transporter) and divalent metal transporter 1. In addition, two other mechanisms, endocytosis of lacto-TF and heme-mediated iron absorption, provide additional iron sources for cells (12). On the other hand, SLC40A1-mediated iron export and exosome-mediated ferritin export are primarily mechanisms of iron export (13). Ferroptosis could be induced through modulation on multiple stages of iron metabolism, such as, increasing iron uptake, decreasing iron storage, or restricting iron ion efflux, mediated by the corresponding signaling pathways, which all lead to the augmentation of intracellular iron ion content and induce ferroptosis (14). At least two processes cause membrane lipid peroxidation when there is excessive iron in cells. One of them involves the Fenton reaction, which produces hydroxyl radicals and takes part in the peroxidation of phospholipids, converting non-toxic phosphatidyl alcohol into harmful phospholipid hydroperoxides (PLOOHs). In the other process, extra iron activates iron-containing enzymes such as lipoxygenase, to directly catalyze the deoxygenation of free and esterified polyunsaturated fatty alcohol and generate PLOOH (2,15). Additionally, excess iron will catalyze the creation of ROS in cells, triggering lipid peroxidation and eventually ferroptosis.

Ferritin, a protein complex that stores iron, prevents ROS from oxidizing Fe^{2+} and is essential for maintaining iron levels in cells. Ferritin is primarily made up of the ferritin heavy chain 1 (FTH1) and the ferritin light chain (FTL); the former is primarily in charge of reducing Fe^{3+} to Fe^{2+} at the iron oxidase active site, whereas the latter serves to stabilize ferritin. In 2016, Hou *et al* (9) demonstrated that autophagy can induce ferroptosis by degrading ferritin in fibroblasts and cancer cells. Gao *et al* (16) discovered that deleting autophagy-associated proteins ATG3, ATG5, ATG7 and ATG13 prevented ferroptosis. Although it was previously suggested that autophagy is unrelated to ferroptosis, autophagy-dependent ferroptosis has been observed in cancer cells. Furthermore, nuclear receptor coactivator 4-mediated ferritin autophagy raised intracellular Fe^{2+} levels and accelerated cellular ferroptosis. Kremer *et al* (17) showed that inhibiting glutamate-oxaloacetate transaminase 1 (GOT1) increased ferritin autophagy, causing an increase in labile iron pools and promoting ferroptosis in cells.

Iron-sulfur clusters (ISCs) are crucial cofactors in the maintenance of redox and iron homeostasis. They are primarily produced by a number of mitochondrial proteins involved in cysteine desulfurase (NFS1) and ISC assembly, and their associated regulatory pathways can assist cancer cells in avoiding ferroptosis by lowering the level of labile iron pools. The iron-sulfur protein family member CDGSH iron-sulfur domain 2 (CISD2) is highly expressed in head and neck cancer. According to a previous study (18), CISD2 may prevent ferroptosis in cancer cells *in vitro* by controlling ISCs and lowering the concentration of free iron. NFS1 is necessary for the progression of lung cancer *in vivo*. The ability of NFS1 to engage in the creation of ISCs, resulting in lower levels of labile iron pools and protecting cancer cells from ferroptosis, may be connected to its overexpression in lung cancer. Alvarez *et al* (19) also demonstrated that inhibition of NFS1 increases the intracellular level of labile iron pools, making lung cancer cells susceptible to ferroptosis and thus limiting the development of lung cancer *in vivo*. Moreover, when under low ISC conditions, iron-sulfur regulatory proteins (IRPs) such as IRP1 (also known as ACO1) and IRP2 (also known as IREB2) (20), will translationally regulate iron metabolism-related proteins, including TFRC, SLC11A2, SLC40A1, FTH and FTL, raising iron levels in the cell and thus promoting ferroptosis (14).

Lipid peroxidation. Ferroptosis is often characterized by high levels of lipid peroxides. The accumulation of lipid peroxides in the cell membrane change the membrane permeability and stability, and provoke the cell membrane ruptures, finally leading to cell ferroptosis (21). Acyl-CoA synthase long chain family member 4 (ACSL4) and lyso-phosphatidyl-choline acyltransferase 3 (LPCAT3), are considered to be key mediators of PUFA-PL synthesis. The accumulation of PLOOH relies on both enzymatic and non-enzymatic processes. In enzymatic reactions, PLOOH production is mainly mediated by lipoxygenases (LOXs) and cytochrome P450 oxidoreductases (POR) (11). LOXs can directly catalyze the deoxygenation of free and esterified PUFA-PL to PLOOH (22). Shah *et al* (23) demonstrated that LOX-5, LOX-12 and LOX-15 overexpression render cells more susceptible to ferroptosis. It was also demonstrated that LOX inhibitors effectively act as an antioxidant and protect cells from lipid peroxidation. A previous study revealed that P450 can accept electrons from POR and thus catalyze the peroxidation of PUFAs (24). In 2020, Zou *et al* (25) discovered that POR coupled to cytochrome P450 generates enhanced levels of lipid peroxidation as well as increased ROS. The aforementioned study provided evidence that POR is necessary for ferroptosis to occur in cancer cells. On the other hand, the non-enzymatic mechanism has been reported that ASCL4 catalyzes the binding of free PUFAs to coenzyme A (CoA) to produce PUFA-CoA, which is subsequently re-esterified by LPCAT3 and bound to PLs to form PUFA-PLs, and finally catalyzed by arachidonic LOX (ALOX) to produce PL-PUFA-OOH (26,27). In addition, NADPH oxidases (NOXs), which catalyze the formation of superoxide, contribute to the iron-dependent accumulation of lipid peroxidation (1,28,29). In the latest research report, 7-dehydrocholesterol (7-DHC), an intermediate metabolite of distal cholesterol biosynthesis, plays an important role in regulating ferroptosis. In a non-enzymatic reaction, 7-DHC exerts its anti-phospholipid autooxidation function through the use of conjugated diene, and protects plasma and mitochondrial membrane from phospholipid autooxidation, inhibiting the occurrence of ferroptosis in cells. Accordingly, when the biosynthesis of endogenous 7-DHC is blocked by drug targeting, the reduction of 7-DHC induced ferroptosis of the cells (30).

Ferroptosis defense mechanisms

Glutathione peroxidase 4 (GPX4)-glutathione (GSH). GPX4 is a member of the GPX family of proteins that are found mainly in the cytoplasm, mitochondria and nucleus of cells (31). A previous study suggested that only cytoplasmic GPX4 plays a role in ferroptosis (32), but a recent study found

that mitochondrial GPX4 also has an important role in ferroptosis (33). GSH is a tripeptide composed of glutamate, cysteine and glycine and is synthesized mainly by glutamate-cysteine ligase (GCL) and glutathione synthase (GSS) catalytic synthesis. Cysteine is the rate-limiting amino acid for GSH synthesis. Cells mainly exchange intracellular glutamate for extracellular cystine through System Xc at a ratio of 1:1, and then reduce the imported cystine to cysteine, thus allowing the synthesis of GSH (34). System Xc is composed of the light chain of solute carrier family 7 member 11 (SLC7A11) and the heavy chain of solute carrier family 3 member 2 (SLC3A2). Inhibiting its expression can also induce ferroptosis (35). In cells, GPX4 is able to use glutathione as a substrate to reduce toxic PL-PUFA-OOH to non-toxic PL-PUFA-OH, thereby inhibiting the occurrence of ferroptosis (36). The inactivation of GPX4 also leads to dysregulation of cellular REDOX and ultimately results in the occurrence of cellular ferroptosis (37). A recent study has discovered that hyperoxidized PRDX3 serves as a specific marker for ferroptosis and induces ferroptosis in cells by inhibiting cystine uptake. During the process of ferroptosis, PRDX3 becomes overoxidized due to exposure to mitochondrial lipid peroxides. Subsequently, the over-oxidized PRDX3 translocates from mitochondria to the plasma membrane, hindering cell uptake of cystine, impacting GSH synthesis, and thereby promoting ferroptosis (38). In cases where there is cystine starvation or deficiency, certain tumor cells can prevent ferroptosis by converting methionine into cysteine through the transamination pathway (39). However, it remains unclear whether metabolites produced by amino acids are independently involved in ferroptosis apart from the cysteine pathway. A recent study has found that tryptophan metabolites serotonin (5-HT) and 3-hydroxyanthranilic acid (3-HA) significantly inhibit ferroptosis in tumor cells. Mechanistically, both 5-HT and 3-HA act as potent free radical trapping antioxidants, effectively eliminating lipid peroxidation and thus inhibiting the occurrence of ferroptosis (40).

Ferroptosis suppressor protein 1 (FSP1)-panthethine alcohol ($CoQ_{10}H_2$). FSP1, also known as flavoprotein apoptosis-inducing factor mitochondrial-associated 2, was originally considered to be a pro-apoptotic gene (41), but it was previously found that this gene is associated with ferroptosis and that it can inhibit ferroptosis caused by GPX4 deletion (42). The reduced form of coenzyme Q (CoQ_{10}), $CoQ_{10}H_2$, is a potent lipophilic antioxidant that is considered to trap oxygen radicals in phospholipids and lipoproteins (43). It was revealed that the plasma membrane protein FSP1 may catalyze the conversion of CoQ_{10} to $CoQ_{10}H_2$ using NAD(P)H to trap free radicals of lipid peroxidation, alleviating cellular lipid peroxidation and preventing ferroptosis (43). Although CoQ_{10} is mainly synthesized in mitochondria, its presence can also be detected in non-mitochondrial membranes, such as the plasma membrane (44,45). Therefore, it has been hypothesized that FSP1 exerts its role in inhibiting ferroptosis by generating $CoQ_{10}H_2$ in non-mitochondrial membranes, thereby trapping the free radicals of lipid peroxidation (42,46). In conclusion, the FSP1-CoQ_{10}-NAD(P)H pathway can efficiently prevent the occurrence of ferroptosis and may have a synergistic effect with GPX4-GSH mechanism.

Dihydroorotate dehydrogenase (DHODH)-$CoQ_{10}H_2$. In addition to the two major ferroptosis defense systems aforementioned, the DHODH-$CoQ_{10}H_2$ system was identified by Mao *et al* (33) as the third one. By using metabolomic research, the authors discovered that treatment of cancer cells with GPX4 inhibitors, such as RSL3 or ML162, leads to the depletion of N-carbamoyl-L-aspartate accompanied by uridine accumulation in cancer cells. Supplementation of cells with dihydroorotate, the substrate of mitochondrial DHODH, resists the inhibitory effect of GPX4; whereas supplementation of its product, orotic acid, enhances the sensitivity of cells to GPX4 inhibitors, suggesting that DHODH may be involved in the regulation of cellular ferroptosis. It was identified that the inhibition of DHODH sensitizes HT-1080 GPX4-high cancer cells to ferroptosis while significantly induces ferroptosis in GPX4-low cancer cells. This research conclusively demonstrated that DHODH inhibits ferroptosis and mitochondrial lipid peroxidation in a CoQ_{10}-dependent way. DHODH is an enzyme that can take part in pyrimidine production and is primarily present on the inner membrane surface of mitochondria (47). When GPX4 is inhibited or inactivated in mitochondria, the level of DHODH increases significantly and is able to reduce CoQ_{10} in the inner mitochondrial membrane to $CoQ_{10}H_2$, thus neutralizing excessive lipid peroxidation in mitochondria and protecting cells from ferroptosis (33). Therefore, the discovery of this pathway offers a fresh concept and method for cancer therapy, with the inhibitors of DHODH as inducers of ferroptosis.

GTP cyclization hydrolase 1 (GCH1)-Tetrahydrobiopterin (BH4). BH4 is a crucial cofactor for enzymes that hydroxylate aromatic amino acids and has a redox function in the processes of enzymes catalysis (48). Additionally, BH4 is a potent antioxidant that may capture peroxyl radicals in lipids, but its ability to inhibit ferroptosis is unrelated to its activity as an aromatic amino acid hydroxylase cofactor (49). GCH1 is not only the initiating enzyme in the GTP synthesis BH4 pathway, but also the rate-limiting enzyme in the BH4 synthesis pathway, controlling the concentration of intracellular BH4 (48,50). It was discovered that GCH1 was able to prevent ferroptosis by starting the synthesis of dihydrobiopterin and tetrahydrobiopterin, as well as by selectively preventing two polyunsaturated fatty acyl tails from consuming phospholipids. These actions trap lipid peroxidation radicals in cells and shield some phospholipids from peroxidation (51). Meanwhile, BH4 can also promote the production of CoQ_{10} by converting phenylalanine to tyrosine, which is further converted into a precursor substance of CoQ_{10}, thus exerting an antioxidant function in another way. In addition, the recycling of BH4 requires the involvement of dihydrofolate reductase (DHFR). Therefore, blocking DHFR combined with GPX4 inhibitor may also play a role in inducing ferroptosis.

Membrane-bound O-acyltransferase domain 2 (MBOAT1/2)-mediated cytosolic phospholipid remodeling pathway. MBOAT2 is a lysine-Pl acyltransferase that selectively transfers monounsaturated fatty acids (MUFA) to lyso-phosphatidyl-ethanolamine, resulting in an increase of cell PE-MUFA and a corresponding decrease of cell PE-PUFA. This process effectively inhibits ferroptosis

independent of the GPX4 and FSP1 pathways, as PE-PUFA is a major determinant of lipid peroxidation and ferroptosis sensitivity (5,52). MBOAT1, another member of this family, also inhibits ferroptosis through similar mechanisms (53). Furthermore, MBOAT2 and MBOAT1 are directly regulated by the androgen receptor (AR) and estrogen receptor (ER), respectively, which upregulate their gene expression. The use of anti-AR drugs enzalutamide or ARV-110 can downregulate MBOAT2 expression in AR(+) prostate cancer cells, making them sensitive to ferroptosis; similarly, Ful, an ER depressant, can downregulate MBOAT1 expression in ER(+) breast cancer cells to achieve a similar effect. These findings suggest that sex hormone signaling can inhibit ferroptosis in cancer cells via phospholipid remodeling mediated by MBOAT1/2 genes with potential implications for targeted treatment (53).

3. Cancer-related pathways in ferroptosis

TP53. The tumor suppressor gene TP53, also known as P53, is also referred to as the 'genomic guardian' because it is essential for tumor inhibition and maintains genomic stability by promoting cancer cell apoptosis and cell cycle arrest (54). TP53 is mainly divided into two types: Wild type and mutant type. The wild type P53 can cause apoptosis of tumor cells and prevent the cells from becoming cancerous; whereas the mutant P53 gene will promote the cells to become cancerous. It has been demonstrated that P53 deletions and mutations are linked to ~50% of all human malignancies (55). In recent years, it has been discovered that P53 can facilitate ferroptosis in cells by controlling the expression of genes connected to ferroptosis. In 2015, Jiang *et al* (3) discovered that P53 binds to the P53 response element in the SLC7A11 promoter region, inhibiting the expression of SLC7A11 and making cancer cells susceptible to ferroptosis. Furthermore, in 2019, Wang *et al* (56) found that P53 reduces the level of monoubiquitination of histone H2B on lysine 120 by promoting nuclear translocation of ubiquitin-specific protease 7 (USP7), thereby epigenetically inhibiting the expression of SLC7A11 (56). The newly discovered impact of p53 on cell metabolism, oxidative responses and ferroptotic cell death through regulation of spermidine/spermine N1-acetyltransferase 1 (SAT1) has been a hot research area (57). When SAT1 is overexpressed, spermidine and spermine are rapidly depleted, which leads to cellular mitochondria-mediated apoptosis and a decline in cell growth (58). Ou *et al* (59) discovered that P53 can stimulate the expression of SAT1 at the transcriptional level, hence enhancing the ability of arachidonate 15-LOX (ALOX15) to sensitize cells to undergo ferroptosis. The aforementioned study identified ALOX15 as a metabolic target of P53 involved in ferroptotic cell death. However, the exact mechanism of how the P53/SAT1/ALOX15 axis is activated remains unclear. Controversially, P53 has been reported to have ferroptosis-suppressor activities, for example by preventing the transcriptional activation of the cyclin-dependent kinase inhibitor P21, thereby promoting GSH production and reducing lipid peroxidation accumulation (60). Xie *et al* (28) demonstrated that TP53 restricts erastin-induced ferroptosis by blocking dipeptidyl-peptidase-4 (DPP4) activity, thereby promoting plasma-membrane-associated DPP4-dependent lipid peroxidation, which ultimately results in ferroptosis (28). Furthermore, Hu *et al* (61) identified glutaminase 2 (GLS2) as a unique P53 target gene. In several cancer cells, including human colorectal carcinoma HCT116 and hepatocellular carcinoma HepG2, GLS2 expression can upregulate GSH and NADH production, improving cellular antioxidant defense capacity. Although GLS2 is suspected to be related to cellular ferroptosis, more evidence is needed to determine whether GLS2 expression mediates P53-regulated ferroptosis. Taken together, these studies suggested that P53 plays a dual role in the occurrence of ferroptosis under various contexts. In order to produce ferroptosis in cells, P53 can either decrease the expression of SLC7A11 or increase the expression of specific target genes, such as SAT1. However, P53 may also protect cells against ferroptosis by inhibiting the activity of DPP4 or promoting the transcriptional activation of P21.

Nuclear Factor erythroid 2-Related Factor 2 (NRF2). NRF2 is a key regulator of oxidative stress; when it is activated, it encourages the iron storage, slows cells' iron uptake and reduces the production of ROS in cells. NRF2 is regulated by KELCH-like ECH-associated protein 1 (KEAP1). KEAP1 can bind to NRF2 in the cytoplasm and be connected to the E3 ubiquitin ligase (Cul3), where NRF2 can be degraded by the proteasome system through ubiquitination. However, in some special cases, such as mutations in NRF2 or KEAP1, a selective autophagy junction protein SQSTM1, also known as P62, could bind and inactivate KEAP1, preventing NRF2 degradation and enhancing subsequent NRF2 nuclear accumulation. In such cases, the antioxidative transcriptional factor NRF2 regulates the expression of numerous cytoprotective genes involved in detoxification, antioxidant and drug metabolism by binding to the antioxidant response element (62). For instance, as a target gene of NRF2, SLC7A11 is essential for cell glutamate exchange and cysteine production. It was found that SLC7A11 expression was upregulated when NRF2 was activated (63). In addition, GCL and GSS, which catalyze glutathione synthesis, are also target genes of NRF2. Overexpression of NRF2 causes elevated cellular GSH levels and promotes the expression of GPX4 (64). Heme-oxygenase 1 (HMOX-1) is also an antioxidant gene targeted by NRF2. In cells, HMOX-1 catalyzes the breakdown of heme to free iron, carbon monoxide and biliverdin. The latter can be further converted to bilirubin, which acts as an antioxidant and inhibits the production of ROS (65). It was discovered that upregulating HMOX-1 has positive antioxidant benefits, whereas knocking down HMOX-1 reduces the response of NRF2 to oxidative stress (66). NRF2 also controls the expression of other genes to protect the cell from ferroptosis, such as GSTs, GCLM and CHAC-1, which are related to ROS detoxifying enzymes and GSH metabolism pathway (14,67), as well as SLC40A1 and MT1G (Metallothionein-1G), which are engaged in the iron metabolism route.

RAS. The main RAS family genes are HRAS, KRAS and NRAS, which are collectively known as oncogenic RAS (68). KRAS mutations are among the most prevalent in human cancers. Dolma *et al* (69) and Yang and Stockwell (70) demonstrated that the ferroptosis inducers erastin and RSL3 were capable of selectively killing RAS-mutated tumor cells long before the discovery of ferroptosis (69,70). In a subsequent

study, it was revealed that numerous RAS-mutated cancer cells are vulnerable to ferroptosis. This might be due to the fact that mutant RAS can control the expression of genes involved in iron metabolism, leading to higher iron levels, which in turn affects the process of ferroptosis in cells. KRASG12D is the most common type of mutations in KRAS. Dai *et al* (71) found that, in pancreatic ductal adenocarcinoma cells, KRASG12D was able to promote macrophage differentiation into the oncogenic M2 phenotype. KRASG12D was released by means of exosomes from cancer cells undergoing ferroptosis and was taken up by macrophages through advanced glycosylation end product-specific receptor. The uptake of KRASG12D activates the STAT3 signaling pathway and selectively upregulates the genes involved in the fatty acid oxidation metabolic pathway, such as carnitine palmitoyl-transferase 1A and acyl-CoA dehydrogenase medium chain, leading to macrophage M2 polarization.

Hypoxia inducible factor (HIF). Hypoxia is a hallmark of tumor formation and is mainly regulated by HIF, which promotes the growth, survival and metastasis of cancer cells (72). HIF consists of an alpha subunit, such as HIF-1α, HIF-2α and HIF-3α, and a beta subunit (HIF1β, also known as ARNT). Under normal oxygen supply conditions, HIF-1α and HIF-2α are hydroxylated by Egl nine homolog family (EGLN) family of HIFs and then recognized by the E3 ubiquitin ligase (Von hippel-lindau, VHL) for eventual degradation in the proteasome. However, under hypoxia, prolyl hydroxylase is inactivated, HIF-1α and HIF-2α accumulate in the cell and bind to ARNT to form the HIF complex, which is able to bind to the hypoxia response element (HRE) of the hypoxia gene promoter region, thereby inducing transcription of genes associated with hypoxia adaptation and triggering a series of hypoxia adaptation responses in tissue cells (73). Numerous studies have reported that HIF is able to regulate the occurrence of ferroptosis in cells, that a number of genes related to iron metabolism are regulated by HRE, and that HIF has a dual role for ferroptosis in cancer cells. Li *et al* (74) found that, in human myeloma cells, carbonic anhydrase 9 (CA9) is overexpressed as a result of hypoxia. Consequently, CA9 overexpression prevents ferroptosis in cells via modulating TFR and FTH, leading to the decreased iron intake and increased ferritin storage. Yang *et al* (75) also discovered that hypoxia stimulated the expression of HIF-1 in HT-1080 fibrosarcoma cells and increased the expression of fatty acid binding proteins 3 and 7, which enhanced the ability of the cells to take in and store fatty acids and prevented the development of ferroptosis. In addition, HIF-2α activation upregulates hypoxia-inducible lipid. Droplet associated protein overexpression in renal clear-cell carcinoma, causes a rise in the synthesis of polyunsaturated fatty acids and lipid peroxidation levels, which finally results in ferroptosis (76).

Non-coding RNAs. Non-coding RNAs, including long non-coding RNA, microRNA (miRNA or miR), circular RNA, and others, are RNA molecules that are present in the gene transcriptome but do not undergo translation to produce proteins. It was previously suggested that certain non-coding RNAs could be involved in the ferroptosis. For instance, ELAV-like RNA-binding protein 1 (ELAVL1) is known to be able to bind and stabilize LINC00336. And it was reported that P53 in lung cancer cells could bind to the ELAVL1 promoter region and suppress ELAVL1 expression. As a result, LINC00336 was freed from its association with ELAVL1 and could interact with miR-6852, which would then bind directly to cystathionine-β-synthase (CBS) and suppress CBS expression, ultimately promoting the occurrence of ferroptosis (77). Furthermore, another study by Wang *et al* (78) identified LINC00618 as a potential regulator of ferroptosis. It was found that LINC00618 was able to increase the production of ROS and affect iron metabolism. Moreover, LINC00618 could downregulate the expression of SLC7A11, a gene involved in cellular antioxidant defense. In the melanoma cell lines G-361 and A375, Zhang *et al* (79) demonstrated that miR-9 could prevent ferroptosis by targeting GOT1 which catabolizes glutamine and eventually converts it to α-ketoglutarate, promoting the accumulation of cellular ROS (79). On the other hand, Luo *et al* (80) identified that miR-137 directly suppressed the expression of SLC1A5 in melanoma cells, resulting in a slower uptake of glutamine and decreased levels of malondialdehyde accumulation, which prevented the incidence of ferroptosis in cells (80). In addition, it has also been reported that miRNAs are also involved in the regulation of other genes implicated in ferroptosis, such as SLC7A11, TFR and FTH. These miRNAs target and regulate factors such as NRF2 and ACSL4, which are known to promote or inhibit the occurrence of ferroptosis in cells.

4. Ferroptosis in the combination therapy of cancer

Lung cancer. Lung cancer, which accounted for 17.09% of new cancer cases countrywide in China, was first in the prevalence of malignant tumors and second most frequent disease worldwide (81). Most prevalently in the non-small cell lung cancer (NSCLC) form, lung cancer is characterized by a poor survival rate (82). Chemotherapy drugs such as acetaminophen (APAP) and cisplatin (CDDP) are currently used in the treatment of lung cancer, but often cause drug resistance in tumor cells, resulting in unsatisfactory therapeutic efficacy (82,83). Recent studies suggested that the combined application of these drugs with ferroptosis inducers might help to improve the therapeutic efficacy of lung cancer (84,85). APAP is a kind of analgesic and detoxification drug. When combined with erastin, it could induce ferroptosis of cells by regulating the nuclear translocation of Nrf2 and inhibit the growth of lung cancer xenografts (84). Meanwhile, it has also been reported that erastin or sorafenib combined with low-dose CDDP can effectively inhibit the growth of CDDP-resistant NSCLC cells *in vivo* and promote ferroptosis by inducing the accumulation of intracellular lipid peroxides (85). Although few studies of these combined drugs have yet been performed, and specific mechanisms of action have not yet been fully understood, this allowed the opening of new perspectives for lung cancer therapy in the future.

Gastric cancer. Gastric cancer, a prevalent malignancy in China, ranks fifth in incidence and fourth in fatality (86). Adriamycin, platinum drugs, 5-fluorouracil and paclitaxel are widely used in clinical treatment of gastric cancer (87,88); although these drugs demonstrated antitumor effects in

gastric cancer, they are often limited by drug resistance. Cai *et al* (89) detected that SIRT6 silencing could induce ferroptosis in gastric cancer cells and overcome the resistance to sorafenib. Precisely, inhibition of SIRT6 leads to the inactivation of Keap1/Nrf2 signaling pathway and downregulation of GPX4, while overexpression of GPX4 or activation of Keap1/Nrf2 signaling pathway can reverse the effect of downregulation of SIRT6 on sorafenib-induced ferroptosis. Therefore, targeting the SIRT6/Keap1/Nrf2/GPX4 signaling pathway may be a potential strategy to overcome sorafenib resistance in gastric cancer cells. A previous study showed that during sorafenib-induced ferroptosis in gastric cancer cells, Activating Transcription Factor 2 (ATF2) can inhibit SLC7A11 protein degradation by promoting the expression of heat shock protein 110, thereby protecting gastric cancer cells from sorafenib-mediated ferroptosis. Accordingly, ATF2 knockout promoted sorafenib-induced ferroptosis (90). Therefore, ATF2 could be a potential target for effective treatment of gastric cancer. Cancer-associated fibroblasts (CAF) are the main stromal cell type in the tumor microenvironment. Zhang *et al* (91) found that the exosome miR-522 secreted by CAF inhibited the occurrence of ferroptosis and promoted acquired drug resistance in gastric cancer cells. This occurs through the selective packaging of miR-522 into CAF-derived exosomes by heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1)/ USP7 axis. Subsequently, miR-522 directly targets ALOX15, preventing the accumulation of lipid peroxidation and inhibiting ferroptosis in gastric cancer cells. Some chemotherapeutic drugs may promote the secretion of miR-522 by CAF through activating the expression of USP7 and hnRNPA1, making gastric cancer cells resistant to these drugs.

Breast cancer. Breast cancer is one of the most common types of cancer in women, second only to uterine cancer, and represents a significant threat to the health of women (92). Tumor-infiltrating neutrophils (TIN) are the population of immune cells most strongly associated with poor prognosis for 25 cancer types, including breast cancer (93). For breast cancer, the TIN-rich tumor microenvironment is resistant to immune checkpoint blocking therapy (94). When combined with immunotherapy, clearing or targeting immunosuppressive TIN is expected to produce synergistic effects (95). Zhao *et al* (96) found through single-cell RNA sequencing that aconite decarboxylase 1 (Acod1) was the metabolic enzyme with the highest degree of upregulation in TIN. It was also demonstrated that TIN inhibits the occurrence of ferroptosis and promotes breast cancer metastasis through high expression of Acod1. Knockout of Acod1 reduces the survival and accumulation of TIN and improves the efficacy of immune checkpoint blocking therapy in the treatment of breast cancer metastasis. The aforementioned study showed that Acod1 is a promising immune-metabolic target that can induce ferroptosis in TIN and protect neutrophils that do not express Acod1 outside the tumor, thus addressing the safety issue of targeting TIN in the treatment of tumor metastasis.

Hepatocellular carcinoma. Hepatocellular carcinoma is a common type of liver cancer, with a five-year survival rate of only 12%. It ranks as the second leading cause of cancer-related deaths worldwide (97). Sorafenib is currently used as a first-line agent for hepatocellular carcinoma whose mechanism of action has been associated to ferroptosis. However, it is unable to induce extensive ferroptosis in all hepatocellular carcinomas (98). Interestingly, it was found that in the process of REDOX homeostasis maintenance, the RNA-binding protein PNO1 promoted the occurrence of autophagy, which enabled macromolecules and organelles to resynthesize new amino acids, thereby increasing the content of glutamate in cells. With the increase of glutamate content in the cell, system Xc is activated, resulting in the increase of cystine uptake and the promotion of GSH biosynthesis, thus protecting hepatocellular cancer cells from ferroptosis. Accordingly, inhibition of PNO1 expression will inhibit the transcription of SLC7A11 and ultimately promote the occurrence of ferroptosis (99). The discovery identified PNO1 as a new candidate therapeutic target in the treatment of hepatocellular carcinoma. The combination of PNO1 inhibitors with agents that induce ferroptosis, such as sorafenib, would provide a potential therapeutic strategy for antitumor therapy based on ferroptosis. NADPH is an important cofactor regulating cystine reduction or reducing/oxidizing GSH (GSH/GSSG) cycle reaction (100). Decreased NADPH abundance can be used as a biomarker to detect ferroptosis in the liver (101,102). Fang *et al* (103) found in their study that cytoplasmic NADPH provider malidase 1 (ME1) is a novel inhibitor of ferroptosis in the liver. They specifically knocked out the ME1 gene in mouse liver cells and observed that this increased the susceptibility to ferroptosis and aggravated liver damage after liver ischemia/reperfusion surgery. By contrast, supplementation with ME1 substrate L-malate increased NADPH abundance to protect the liver from ferroptosis and tissue damage. The study suggested that ME1 is a potential therapeutic target for the treatment of liver ischemia-reperfusion injury or other diseases associated with ferroptosis. Using drug screening, Tang *et al* (104) found that USP8 small molecule inhibitors can significantly inhibit the activity of liver cancer cells. Mechanism studies have shown that USP8 modified OGT protein through deubiquitination to improve the stability of the protein, which subsequently regulated SLC7A11 through glycosylation and inhibited ferroptosis in hepatocellular carcinoma, thus promoting the occurrence of liver cancer.

Ovarian cancer. Ovarian cancer is the third most common and second most deadly female cancer globally, with ~240,000 new cases diagnosed each year and 150,000 deaths (97,105). Currently, the first-line chemotherapy for ovarian cancer is platinum-based drugs combined with paclitaxel, while targeted drugs bevacizumab and poly(adp-ribose) polymerase (PARP) inhibitors are often used for maintenance therapy (106). However, because of the primary or secondary resistance to these drugs developed in the patients receiving the treatment, the therapeutic efficacy remains to be desired. Recent studies have revealed that ferroptosis inducers, such as erastin, in combination with CDDP or PARP inhibitors, inhibit the growth and metastasis of ovarian cancer cells (107,108). Therefore, the development of new drugs for the relevant targets of ferroptosis can help to overcome the shortcomings of current first-line chemotherapy drugs and provide a new strategy for the treatment of ovarian cancer.

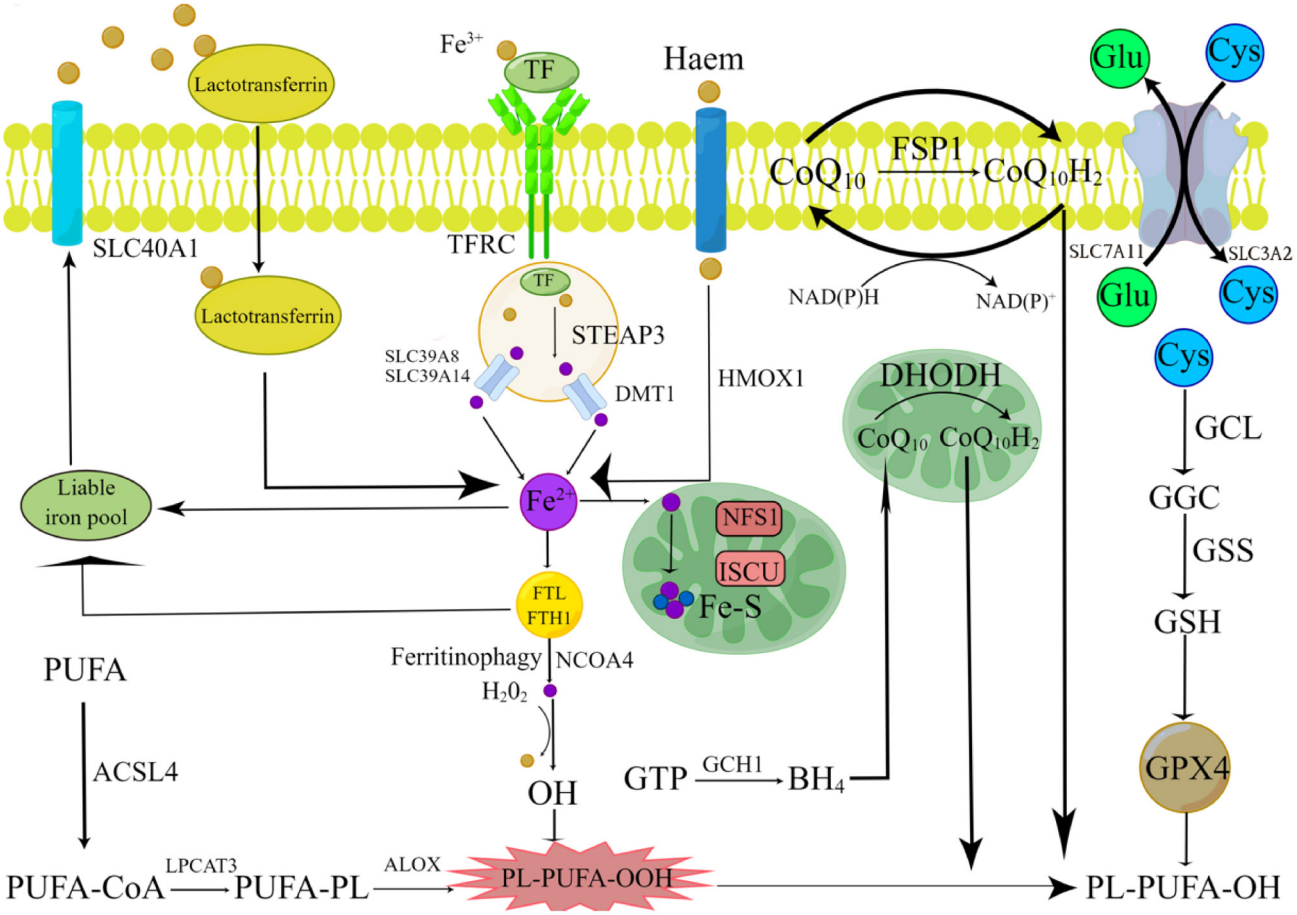

Figure 1. Molecular mechanisms of ferroptosis. Lipid peroxidation, iron metabolism and ferroptosis defense mechanisms are the three primary mechanisms of ferroptosis. The principal ferroptosis-related antioxidant defense mechanisms include the GPX4-GSH pathway, the FSP1-$CoQ_{10}H_2$ pathway, the DHODH-$CoQ_{10}H_2$ pathway and the GCH1-BH4 pathway. The figure was created by Figdraw (2.0). GPX4, glutathione peroxidase 4; GSH, glutathione; FSP1, ferroptosis suppressor protein 1; $CoQ_{10}H_2$, pantethine alcohol; DHODH, dihydroorotate dehydrogenase; GCH1, GTP cyclization hydrolase 1; BH4, tetrahydrobiopterin.

A recent study has also shed light on the role of miRNAs associated with ferroptosis in the development of ovarian cancer. Ma *et al* (109) discovered that the tumor suppressor miR-424-5p might target ACSL4 in ovarian cancer cells to regulate the initiation of ferroptosis. When miR-424-5p was expressed, it could directly bind to the 3'-UTR of ACSL4 and decrease its production, reducing the ferroptosis triggered by erastin in ovarian cancer cells. Conversely, when miR-424-5p expression was decreased, the ovarian cancer cells became more sensitive to erastin induction. Another key enzyme involved in malignant tumor cells, including ovarian cancer, is Stearoyl CoA desaturase 1 (SCD1). It is a rate-limiting enzyme that catalyzes the conversion of saturated fatty acids to monounsaturated fatty acids (110). Tesfay *et al* (111) indicated that inhibiting SCD1 expression led to a decrease in CoQ_{10} expression, resulting in lipid peroxidation and ferroptosis in ovarian cancer cells.

The Hippo pathway, a major signaling pathway regulating cell proliferation, differentiation and metastasis, has recently been shown to be associated with ferroptosis in ovarian cancer cells. Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), the two key factors in this pathway, are significant transcriptional coactivators and are considered to act as receptors for cell density (111). When cells proliferate at high densities, YAP and TAZ are phosphorylated and degraded by proteasomes; conversely, when cells proliferate at low densities, YAP and TAZ are dephosphorylated and translocated from the cytoplasm to the nucleus, where they bind to the transcription factor TEAD to drive gene expression and regulate cell proliferation and differentiation (112). A number of studies revealed that YAP and TAZ exerted regulatory functions in the initiation of cellular ferroptosis. In 2020, Yang *et al* (29) discovered that TAZ directly promoted the expression of angiopoietin-like 4, which enhanced the activity of NOX2, leading to ferroptosis in ovarian cancer cells. Another study revealed that YAP regulated the expression of its target gene, S phase kinase-associated protein 2 (SKP2), contributing to ferroptosis in ovarian cancer cells (113). When YAP was overexpressed, it increased the expression of SKP2, resulting in increased lipid peroxidation. Conversely, when YAP was silenced, SKP2 expression was inhibited, resulting in a decrease in iron concentration and protection from ferroptosis in ovarian cancer cells. Furthermore, Li *et al* (114) demonstrated that inhibiting acetyl-galactosyl-transferase 14 expression decreased mTOR activity by controlling EGFR glycosylation, which in turn inhibited the expression levels of SLC7A11 and GPX4, ultimately inducing ferroptosis in ovarian cancer cells.

5. Conclusions and perspectives

Ferroptosis, a novel form of cell death that relies on iron and ROS, offers new therapeutic perspectives for the treatment of cancer.

Lipid peroxidation is hallmark of ferroptosis and takes place in both enzymatic and non-enzymatic reactions. To date, five principal ferroptosis-related antioxidant defense mechanisms have been described in the literature, including the GPX4-GSH pathway, the FSP1-$CoQ_{10}H_2$ pathway, the DHODH-$CoQ_{10}H_2$ pathway, the GCH1-BH4 pathway and the newly discovered MBOAT1/2-mediated cellular phospholipid remodeling pathway (Fig. 1).

Iron metabolic processes in ferroptosis have been widely explored, primarily involving the dynamic balance of labile iron pools in cells, autophagic ferritin degradation and mitochondrial ISC production.

Multiple key factors (and the related signaling pathways), such as TP53, NRF2 and RAS, which have been significantly investigated in the previous studies for their critical roles and intricate mechanisms of action in various cancer development and metastasis, have been revealed to be associated with the process of cell ferroptosis. These findings, on one hand, show that cell ferroptosis is, as well as other programmed cell death processes previously discovered (such as apoptosis, autophagy or pyroptosis), part of the essential regulating mechanisms of cell growth, survival and response to external signaling; on the other hand, they provide new clues for the understanding of cancer physiopathology, and open new therapeutic perspectives for cancer, particularly in the field of drug combination therapy.

Considerable advances in the research on ferroptosis have been achieved in the past decade. A total of >10,000 published articles retrieved by Pubmed (https://pubmed.ncbi.nlm.nih.gov/) have been devoted to ferroptosis. However, further research on this subject is still required. Particularly, deeper comprehension of the roles of ferroptosis in cancer development and drug resistance in association with specific cancer types, would allow to devise more efficient drugs and treatments based on the new concept.

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Authors' contributions

LZ, XL and CG designed the project and drafted the manuscript, XG and LL revised and edited the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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