Review Article

Ferroptosis: a promising target for fumarate hydratase-deficient tumor therapeutics literature review

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Background and Objective: This review aims to investigate the ferroptosis mechanism of fumarate hydratase (FH)-related tumors for the purpose of possible treatment of tumors. Ferroptosis is an iron (Fe)-dependent form of regulated cell death caused by lipid peroxidation on the cell membrane. Studies have implicated FH in tumorigenesis. As mutations in the FH gene alter cellular metabolism and increase tumorigenesis risk, particularly in the kidneys. As most tumor cells require higher amounts of ferrous ions (Fe^{2+}) than normal cells, they are more susceptible to ferroptosis. Recent studies have indicated that ferroptosis is inhibited the pathogenesis and progression of FH-deficient tumors by regulating lipid and iron metabolism, glutathione-glutathione peroxidase 4 (GSH-GPX4), nuclear factor-erythroid 2-related factor 2 (NRF2)/heme oxygenase-1 (HO-1) pathways. While the Fe^{2+} content is significantly lower in FH-deficient tumor cells, than that in normal cells. It is promising to promote ferroptosis by increasing the concentration of Fe^{2+} in cells to achieve the purpose of tumor treatment.

Methods: In this study, we searched for relevant articles on ferroptosis and FH-deficient tumors using PubMed database.

Key Content and Findings: FH is a tumor suppressor. A number of basic studies have shown that the loss of FH plays an important role in hereditary leiomyomas and tumors such as renal cell carcinoma, ovarian cancer, and other tumors. This type of tumor cells can through induce ferroptosis, inhibit proliferation, migration and invasion of tumor cells, increase the sensitivity of tumor cells to chemotherapy, and reverse the drug resistance through various molecular mechanisms. At present, the research on ferroptosis in FH-related tumors is still in the basic experimental stage.

Conclusions: This article reviews the anti-tumor effects and mechanisms of FH and ferroptosis, in order to further explore the medical value of ferroptosis in FH-related tumor therapy.

Keywords: Fumarate hydratase (FH); tumors; ferrous ions (Fe^{2+}); ferroptosis

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Introduction

The mode of ferroptosis-mediated death is unique form that of pyroptosis, autophagy, and programmed cell death. It depends on the intracellular ferrous ions (Fe^{2+}) concentration (1). Ferroptosis is mainly caused by increased concentration of intracellular oxidation of polyunsaturated fatty acids (PUFAs), iron metabolism disorders, and imbalance in the oxidative system (2). As ferroptosis is involved in many pathological processes (3,4), it provides a novel target for preventing and treating related human diseases, including cardiovascular diseases, fibrotic diseases, and tumors. Cancer is the leading cause of human death worldwide (5,6). However, destroying tumor cells effectively while maintaining normal cells are challenging. As most tumor cells contain higher concentrations of Fe^{2+}, it is easier to induce ferroptosis in these cells than in normal cells (7,8). Therefore, ferroptosis can be a potential cancer therapy approach. Yang *et al.* found that promoting ferroptosis by inhibiting glutathione peroxidase 4 (GPX4) activity and/or promoting glutathione (GSH) metabolism is effective for treating triple-negative breast cancer. This not only induced tumor cells death but also attenuated tumor drug resistance (9). Gao *et al.* demonstrated that during lipstatin-1 mediated degradation of nuclear factor-erythroid 2-related factor 2 (NRF2), ferroptosis occurs in colorectal cancer cells, which inhibits tumor growth *in vivo* (10). Specific knockout of the transferrin gene in the hepatocyte of mice with high Fe^{2+} diet increased the possibility of liver fibrosis induced by ferroptosis, and knockout of solute carrier family 39 member 14 (SLC39A14) expression in the ferroptosis pathway or treatment with ferroptosis inhibitors could effectively alleviate liver fibrosis (11). Insufficient GPX4 promoted ferroptosis including bronchial and kidney epithelial cells and neurons (12). Taken together, the results suggest that weather tumor cells or normal cells are sensitive to ferroptosis. Hence, mediating ferroptosis in cancer cells combined with current treatment methods can improve the efficacy of cancer therapy.

Fumarate hydratase (FH) is a key enzyme in the tricarboxylic acid (TCA) cycle, regulation of FH activity is therefore life-or-death for fumarate turn over and mitochondrial metabolism (13). Its deficiency causes several changes in cellular metabolism (14), such as decreased expression of divalent metal transporter 1 (DMT1) and reduced cytoplasmic Fe^{2+} content (15,16). Renal carcinoma with FH deficiency is resistant to ferroptosis (17), and hence, its tumorigenesis and metastasis have attracted attention (18,19). FH deficiency-induced tumors, which are mostly hereditary (20), are highly malignant and show early metastasis, which significantly affects the patient's quality of life (21). There is no effective treatment for FH-related tumors except early prevention and surgery (22). Therefore, triggering ferroptosis in these tumor cells might be a promising approach for cancer treatment. However, FH-deficient tumor cells are resistant to ferroptosis, which requires a better understanding of the ferroptosis pathway for the treatment of FH-associated tumors. We present this article in accordance with the Narrative Review reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-21/rc).

Methods

We searched the PubMed databases for original research and review articles published in English languages between January 2000 and March 2024. The following search terms were employed: ("Cancer") AND ("FH-deficient cancer" OR "Fe^{2+}" OR "Ferroptosis" OR "Pathology" OR "Prognosis"). Articles cited in the relevant articles were also examined as potential sources of information. The database resources are summarized in *Table 1*.

Ferroptosis and tumors

Ferroptosis is a recently discovered non-apoptotic cell death program, which is catalyzing the lipid peroxidation of unsaturated fatty acids in the cell membrane leading to cell death, and is closely related to Fe^{2+} content (23,24). The main underlying mechanism of ferroptosis is the induction of unsaturated fatty acid peroxidation by excessive Fe^{2+} concentrations, leading to cell membrane damage (25). To increased concentration of reactive oxygen species (ROS) and reduced antioxidant (such as GSH) levers are prerequisites for ferroptosis (26). Increased Fe^{2+} cause excess production of ROS, by participating in the H_2O_2 reaction (Fenton reaction), which causing DNA and protein damage, disrupting cell membranes, and causing cell death (27). ROS accumulation promotes the translocation of NRF2 from the cytoplasm to the nucleus, where it activates antioxidant enzymes, such as heme oxygenase-1 (HO-1), to exert antioxidant effects and inhibit ferroptosis (28). Mitochondria, the core organelles that control metabolism and produce ROS, are closely related to ferroptosis (17). Ferroptosis involvement in multiple human diseases has

Table 1 The search strategy summary

Items	Specification
Date of search	January 12th 2023–March 31st 2024
Databases	PubMed
Search terms used	("Cancer") AND ("FH-deficient cancer" OR "Fe^{2+}" OR "Ferroptosis" OR "Pathology" OR "Prognosis")
Timeframe	2000–2024
Inclusion and exclusion criteria	Inclusion criteria: original articles, review articles, case reports, written in English only
	Exclusion criteria: letters to the editor, non-English language
Selection process	P.C. independently conducted the search

FH, fumarate hydratase.

been established, such as gastric cancer, kidney diseases, and Alzheimer's disease (29-31). These findings suggest that modulating ferroptosis might be potential therapeutic approaches in treating cancer or other diseases. According to the existing literature, promoting ferroptosis can inhibit tumors development through different pathways, such as consume intracellular GSH, and induce ferroptosis in non-small cell lung cancer cells (32). It promotes lipid peroxidation and fosters ferroptosis by inducing ROS accumulation, when gemcitabine is used to treat pancreatic cancer (33). Increasing Fe^{2+} in the labile iron pool (LIP) induces ferroptosis in breast cancer cells (34). As the survival, metastasis, and drug-resistance mechanisms of tumor cells are closely related to ferroptosis (35-37), regulating ferroptotic signaling pathways can control tumors growth.

FH and tumors

Molecular structure and function of FH

FH is widely distributed in living body, one is the dimer containing iron-sulfur clusters (4Fe-4S), which is oxygen sensitive, heat stable and iron dependent. The second class, tetramers, present in human and other eukaryotic cells, have a molecular weight of approximately 200 kDa and do not carry the cofactor iron (38). FH, involved in the TCA cycle, catalyzes reversible hydration between fumarate and malate in both plants and animals (39,40). In the mitochondria and cytosol of mammalian cells, there are two isoenzymes with the same amino terminus. FH is located in the mitochondria in a tetramerc form, where it is crucial for the TCA cycle and cellular respiration. In the nucleus, FH is involved in a nonclassical TCA cycle of metabolic-epigenetic circuits (40). Although different domains on FH have been shown to have different functions, it has not been fully explored yet.

Mutations in FH, which are hereditary, can terminate protein synthesis (41). Mutations in FH genes are not necessarily pathogenic (42), but FH tetramerization is necessary for enzymatic activity (43). Pathogenic FH mutations might occur via the following two mechanisms: first, a mutation in the catalytic site most likely in Llys477, can affect enzyme binding and/or decrease the FH activity (41,44). Second, a mutation can interfere with the formation of quaternary structure, can impair FH activity. Experiments reconstructing human recombinant FH mutants have shown that defects in the quaternary structure render the enzyme inactive (45). This indicates that the enzyme is inactivated due to improper folding in the quaternary structure, which affects the binding site in the enzyme's active center, than changes in the primary structure.

FH in the TCA cycle

During the TCA cycle, acetyl-CoA (come from glucose, fatty acids as well as amino acids) is completely oxidized into water and carbon dioxide by cells to release large amounts of energy and produce adenosine-5'-triphosphate (ATP), flavin adenine dinucleotide (FADH2), and produce nicotinamide adenine dinucleotide (NADH) to support different physiological activities (46). Although energy is mainly produced via glucose metabolism in both tumors and normal cells, in tumor cells, it resembles the glycolysis pathway—and produces lactate as the end product. That is known as the Warburg effect (47). This causes a high accumulation of lactate in most tumor cells, which is associated with increased resistance to ferroptosis inducers,

such as RSL3, erastin, and sorafenib (48). Research results suggest that the diminished FH activity renders the cellular ATP production less dependent on the TCA cycle and more dependent on glycolysis (13). Moreover, FH-deficient cells exhibit increased lactate accumulation due to disruption in the TCA cycle pathway. Lactate significantly enhances oxidative stress resistance and promotes DNA damage repair through ROS signaling (49). Meanwhile, as aerobic glycolysis produces less energy, tumor cells can only meet their metabolic demands by increasing glucose intake. Simultaneously, this produces excessive reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is an antioxidant. This prompts the conversion of oxidated GSH to its reduced form, protect cells from the damage of ROS (50). NADPH suppresses oxidizers and inhibits ferroptosis (51). Inhibition of NADPH production can promote ferroptosis (52). Further, *in vitro* experiments using UOK262 cell culture have shown that suppression of UOK262 cell proliferation in the absence of phosphogluconate dehydrogenase (PGD). When the pentose phosphate pathway (PPP) was impaired, and FH is essential for cell growth (53). The conversion of glucose to ribose-5-phosphate (R-5-P) which is essential for the synthesis of genetic material, also promotes the rapid growth of tumor cells. R-5-P can also be converted to fructose-6-phosphate (F-6-P) and glyceraldehyde-3-phosphate (G-3-P), further providing energy for tumor cells.

The conversion of fumarate to malate by FH occurs in the mitochondria, which also promotes succinate accumulation during FH deficiency (54) (*Figure 1A*). Increased concentrations of succinate and fumarate, particularly the latter, are genetically toxic to cells, dramatically leading to the activation of oncogenic (55). Compared to 2-oxoglutarate, fumarate and succinate can competitively inhibit α-ketoglutarate (α-KG)-dependent dioxygenase, which are essential for DNA repair and promotes demethylation of DNA, RNA, and histones. The family of α-KG-dependent dioxygenase includes Jumonji C-domain lysine demethylases (JmjC-KDMs), ten-eleven translocation (TET) DNA cytosine-oxidizing enzymes, and others. TET catalyze oxidation of methylated cytosines on DNA, thereby facilitating DNA demethylation (56,57). JmjC-KDMs can demethylate on histone tails. DNA hypermethylation contributes to tumorigenesis, which is associated with abnormal gene expression (58). TET also can inhibition of RNA demethylation enhances tumor cell migration (59,60). Taken together, FH deficiency inhibits DNA repair and promotes tumor development.

FH in the amino acid metabolism

Citrulline and aspartic acid can be generated to argininosuccinate by argininosuccinate synthetase, and then fumarate and arginine can be catalyzed by argininosuccinate lyase. It has been widely reported that fumarate can directly bind to GSH, resulting in decrease in reactive GSH (61). Knockdown of argininosuccinate lyase (*ASL*) gene prevents fumarate biosynthesis, leading to an elevated GSH level and counteracting ferroptosis in the presence of arginine. We identify a shared metabolic signature of TCA cycle dysfunction in mouse, whereby enhanced GSH synthesis is accompanied by a concomitant impairment in *de novo* proline and aspartate synthesis (62). NADPH has been implicated as the major cofactor supporting mitochondrial proline biosynthesis, mitochondrial NADPH is essential to enable proline biosynthesis and short of FH could partly explain the decrease in proline (63). Altogether, these findings suggest that arginine deprivation elevates GSH and protect cells against ferroptosis through reducing fumarate biosynthesis (64). Arginine presence reduces intracellular GSH, without affecting GPX4 expression. In the current study, which reveals the ferroptosis regulation by the arginine metabolism (64). In the absence of FH, fumarate accumulation inhibits this metabolism. Further, exogenous synthesis of arginine and fumarate form argininosuccinate reverses this reaction and reduces cytotoxicity (*Figure 1B*). In summary, the data implying that arginine manipulated ferroptosis in a GPX4-independent manner (64). Re-expression of FH in FH-deficient mice is essential for reducing intracellular fumarate concentration. Arginine is essential for FH-deficient cells as it alleviates increased GSH concentrations in these cells. In mice tumor allogeneic grafts, the reduction of arginine inhibits tumor cells growth (65). Moreover, the uptake of arginine efficient in FH-deficient cells than in wild-type cells (66). Based on this, reducing the intracellular arginine content can alleviate FH-deficient conditions.

In the study, we found that growth of UOK262 cells with glutamine (Gln) and asparagine (Asn) resulted in a nearly 2-fold increase in intracellular fumarate levels. We propose that Gln and Asn are important carbon or nitrogen source for the production of other amino acids and for the TCA cycle (67). The lack of energy or glutamate further suppresses tumor growth. Gln uptake flux was markedly

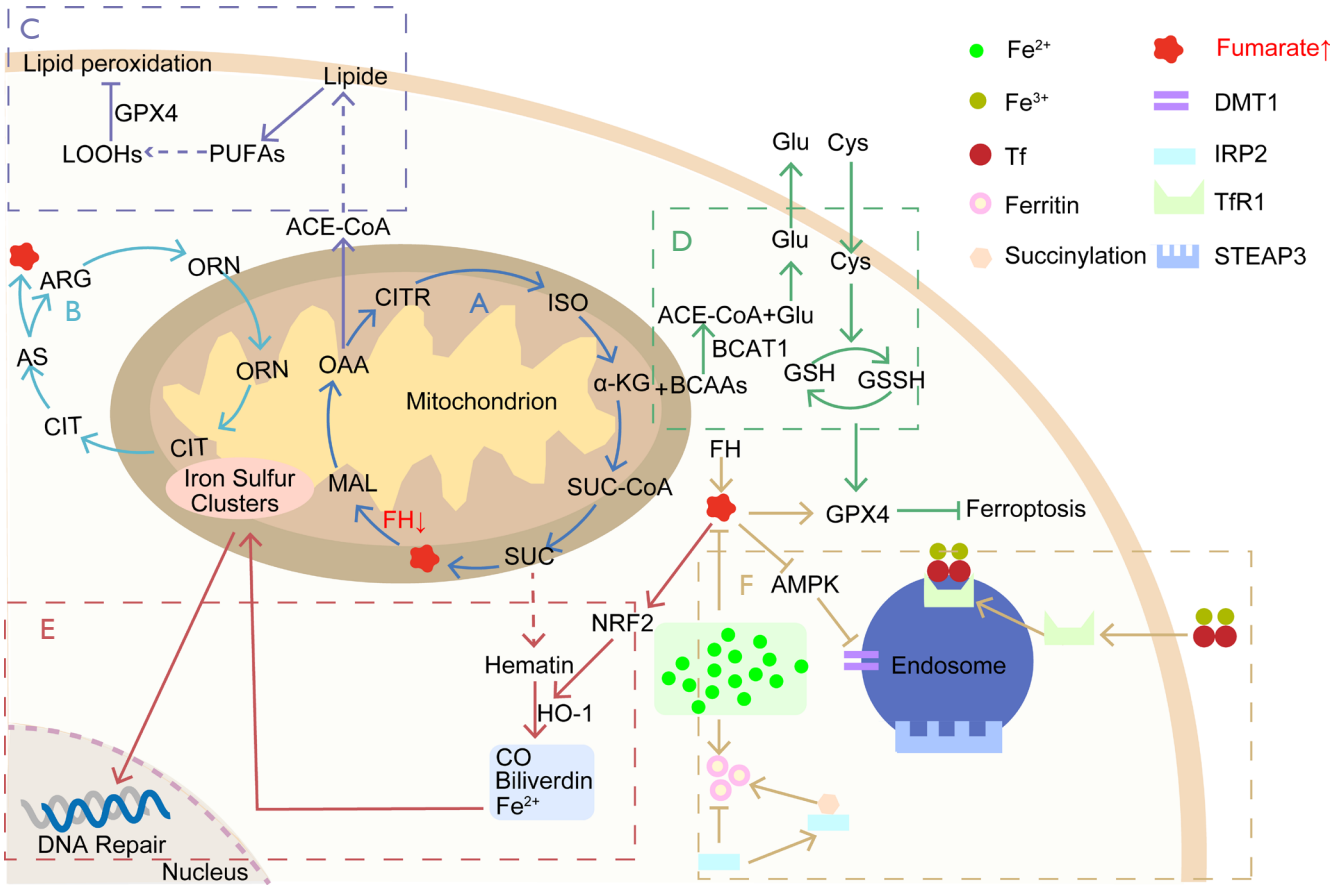

Figure 1 Possible mechanism of ferroptosis and fumarate hydratase-related tumors. (A) Regulation mechanism of FH in TCA cycle. When the activity of FH decreases, the TCA cycle is inhibited, the concentration of fumarate increases, the concentration of succinate increases, and the concentration of malate decreases. (B) Regulation mechanism of FH in UC. The increase in fumarate concentration, in turn, disrupts the UC and prevents the conversion of argininosuccinate to arginine and fumarate. Fumarate increases cell toxicity and promotes tumors formation. (C) Lipid peroxidation. The TCA cycle is blocked, the ACE-CoA content increases, promoting the conversion of PUFAs to LOOHs, and GPX4 promotes the conversion of LOOHs to LOHs, thus inhibiting the occurrence of ferroptosis. (D) GSH-GPX4 pathway. The inactivation of FH inhibits the metabolism of α-KG and promotes BCAAs to produce nitrogen sources and carbon sources for tumor cells through BCAT1 and provides energy for them to compete with the body for the utilization of amino acids; also, the glutamate produced by GSH-GPX regulates the axis to produce glutathione, which inhibits the occurrence of ferroptosis and provides necessary nutrients for tumors growth and proliferation. (E) NRF2/HO-1 pathways. In the TCA cycle, heme synthesis is promoted. In the cytoplasm, fumarate increases the activity of HO-1 and accelerates the degradation of hemoglobin, and leads to the increase of Fe^{2+} in the LIP, which leads to the synthesis of iron-sulfur clusters and promotes DNA repair. (F) Ferrous ions metabolism. Fumarate reduces the concentration of Fe^{2+} in the LIP and inhibits the occurrence of ferroptosis by inhibiting DMT1. At the same time, the increase of ferritin synthesis and the decrease of Fe^{2+} production inhibit ferroptosis and further synthesis of fumarate, which further affect the process of the TCA cycle and the UC. FH, fumarate hydratase; TCA cycle, tricarboxylic acid cycle; UC, urea cycle; ACE-CoA, acetoacetyl coenzyme A; PUFAs, polyunsaturated fatty acids; LOOHs, lipid peroxides; GPX4, glutathione peroxidase 4; LOHs, lipid hydroperoxides; GSH, glutathione; α-KG, α-ketoglutarate; BCAAs, branched-chain amino acids; BCAT1, branched-chain aminotransferase 1; NRF2, nuclear factor E2-related factor 2; HO-1, heme oxygenase-1; LIP, labile iron pool; DMT1, divalent metal transporter 1; AMPK, AMP-activated protein kinase; ARG, arginine; AS, argininosuccinate; CIT, citrulline; CITR, citrate; CO, carbon monoxide; Cys, cysteine; Glu, glutamate; GSSH, oxidized glutathione; IRP2, iron regulatory protein 2; ISO, isocitrate; MAL, malate; OAA, oxaloacetic acid; ORN, ornithine; STEAP3, six-transmembrane epithelial antigen of prostate 3; SUC, succinate; SUC-CoA, succinyl-coenzyme A; Tf, transferrin; TfR1, transferrin receptor 1.

decreased by 36% in knockdown of FH cells, research suggesting the occurrence of alterations of amino acid metabolism or TCA cycle metabolism (13). During amino acid metabolism, fumarate is an intermediate product of phenylalanine and tyrosine metabolism. Therefore, FH deficiency can block these pathways, affecting amino acid metabolism and nucleotide synthesis, resulting in hyperammonia. Increased fumarate concentrations affect the metabolism of α-KG and Gln. α-KG acts as a key substrate for the TCA cycle to produce ATP, amino acids, nucleotides, and lipids in cancer cells (68). Through glutaminase (GLS1) and glutamate dehydrogenase (GDH), it produces glutamate and ammonia (NH3), which can enter the mitochondria to produce energy. Further evidence of Gln is the major energy source in some malignancies (69-71). FH-deficiency disrupts the TCA cycle. Tumors can be suppressed by controlling the Gln concentration in cells and the uptake of branched-chain amino acids (BCAAs).

Potential mechanism of action of FH in tumors

The presence of fumarate makes cells highly dependent on glucose and creates a pseudo-hypoxic environment. This enhances the cells' antioxidant capacity, providing suitable environment for tumor cells and promoting tumor growth (72). Further, the increase in intracellular glutamate concentration can inhibit ferroptosis. It promotes cystine into the cell through system Xc, which increases the activity of GPX4 (73). GSH acts as a co-factored for GPX4, which promotes the survival of tumor cells by converting lipid hydroperoxide into non-toxic fatty alcohols.

Renal tumors caused by mutations of the FH gene, occur primarily in young adults, who have a short survival rate (74,75). The loss of FH is associated with the development of hereditary leiomyomas and renal cell carcinomas (HLRCCs). It is uncertain whether other carcinogenic factors are required (76). FH-related tumors can be caused by mutations in the FH gene. R190H mutation is the most commonly described FH variant in renal cell cancer (RCC) (42). On the cBioPortal website, a mutation rate of 6.45% in the FH gene was observed in 62 patients with unclassified renal cell carcinomas. Most of these mutations occurred at G401V and S41T. However, a study consisting of 35 patients with clear cell carcinomas showed a mutation rate of only 2.9% for the FH gene, and the mutation site was N64C (*Table 2*).

A homozygous mutation in FH causes fumarateuria, an autosomal recessive condition (96), characterized by impaired growth, dystonia, epilepsy, and cerebral atrophy, eventually causing death (97). This disease can only be treated symptomatically as there is no proper treatment for it.

FH chromosome 1q42.3-43 mutations cause HLRCC, such as uterine leiomyomas (ULMs), RCC, cutaneous leiomyomas (CLMs), bladder cancer, and Leydig cell tumors. However, the last two tumors are less common (72). HLRCC varies widely among families and even among individuals within a family (98). There are various histopathologic features associated with FH-deficient tumors, including peri-nucleolar halos, bizarre nuclei, eosinophilic inclusions, and stag-horn vasculature (99). Early diagnosis of cancer is an efficient strategy to improve the survival rate of patients and prevent tumor progression (100). Therefore, it is imperative to prevent, diagnose, and treat HLRCC at an early stage.

In most cases, women with HLRCC syndrome present with ULMs, a dominant genetic disorder (101), associated with atypical pathological characteristics (102). In women of childbearing age, uterine-like mass is the most common benign tumor (103). FH-mutated ULM accounts for 1% of all leiomyomas, while FH-mutated uterine fibroids are often associated with loss of FH expression, as shown by immunohistochemistry (104). However, HLRCC cannot be diagnosed solely based on immunohistochemical staining as mutations in some parts of the FH gene do not affect the expression of the corresponding protein in more than 1/3rd of patients (105). The cells exhibit various growth patterns, such as papillary, tubular papillary, and infiltrative types. Morphologically, the nucleoli have a body-like appearance with gaps surrounding them (21,106,107). In FH sub-type leiomyomas, NRF2 target genes are activated, and NRF2 plays a vital role in fighting ferroptosis. In order to prevent misdiagnosis and to delay the progression of the disease, patients with high suspicion of HLRCC should undergo genetic testing. Earlier medical and surgical treatment is recommended for women with HLRCC syndrome (108), in order to prevent further pathogenicity of FH gene mutations.

CLM is a benign tumor that usually occurs in people approximately 25 years of age. Both single and multiple lesions can be seen, rarely occurring in the genital area, areola smooth muscles, and vascular smooth muscles (109). They are light brown protuberant nodules that measure approximately 1 cm. Although the symptoms are not

Table 2 Relationship between fumarate hydratase and tumors

FH-deficient tumors	Patient number	Frequent (%)	Mutation site	Potential mechanisms	Related molecules	References
URCC	62	6.45	G401V, S41T, 413DUB	Invasion mucin production, EMT	CD10, PAX2, CAIX, CK7, AMACR	(77-79)
ccRCC	35	2.9	N64C	DNA/RNA methylation, abnormal histone modifications, migration, tumor metastasis, angiogenesis, cell adhesion	IL-17, BCAM, BAP1, MECP2, MBD3, TET2, PBRM1, VHL	(80,81)
pRCC	283	1.42	X464-splice, Q439	EMT, VEGF, mTOR pathway, chemotaxis, cell proliferation	SETD2, Merlin, KDM6A, FAT1, BAP1, PBRM1, TP53, SMARCB1, NRF2, STAG2, TFE3, CD133	(82,83)
WT	657	0.0	–	MAPK pathway, EMT	TP53, CD56, CCT, IGF2, STAT3, CCT4	(84,85)
csCC	39	7.69	H204N	Invasive, metastatic, MAPK pathway, JAK/STAT pathway, SHH pathway	PD-1, EGFR, HER-2, TP53, NF-κB, RAS, FGFR, PDGFR, SHHP	(86,87)
CM	444	4.74	S49F, F497L	HIF-1α/NOL7/RAS/PI3K/PKB/ERK pathway, chemokine signaling pathway, apoptosis, Toll-like receptor signaling pathway, MAPK pathway, migration	DR6, TP53, RAS, AIM2, MMP2, CASP3, GSDMD, IFN, EGFR	(88,89)
UCEC	529	6.05	R87H, D179N, E488K, P369S, A104T, R343Q, P359S, A104T, R343Q, P359S, H235R, A320T, S399I, V435M, G97S, E224Q, V255L, N284H, E499K, X413-splice	Migration, mTOR/4EBP1 pathway, metastasis, invasion, RTK-RAS pathway	USP5, ARID1A, MUC16, PTEN, ALDH, TP53, KMT2D, ER, OBSCN, GATA3, PAX2, FDX1, CD10, DLAT, TTN	(90,91)
UCS	22	4.55	V206L	EMT, phosphatidylinositol-3-kinase pathway	POLE, TP53, PTEN, HER-2, RAS	(92,93)
EC	197	3.05	K61N, Y110H	Defects in DNA repair PI3K-PKB-mTOR pathway	POLE, TP53, HER-2, PAX2, PTEN, TLRs	(94,95)

FH, fumarate hydratase; URCC, unclassified renal cell carcinoma; EMT, epithelial-mesenchymal transition; CD10, cluster of differentiation 10; PAX2, paired box 2; CAIX, carbonic anhydrase IX; CK7, cytokeratin 7; AMACR, alpha-methylacyl-CoA racemase; ccRCC, clear cell renal cell carcinoma; IL-17, interleukin 17; BCAM, basal cell adhesion molecule; BAP1, BRCA1-associated protein 1; MECP2, methyl CpG binding protein 2; MBD3, methyl CpG binding domain 3; TET2, Tet methylcytosine dioxygenase 2; PBRM1, polybromo 1; VHL, Von Hippel-Lindau; pRCC, papillary renal cell carcinoma; VEGF, vascular endothelial growth factor; mTOR pathway, mammalian target of rapamycin pathway; SETD2, SET domain containing 2; Merlin, moesin-ezrin-radixin-like protein; KDM6A, lysine-specific demethylase 6A; FAT1, FAT atypical cadherin 1; TP53, tumor protein 53; SMARCB1, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily B, member 1; NRF2, nuclear factor-erythroid 2-related factor 2; STAG2, stromal antigen 2; TFE3, transcription factor E3; CD133, cluster of differentiation 133; WT, Wilms' tumor; MAPK pathway, mitogen-activated protein kinase pathway; CD56, cluster of differentiation 56; CCT, chaperonin-containing tailless; IGF2, insulin-like growth factor 2; STAT3, signal transducer and activator of transcription 3; CCT4, chaperonin containing tailless complex polypeptide subunit 4; csCC, cutaneous squamous cell carcinoma; JAK/STAT pathway, Janus kinase/signal transducer and activator of transcription pathway; SHH pathway, Sonic Hedgehog pathway; PD-1, programmed cell death protein 1; EGFR, epidermal growth factor receptor; HER-2, human epidermal growth factor receptor-2; NF-κB, nuclear factor-κ-binding; RAS, renin-angiotensin system; FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; SHHP, Sonic Hedgehog protein; CM, cutaneous melanoma; HIF-1α, hypoxia inducible factor-1α; NOL7, nucleolar protein 7; PI3K, phosphoinositide3-kinases; PKB, protein kinase B; ERK, extracellular signal-regulated kinase; DR6, death receptor 6; AIM2, absent in melanoma 2; MMP2, matrix metallopeptidase 2; CASP3, caspase 3; GSDMD, gasdermin D; IFN, interferon; UCEC, uterine corpus endometrial carcinoma; 4EBP1, 4E-binding protein 1; RTK, receptor tyrosine kinase; USP5, ubiquitin specific peptidase 5; ARID1A, AT rich interaction domain 1A; MUC16, mucin 16; PTEN, phosphatase and tensin homolog deleted on chromosome ten; ALDH, acetaldehyde dehydrogenase; KMT2D, histone-lysine N-methyltransferase 2D; ER, estrogen receptor; OBSCN, obscurin; GATA3, GATA binding protein 3; FDX1, ferredoxin 1; DLAT, dihydrolipoamide acetyltransferase; TTN, titin; UCS, uterine carcinosarcoma; POLE, polymerase-epsilon; EC, endometrial cancer; TLRs, Toll-like receptors.

obvious, these nodules on the skin can be painful under harsh environments, such as emotional stress and cold temperatures (110-112).

FH-RCC tumors account for 19–30% of all HLRCC. Although the number of FH-RCC mutations usually low, widespread genomic changes are observed and some genes are missing entirely (113). Currently, due to the lack of effective treatment for FH-RCC, DNA sequencing is recommended for patients who are suspected of this condition. Further, early prevention and surgery should be performed immediately after the diagnosis (114). As RCC is aggressive during early metastasis, treatment is usually ineffective (67,115,116), and immediate surgical resection should be performed (117). FH-RCC is highly immunogenic, involving high T-cell infiltration. Therefore, detecting immune targets in tumors, developing drugs for these targets and promoting immune cells in the body to recognize the corresponding tumor cells for clearance are some therapeutic approaches (67,118). We will discuss how ferroptosis can influence the growth of FH-associated tumors in the following section.

Ferroptosis in FH-deficient tumors

FH-deficient tumors inhibit lipid peroxidation in ferroptosis

Lipids are essential for maintaining cell function and membrane structure (119). During ferroptosis, the lipid bilayer is disrupted due to increased peroxidation PUFAs, affecting normal cell membrane functions. GPX4 reduces cytotoxic peroxides to their corresponding alcohols, and regulates lipid peroxidation. Inhibition of GPX4 activity can exacerbate lipid peroxidation and promote ferroptosis (120). Contrarily, it suppresses ferroptosis.

In FH-deficient cells, the acetyl-CoA left over after the TCA cycle is used for fatty acid synthesis. Peroxidation of PUFAs on the membrane phospholipids contributes to ferroptosis. Studies have shown that the abundance and localization of PUFAs in cells influence the degree of lipid peroxidation, thus affecting ferroptosis (120-122). Simultaneously, lipid peroxidation can directly cooperate with GPX4 synthesis, which eventually inhibits ferroptosis (*Figure 1C*) (123). In conclusion, to varying degrees, metabolic pathways can influence the effect of ferroptosis on tumor cells.

FH-deficient tumors inhibit ferroptosis through the GSH-GPX4 pathway

System Xc consists of heterodimers [the light chain subunit solute carrier family 7 member 11 (SLC7A11) and the heavy chain subunit solute carrier family 3 member 2 (SLC3A2)]. Ferroptosis on the cell membrane can be prevented via a 1:1 exchange of cystine and glutamate in and out of the cell through system Xc. The amino acids glutamate, cysteine, and glycine participates in GSH synthesis and functions as a major antioxidant to neutralize cellular stress caused by ROS (124). As GSH is a key intracellular antioxidant, its depletion triggers ferroptosis. NADPH is known to inhibit ferroptosis by mediating the conversion of glutathione disulfide (GSSG) to GSH via GSSG reductase (52). It also promotes GPX4 activity to inhibit ferroptosis.

In FH-deficient cells, accumulate fumarate that complexes with GSH, thus depleting cells of NADPH and ultimately elevating ROS level (125). It is well-known that ROS can be detoxified by GSH. Meanwhile, Fe^{2+} levels decrease and NRF2 is elevated to promote overexpression of GPX4, which is the only known enzyme capable of scavenging lipid peroxides. It requires NADPH via GSH to degrade lipid peroxides (124). Inactivation of GPX4 is fatal in RCCs (126). In addition, cellular experiments have demonstrated that $FH^{-/-}$ tumor cells are more sensitive to ferroptosis inducers and regulate tumor cells growth through multiple pathways. Down regulation of GPX4 activity accelerates tumor cell death through succinylation of GPX4 in $FH^{-/-}$ tumor cells. However, it also protects against ferroptosis by activating NRF2 and GSH activities and reducing Fe^{2+} concentration. Overall, multiple pathways are involved in the inhibition of ferroptosis in $FH^{-/-}$ tumor cells (127). These toxic lipid peroxides can be converted into non-toxic phospholipids by GPX4 to protect the cell membrane. Thus, system Xc is an effective antioxidant system for regulating ferroptosis, which reduces GSH and GPX4 production and promotes ferroptosis (128). The reduction of intracellular glutamate concentration inhibits system Xc, reducing GSH production, and promoting ferroptosis (129). Oxidative stress promotes ROS production and excess ROS cause mitochondrial oxidative damage (130). The SLC7A11-GPX signaling axis is the core defense mechanism for ferroptosis in cells. After entering through system Xc, the intracellular cystine is used to synthesize GSH, which can promote GPX4 and inhibit lipid peroxidation (*Figure 1D*). Reducing the protein expression

level of SLC3A2 and SLC7A11 has been shown to inhibit system Xc activity. The overexpression of SLC7A11 promoted GSH synthesis and inhibited ferroptosis, indicating that system Xc is integral in ferroptosis-treated diseases (41). System Xc activity is closely related to the intracellular antioxidant capacity. The lower the activity, the weaker the cellular antioxidant capacity (131). Previous studies have shown that the increase in GPX can promote chemotherapeutic resistance in tumor cells. Whereas the inactivation of GPX causes ferroptosis in tumor cells, restraining tumor metastasis and recurrence (132). Therefore, when GPX4 is reduced, it can inhibit the GSH-GPX4 pathway, which suppresses drug resistance and increases ferroptosis in tumor cells.

FH-deficient tumors inhibit ferroptosis through NRF2/HO-1 pathway

To control the high ROS level, FH-deficient cells increase the antioxidant transcription factor NRF2 (133). NRF2, a crucial regulator of ferroptosis, also maintains intracellular redox status. NRF2 target genes act through the p62-Kelch-like ECH-associated protein 1 (KEAP1)-NRF2 pathway to prevent lipid peroxidation and iron accumulation, thus inhibiting cellular ferroptosis. When translocated into the nucleus, NRF2 binds to small Maf (sMaf) and thus increases antioxidant response elements (AREs) transcription and promotes oxide metabolism (134). Tumor cells lacking NRF2 are sensitive to ferroptosis (135). NRF2 activity is mainly regulated by the redox sensor protein KEAP1, which is usually hyperactive in human tumors and contributes to tumor progression (136). In HLRCC, NRF2 coordinates cellular responses to oxidative stress (137) and is critical for tumor growth and survival. Fumarate accumulation elevates the levels of NRF2, which acts on HO-1 and increases the synthesis of iron-sulfur clusters to promote DNA damage repair (*Figure 1E*). Wang *et al.* have shown that SLC7A11, a transcriptional target of NRF2, along with other related genes might be involved in preventing ferroptosis by transporting cystine into cells and increasing GSH concentration (138). In HK-4 cells overexpressing NRF2, the expression of GPX4 increased at both transcription and translation levels (139). A similar situation in HEK293T cells upregulated NRF2 binds to the antioxidant response elements in GPX4 and SLC7A11, subsequently increasing their expression and inhibiting ferroptosis (140). Therefore, reducing NRF2 expression might be a potential approach to treat FH-related tumors.

In bladder cancer cells, HO-1 expression increased with increasing abietic acid concentration both *in vitro* and *in vivo*. This promoted ferroptosis and inhibited tumor growth. However, the HO-1 gene knockout increased GPX4 expression, which promoted tumor cells growth. HO-1 activity is essential for cells death (141). Certain stimuli can upregulate NRF2 in the nucleus, which can bind to the HO-1 promoter and increase HO-1 expression (142). However, in ovarian cancer cells, the carboxymethylated pachyman induces ferroptosis by inhibiting HO-1 expression to promote tumor cell death (143). Therefore, the effect of HO-1 on ferroptosis varies in different cells. NRF2 is upregulated in both FH-deficient cells and mouse models. Translocation of activated NRF2 from the cytoplasm to the nucleus increases HO-1 expression, which is a transcriptional target of NRF2. Knocking down NRF2 prominently decreases HO-1 expression, regulating lipid peroxide production (144). Further, silencing HO-1 results in the upregulation of GPX4, which further inhibits ferroptosis to promote tumor growth. HO-1 is a powerful antioxidant enzyme that effectively clears ROS activity by producing porphobilinogen (reduced to bilirubin), which is beneficial for various diseases and normal cells (28,142,145). In FH-deficient cells, knocking out HO-1 using siRNA causes cell death (146). Studies showed that inhibiting of HO-1 activity accelerated FH-deficient cell death in FH-deficient mouse models. Elevated expression of HO-1 is observed in various types of tumors. Therefore, inhibition of HO-1 expression can be a breakthrough point in cancer treatment (146). HO-1 promotes ferroptosis by increasing Fe^{2+} production under certain conditions (119). These results suggest that inhibition of HO-1 activity might control FH-deficient tumors. In HLRCC, overexpression of HO-1 increased the Fe^{2+} content in the LIP, which activated ferroptosis and caused tumor cells death. This information indicates that the high expression of HO-1 is crucial for the survival and development of tumor cells. Simultaneously, elevated HO-1 promotes the production of antioxidants to facilitate cells to resist oxidative stress. Therefore, this therapeutic approach is still worth considering.

FH-deficient tumors inhibit ferroptosis through ferrous ion metabolism

Elevated Fe^{2+} concentration is the primary causative factor. This can be achieved by increasing the uptake and decreasing the consumption of Fe^{2+} (138). In FH-deficient tumors, fumarate has been shown to increase

AMP-activated protein kinase (AMPK) phosphorylation. Lowering AMPK levels in cells (147), results decreased expression of DMT1, which, in turn, reduces the Fe^{2+} content in the LIP. While accelerating the transport of Fe^{2+} from the LIP to the mitochondria to produce heme and iron-sulfur clusters to reduce Fe^{2+}. Iron-sulfur clusters act as cofactors for DNA repair (148), contributing to the repair of the genetic material in tumor cells and promote tumor growth and metastasis.

Iron regulatory proteins 1 and 2 (IRP1/2) can regulate LIP. IRP2 inhibits the expression of ferritin light chain and heavy chain1 (FTL/FTH1). In FH-deficient cells, it promotes the succinylation of IRP2 and increases the expression of FTL and FTH1. Further, increased NRF2 promotes the downstream factors FTL and FTH1 resulting in increased ferritin in HLRCC, decreased cytoplasmic Fe^{2+} content, and inhibition of iron-sulfur-containing metabolic (*Figure 1F*) (41). This pathway further lowering the activity of ferroptosis and promoting the proliferation and growth of tumor cells.

Conclusions

Promoting ferroptosis is an effective way to kill tumor cells. FH deficient cells resist ferroptosis through the activities of various factors, such as GSH, SLC7A11, GPX4, FTL, FTH1, p62, KEAP1, and HO-1. Treatment approaches for FH-deficient tumors should include inducing lipid peroxidation, inhibiting the activities of GPX4 and HO-1, increasing Fe^{2+} concentration, and promoting ferroptosis.

Recent studies on the roles of ferroptosis in tumors have provided broad prospects for the diagnosis and treatment of cancer. However, several limitations remain to be addressed (8,149). (I) Given the complexity of ferroptosis and its function in cancer cells, it is necessary to further explore the roles of specific upstream mechanisms. As tumor cells can directly or indirectly reshape the tumor microenvironment through ferroptosis. (II) It is challenging to determine the mechanism of ferroptosis based on the indicators in the internal environment. Moreover, the interaction of ferroptosis with other cell death pathways, synergistic or antagonistic, remains unknown. Therefore, finding common regulatory factors affecting cell survival is important. (III) Several biological nanomaterials, such as an iron-metal-organic framework, have been shown to release high concentrations of Fe^{2+} and accelerate cell membrane rupture upon reaching the tumor site. Both *in vitro* and *in vivo* experiments have shown that increasing the intracellular Fe^{2+} can induce tumor cells death. Therefore, effectively enhancing ferroptosis in tumor cells can significantly improve the therapeutic effect (150). However, the cancers in which ferroptosis has a significant effect are still unclear.

More relevant studies are needed to explore the mechanism between ferroptosis and FH-deficient tumors in the future. Nevertheless, our findings provide ideas for clinical diagnosis and treatment.

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Footnote

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