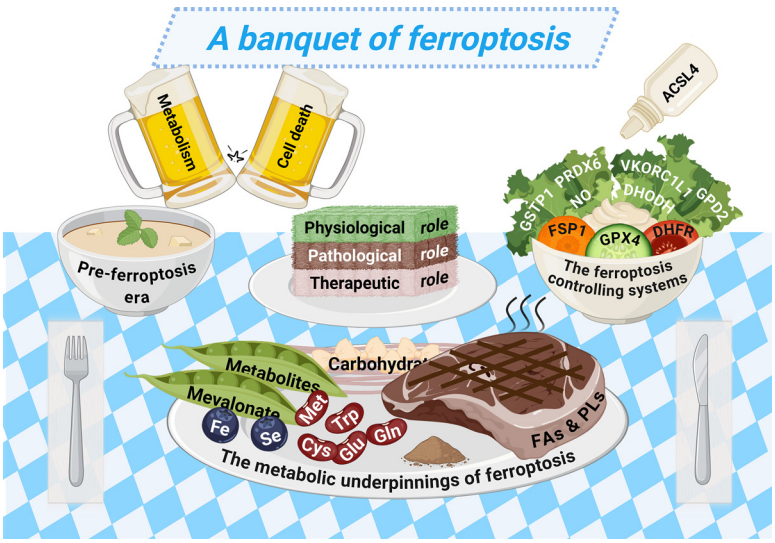
american physiological society
PHYSIOLOGICAL REVIEWS
REVIEW ARTICLE

FERROPTOSIS: WHEN METABOLISM MEETS CELL DEATH



AUTHORS

Jiashuo Zheng (郑嘉烁), Marcus Conrad

CORRESPONDENCE

marcus.conrad@helmholtz-munich.de

KEY WORDS

cancer therapy; cell metabolism; ferroptosis; pathology; physiology

CLINICAL HIGHLIGHTS

- Ferroptosis is a distinct form of cell death marked by iron-dependent, overwhelming lipid peroxidation. Mounting evidence suggests its involvement in aging, tumor suppression, and infection control, indicating that it may be an innate cell death mechanism.
- Ferroptosis has been implicated in a variety of diseases such as ischemia-reperfusion injury, neurodegeneration, and autoimmune diseases. Moreover, ferroptosis may be the underlying mechanism of tissue damage inflicted by certain pathogens and drugs. Inhibition of ferroptosis in these contexts may therefore offer unprecedented therapeutic benefits.
- Ischemia-reperfusion injury is an unavoidable process in organ transplantation. Administering ferroptosis inhibitors during organ preservation may alleviate tissue detriment to donor organs.
- Certain types of cancers, such as clear cell renal cell carcinoma and *MYCN*-amplified neuroblastoma, along with drug-tolerant persister cells and those undergoing epithelial-mesenchymal transition, exhibit high vulnerability to ferroptosis. This suggests the induction of ferroptosis as a potential therapeutic strategy.
- Some existing drugs and irradiation treatments have been shown to induce ferroptosis and eradicate tumor cells. Combining different methods to induce ferroptosis could offer a promising approach to cancer therapy.

ZHENG AND CONRAD, 2025, *Physiol Rev* 105: 651–706
December 11, 2024; Copyright © 2025 The Authors. Licensed under Creative Commons Attribution CC-BY 4.0.
Published by the American Physiological Society.
https://doi.org/10.1152/physrev.00031.2024

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

american physiological society
PHYSIOLOGICAL REVIEWS
Physiol Rev 105: 651–706, 2025
First published December 11, 2024; doi:10.1152/physrev.00031.2024
REVIEW ARTICLE

FERROPTOSIS: WHEN METABOLISM MEETS CELL DEATH

Jiashuo Zheng (郑嘉烁)[1] and Marcus Conrad[1,2]

[1]Institute of Metabolism and Cell Death, Molecular Targets and Therapeutics Center, Helmholtz Zentrum München, Neuherberg, Germany and [2]Translational Redox Biology, Technical University of Munich (TUM), TUM Natural School of Sciences, Garching, Germany

Abstract

We present here a comprehensive update on recent advancements in the field of ferroptosis, with a particular emphasis on its metabolic underpinnings and physiological impacts. After briefly introducing landmark studies that have helped to shape the concept of ferroptosis as a distinct form of cell death, we critically evaluate the key metabolic determinants involved in its regulation. These include the metabolism of essential trace elements such as selenium and iron; amino acids such as cyst(e)ine, methionine, glutamine/glutamate, and tryptophan; and carbohydrates, covering glycolysis, the citric acid cycle, the electron transport chain, and the pentose phosphate pathway. We also delve into the mevalonate pathway and subsequent cholesterol biosynthesis, including intermediate metabolites like dimethylallyl pyrophosphate, squalene, coenzyme Q (CoQ), vitamin K, and 7-dehydrocholesterol, as well as fatty acid and phospholipid metabolism, including the biosynthesis and remodeling of ester and ether phospholipids and lipid peroxidation. Next, we highlight major ferroptosis surveillance systems, specifically the cyst(e)ine/glutathione/glutathione peroxidase 4 axis, the NAD(P)H/ferroptosis suppressor protein 1/CoQ/vitamin K system, and the guanosine triphosphate cyclohydrolase 1/tetrahydrobiopterin/dihydrofolate reductase axis. We also discuss other potential anti- and proferroptotic systems, including glutathione *S*-transferase P1, peroxiredoxin 6, dihydroorotate dehydrogenase, glycerol-3-phosphate dehydrogenase 2, vitamin K epoxide reductase complex subunit 1 like 1, nitric oxide, and acyl-CoA synthetase long-chain family member 4. Finally, we explore ferroptosis's physiological roles in aging, tumor suppression, and infection control, its pathological implications in tissue ischemia-reperfusion injury and neurodegeneration, and its potential therapeutic applications in cancer treatment. Existing drugs and compounds that may regulate ferroptosis in vivo are enumerated.

cancer therapy; cell metabolism; ferroptosis; pathology; physiology

1. INTRODUCTION 651
2. FERROPTOSIS IN THE PREFERROPTOSIS ERA 652
3. METABOLIC PROCESSES IMPINGING... 653
4. THE MAIN FERROPTOSIS CONTROLLING... 669
5. POTENTIAL PHYSIOLOGICAL ROLE... 677
6. FUTURE PERSPECTIVE AND CONCLUDING... 680

1. INTRODUCTION

Cell death is a common occurrence in the human body, with millions of cells dying every second. Under physiological conditions, the vast majority of these cells undergo apoptosis, a process where cells die without rupturing their membranes. However, in pathological conditions, different necrotic cell death modalities, such as necroptosis, pyroptosis, and ferroptosis, may be actively involved. Although all these modalities, including apoptosis, are classified as forms of regulated cell death (1), ferroptosis stands out because of the absence of a cognate executioner protein. In apoptosis, the executioner protein caspase-3 orchestrates the breakdown of cellular components like DNA upon activation. In necroptosis and pyroptosis, phosphorylated mixed-lineage kinase domain-like (MLKL) protein and cleaved gasdermin proteins, respectively, form pores in the plasma membrane. By contrast, ferroptosis is driven by iron-dependent overwhelming lipid peroxidation resulting from metabolic dysfunctions (2). In this regard, ferroptosis is more like a sabotage than a programmed suicide process (3). The uniqueness of ferroptosis is further highlighted by its intricate connections to different metabolic processes involving essential trace elements, amino acids, carbohydrates, mevalonate pathway metabolites, fatty acids, and phospholipids (PLs), which are extensively reviewed in the following. We introduce the main systems and key players controlling ferroptosis and explore its physiological, pathological, and therapeutic roles. Our goal therefore is to provide a detailed overview of the current state of the art in the field of ferroptosis. Before delving into this, however, we briefly discuss the preferroptosis era because it is a common misconception that research

0031-9333/25 Copyright © 2025 The Authors. Licensed under Creative Commons Attribution CC-BY 4.0.

Published by the American Physiological Society.
Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum München (146.107.213.240) on March 3, 2025.

CLINICAL HIGHLIGHTS

- Ferroptosis is a distinct form of cell death marked by iron-dependent, overwhelming lipid peroxidation. Mounting evidence suggests its involvement in aging, tumor suppression, and infection control, indicating that it may be an innate cell death mechanism.
- Ferroptosis has been implicated in a variety of diseases such as ischemia-reperfusion injury, neurodegeneration, and autoimmune diseases. Moreover, ferroptosis may be the underlying mechanism of tissue damage inflicted by certain pathogens and drugs. Inhibition of ferroptosis in these contexts may therefore offer unprecedented therapeutic benefits.
- Ischemia-reperfusion injury is an unavoidable process in organ transplantation. Administering ferroptosis inhibitors during organ preservation may alleviate tissue detriment to donor organs.
- Certain types of cancers, such as clear cell renal cell carcinoma and *MYCN*-amplified neuroblastoma, along with drug-tolerant persister cells and those undergoing epithelial-mesenchymal transition, exhibit high vulnerability to ferroptosis. This suggests the induction of ferroptosis as a potential therapeutic strategy.
- Some existing drugs and irradiation treatments have been shown to induce ferroptosis and eradicate tumor cells. Combining different methods to induce ferroptosis could offer a promising approach to cancer therapy.

on ferroptosis began only in 2012 when the term was coined (4); in fact, the different facets of this form of cell death had been observed and studied long before then.

2. FERROPTOSIS IN THE PREFERROPTOSIS ERA

2.1. Diet-Induced Hepatic Injury—Perhaps the First Reported Ferroptosis Animal Model?

The story begins with T. E. Weichselbaum's report in 1935 that the majority of rats maintained on a cystine-deficient diet died from "hemorrhages" in the liver (later identified as "necrosis") (5). Intriguingly, earlier researchers conducting the very same experiment observed only growth retardation and no significant number of deaths. These seemingly opposing findings were finally resolved in 1947, when it was found that varying levels of tocopherol in the diet were key (6). Furthermore, the content of unsaturated fatty acids also has a decisive impact on the development of necrosis (7). In the meantime, K. Schwarz (8, 9) independently discovered the protective effect of α-tocopherol (the most abundant form of vitamin E) on liver necrosis, using a different model in which the crude casein in diet was replaced by alkali-treated casein. In further research into the cause of necrosis, K. Schwarz and C. M. Foltz (10) identified selenium as the "factor 3," independent of cystine and vitamin E, efficiently protecting the liver. In 1962, the development of the thiobarbituric acid assay allowed demonstration of lipid peroxidation in the necrotic tissue (11). The story of diet-induced hepatic injury was coming to an end (**FIGURE 1**), but these pioneering studies just kick-started ferroptosis research by outlining its key players and principles, including cystine as the source of cysteine and ultimately glutathione (GSH), selenium as a crucial constituent of glutathione peroxidase 4 (GPX4), unsaturated fatty acids as proximate substrates for lipid peroxidation, and tocopherol as a representative of naturally occurring radical trapping antioxidants (RTAs).

2.2. Cellular Cyst(e)ine Deprivation—the Primitive Way to Induce Ferroptosis

The first time that ferroptotic cell death was observed under the microscope probably dates back to the 1950s: H. Eagle (12, 13) found that mouse fibroblasts and HeLa cells underwent cell death within a few days in cystine-deficient medium. Intriguingly, some atypical cell lines such as mouse lymphoma L1210 even died from cyst(e)ine deprivation in normal medium (14), owing to the extremely low activity of the cystine transport system, i.e., system x_c^- (15). System x_c^- is a cystine/glutamate antiporter exchanging extracellular cystine for intracellular glutamate in a 1-to-1 ratio (16, 17). High levels of extracellular glutamate (>1–3 mM) inhibit the uptake of cystine and thus lead to cell death of fibroblasts (18). Compared to fibroblasts, neuronal cells are by far more sensitive to glutamate-induced toxicity (usually in the 2-digit micromolar range) (19). To distinguish cell death induced by glutamate-induced excitotoxicity and oxidative toxicity to neurons, the latter form of cell death was coined "oxytosis" in 2001 and depicts a novel form of cell death characterized by GSH depletion, lipoxygenase (LOX) activation, production of reactive oxygen species (ROS), and calcium influx (20). We now know that oxytosis and ferroptosis present the same phenomenon, yet for over a decade oxytosis was thought to be neuron specific and only studied in one given cell line (i.e., HT-22 cells). The two subunits that make up system x_c^- were eventually elucidated in 1999 by S. Bannai and H. Sato (21), and mice lacking xCT (SLC7A11), the substrate specificity-conferring subunit of system x_c^-, were reported in 2005. Interestingly, these mice appear healthy, whereas embryonic fibroblasts (MEFs) derived from these mice cannot survive under normal cell culture conditions, unless being supplemented with β-mercaptoethanol (22). In the meantime, a chemical compound targeting system x_c^-, sulfasalazine, was identified and extensively investigated (23). Erastin, a more potent and widely used system x_c^- inhibitor, was initially identified as a selective cell death-inducing agent of cells harboring oncogenic RAS (24). It took almost a decade to clarify the main target of erastin,

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

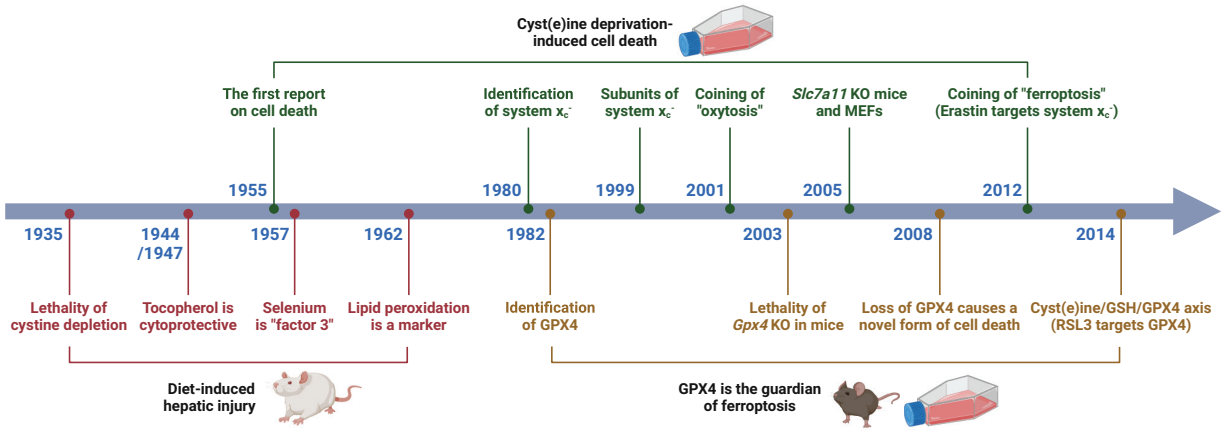


FIGURE 1. Ferroptosis in the preferroptosis era. The rat model of diet-induced hepatic injury, established around nine decades ago, is perhaps the first reported ferroptosis animal model, where cystine, tocopherol, and selenium act as protectors and lipid peroxidation serves as a marker. Cystine deprivation-induced cell death, now known as ferroptosis, was first reported in 1955. It took 25 years to identify the activity of the cystine/glutamate antiporter system x_c^- and another 25 years to genetically disrupt it by deleting its subunit xCT (SLC7A11), discovered in 1999. Meanwhile, the term "oxytosis" was introduced to describe glutamate-induced oxitoxicity in neurons, now recognized as ferroptosis. In 2012, the term "ferroptosis" was coined to describe iron-dependent cell death induced by erastin, a system x_c^- inhibitor. Glutathione peroxidase 4 (GPX4) was discovered in 1982, and two decades later, the first Gpx4 knockout (KO) mouse line, which proved to be embryonic lethal, was established. In 2008, the conditional *Gpx4* knockout mouse model and mouse embryonic fibroblasts (MEFs) were reported, showing that the mere loss of GPX4 causes a novel form of cell death marked by (phospho)lipid peroxidation and rescuable by vitamin E. In 2014, GPX4 was identified as a key player in ferroptosis control, acting downstream of cyst(e)ine and glutathione (GSH), through the investigation of the target of (1*S*,3*R*)-RSL3 (RSL3) and using mice with tamoxifen-inducible deletion of GPX4 in adult mice. Since then, the cyst(e)ine/GSH/GPX4 axis as the core in ferroptosis control has been acknowledged. Figure created with a licensed version of BioRender.com.

which concurrently inspired the coining of "ferroptosis" (4) (**FIGURE 1**).

2.3. *GPX4—the Protagonist in the Control of Ferroptosis*

GPX4 was discovered in 1982 by F. Ursini and coworkers (25) and characterized as a "peroxidation inhibiting protein" that reduces phospholipid hydroperoxides at the expense of GSH. Three years later, GPX4 was found to harbor the rare 21st amino acid selenocysteine (Sec) in its polypeptide chain (26). In 2003, the first *Gpx4* knockout mouse line was established, which proved to be embryonic lethal (27). To circumvent embryonic lethality, our group generated a conditional *Gpx4* knockout mouse line and provided the first evidence that loss of GPX4 in cultured MEFs leads to an as-yet unrecognized form of cell death marked by overwhelming lipid peroxidation. Furthermore, pups with neuron-specific *Gpx4* knockout develop neurodegeneration (28), showing that this novel form of cell death is also relevant in vivo. In parallel, W. S. Yang and B. R. Stockwell (29) explored the mechanism of action of a small molecule compound, named (1*S*,3*R*)-RSL3 (RSL3), which selectively induces an iron-dependent, nonapoptotic form of cell death in RAS-mutant cells. In 2014, the target of RSL3 was unveiled as GPX4 (30). Meanwhile, we reported that deletion of GPX4 in adult mice triggers lipid peroxidation-induced acute renal failure and early death of mice (31). Taken together, all these studies helped to establish the central role of the cyst(e)ine/GSH/GPX4 axis in ferroptosis surveillance (**FIGURE 1**).

3. METABOLIC PROCESSES IMPINGING ON THE VULNERABILITY TOWARD FERROPTOSIS

3.1. *Essential Trace Elements*

3.1.1. Iron.

As the name implies, ferroptosis is "iron dependent." Mechanistically, iron acts as a catalyst in lipid peroxidation, either in the free redox-active form or incorporated in enzymes such as LOXs and cytochrome P450 oxidoreductase (POR) (2). Therefore, changes in cellular iron level may influence the sensitivity of cells toward ferroptosis (32).

Cellular iron metabolism can be divided into four processes: import, intracellular trafficking, storage, and export (**FIGURE 2**). For most tissues, liver-derived transferrin (encoded by the *Trf* gene), which mediates ferric iron (Fe^{3+}) delivery in the blood, is the main source of iron. Cells cultured in transferrin-depleted media are resistant to ferroptosis because of iron deficiency (33). However, global deletion of *Trf* is lethal in mice, whereas ablation of transferrin in hepatocytes

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

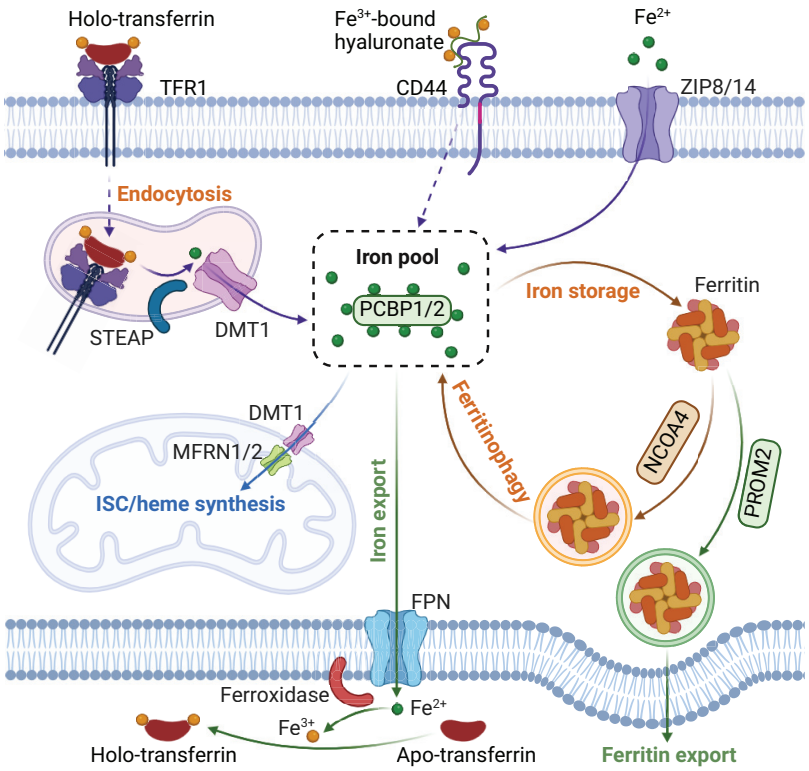


FIGURE 2. Iron metabolism. Cellular iron uptake is primarily mediated by transferrin receptor 1 (TFR1), which binds the transferrin-Fe^{3+} complex (holo-transferrin) and undergoes endocytosis. Fe^{3+} is reduced to Fe^{2+} by the six transmembrane epithelial antigen of prostate (STEAP) family and released into the cytosol via divalent metal transporter 1 (DMT1). In certain contexts, CD44-mediated endocytosis of iron-bound hyaluronates and ZIP8- and ZIP14-mediated non-transferrin-bound iron uptake may make some contributions. Fe^{2+} entering the cytosolic iron pool is distributed with the help of the poly(rC)-binding protein (PCBP) family, with a portion of Fe^{2+} being transported to mitochondria for iron-sulfur cluster (ISC) and heme biogenesis. DMT1 and mitoferrin 1/2 (MFRN1/2) are iron transporters residing on the outer and inner mitochondrial membranes, respectively. Excess iron can be stored in ferritin, with nuclear receptor coactivator 4 (NCOA4)-mediated ferritin degradation (i.e., ferritinophagy) releasing iron when needed. In some cases, ferritin can be exported by prominin 2 (PROM2), although, in most cases, cellular iron export is mediated by the Fe^{2+} transporter ferroportin (FPN). FPN works in concert with a ferroxidase to oxidize Fe^{2+} to Fe^{3+}, allowing it to be loaded onto transferrin. Figure created with a licensed version of BioRender.com.

leads to iron overload in most tissues, likely due to the increase of non-transferrin-bound iron (NTBI) in serum (34). Uptake of the transferrin-Fe^{3+} complex (referred to as holo-transferrin) is mainly mediated by transferrin receptor 1 (TFR1) (**FIGURE 2**). Silencing of *TFR1* renders cells resistant to ferroptosis (29, 33, 35). Consequently, E3 ubiquitin ligases (e.g., HUWE1, NEDD4L, and CHIP), which mediate the degradation of TFR1, protect cancer cells from ferroptosis (36–38). In contrast, oncogenic MYCN and the deubiquitinase OTUD1, which enhance TFR1 expression, sensitize cancer cells to ferroptosis (39, 40). Taken together, these studies highlight the central role of the transferrin-TFR1 system in the control of cellular iron level and ferroptosis susceptibility. Interestingly, the upregulation of TFR1, which is thought to accelerate the death process, is deemed a marker of ferroptotic cells distinct from apoptotic cells (41, 42). The underlying mechanism and physiological role of this alteration, however, remain to be elucidated. Depending on the cell type and the form of extracellular iron source, other iron uptake mechanisms may contribute to ferroptosis sensitivity, such as ZIP14 (aka SLC39A14)- and ZIP8 (aka SLC39A8)-mediated NTBI uptake (34, 43–45) and CD44-mediated endocytosis of iron-bound hyaluronates (46) (**FIGURE 2**).

Iron uptake mediated by the transferrin-TFR1 system is followed by endocytosis. In the acidic endolysosomal compartment, Fe^{3+} is reduced to ferrous iron (Fe^{2+}) by the six transmembrane epithelial antigen of prostate (STEAP) family and then secreted into the cytosol via divalent metal transporter 1 (DMT1) (**FIGURE 2**). Blocking this process by lysosome inhibitors renders cells resistant to ferroptosis (35), whereas ablation of DMT1 can lead to iron accumulation and lipid peroxidation in the lysosome (47, 48). Fe^{2+} entering the cytosolic iron pool is distributed with the help of the poly(rC)-binding protein (PCBP) family (**FIGURE 2**). Mice lacking PCBP1 in hepatocytes exhibit defects in liver iron handling associated with lipid peroxidation and steatosis (49). Cancer cells with gene silencing of *PCBP1* or *PCBP2* are vulnerable to ferroptosis (50, 51). One of the most important intracellular compartments of intracellular iron is mitochondria, the central hub for energy and ROS generation. Restricting iron entry into mitochondria by disrupting DMT1 and mitoferrin 1/2 (MFRN1/2, aka SLC25A37/SLC25A28), which are iron transporters residing on the outer and inner mitochondrial membranes, respectively (**FIGURE 2**), renders cells resistant to ferroptosis (52–55). However, the conflicting reports on lysosomal DMT1 and mitochondrial DMT1 in ferroptosis require further investigation. Mitochondrial iron is mainly used for iron-sulfur cluster (ISC) biogenesis and heme synthesis (**FIGURE 2**). Perturbation of key players such as cysteine desulfurase NFS1 (56), iron-sulfur cluster assembly 2

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

(57), CDGSH iron-sulfur domain-containing proteins (58–61), frataxin (62, 63), glutaredoxin-5 (64), and ferrochelatase (65, 66) can trigger an iron-starvation response and promote ferroptosis. Thus, it appears that iron in the mitochondria promotes ferroptosis, especially when it is not properly utilized.

Excess iron can be stored in ferritin, a heteropolymer composed of heavy chains and light chains (**FIGURE 2**). Loss of ferritin heavy chain in mouse cardiomyocytes and loss of ferritin light chain in pregnant rats lead to ferroptosis-associated cardiomyopathy and preeclampsia, respectively (67, 68). The degradation of ferritin (i.e., ferritinophagy) is mediated by nuclear receptor coactivator 4 (NCOA4) (**FIGURE 2**). Inhibition of ferritinophagy by targeting NCOA4 protects cells against ferroptosis (69, 70). Physiologically, NCOA4 is involved in systemic iron homeostasis and erythropoiesis, as genetic disruption of NCOA4 in mice causes ferritin accumulation in tissues associated with anemia (71). Pathologically, NCOA4-dependent ferroptosis has been implicated in glaucoma (72), ischemic stroke (73), and ionizing radiation-induced intestinal injury (74). In mitochondria, excess iron is stored in mitochondrial ferritin (FTMT), which is structurally and functionally homologous to ferritin heavy chain. Mice lacking FTMT do not show obvious phenotypes (75), whereas under oxidative stress FTMT may play an antiferroptotic role in neurons and macrophages (76, 77). In addition to ferritin, transferrin and lipocalin-2 can also modulate ferroptosis sensitivity by sequestering intracellular iron in certain contexts (78–80). On the other hand, the stress-responsive enzyme heme oxygenase-1 (HMOX-1, HO-1) can increase ferroptosis sensitivity by liberating Fe^{2+} from heme (81–84), albeit conflicting reports claim that loss of HMOX-1 can induce heme toxicity (85, 86).

Cellular iron export is mainly mediated by the Fe^{2+} transporter ferroportin (FPN, aka SLC40A1), which is coupled to a ferroxidase such as ceruloplasmin and hephaestin that oxidizes Fe^{2+} to Fe^{3+} so that the released iron can be loaded onto transferrin (**FIGURE 2**). Ablation of FPN in the neurons of mouse neocortex and hippocampus leads to brain iron accumulation and ferroptosis-associated cognitive impairment, which can be ameliorated by intranasally delivered ferroptosis inhibitors (87). Mice with kidney proximal tubule-specific FPN loss are more vulnerable to folic acid-induced nephrotoxicity accompanied by signs of ferroptosis (88). Consistently, ceruloplasmin depletion in hepatocellular carcinoma cells (HCCs) increases their sensitivity toward ferroptosis (89). In addition to the FPN system, prominin 2 (PROM2)-mediated ferritin export (90), FLVCR1-mediated heme export (91), and TRPML1-mediated lysosomal exocytosis may confer ferroptosis resistance in different contexts by lowering intracellular iron levels (92) (**FIGURE 2**).

3.1.2. Selenium.

In contrast to iron, selenium is the most important trace element in ferroptosis suppression because GPX4, the mainstay in ferroptosis control, is a selenoprotein (30, 31). Therefore, not only the source of selenium but also the regulation of Sec incorporation machinery has crucial impacts on ferroptosis sensitivity (93).

Akin to transferrin in iron metabolism, selenoprotein P (SELENOP) is mainly synthesized in the liver and has the function of distributing selenium to other organs via the circulatory system, although certain organs like brain also express their own SELENOP to maintain the local selenium cycle (94, 95) (**FIGURE 3**). Mice with global *Selenop* deletion display neurological deficits in childhood and reduced fertility in males (96, 97). Coincidently, mice (on a mixed genetic C57BL/6J/129S6SvEv background) with a targeted Sec-to-Cys (U46C) replacement in GPX4 are born but die of seizures ~2–3 wk after birth (98), and male mice with GPX4 ablation in spermatocytes are infertile (99). In vitro, deletion of SELENOP in pancreatic β-cells and glioblastomas leads to decreased GPX4 expression and increased ferroptosis sensitivity, suggesting that SELENOP maintains selenium level in a paracrine/autocrine manner in certain contexts (100, 101). The uptake of SELENOP is mediated by low-density lipoprotein receptor-related protein 8 (LRP8, aka APORE2) and LRP2 (aka megalin) (**FIGURE 3**), which have distinct tissue distributions (102). Mice with global *Lrp8* knockout exhibit defects in brain formation and male infertility (103, 104), mimicking the phenotypes of *Selenop* knockout mice, although these defects are at least partially due to impaired Reelin signaling (105). LRP2 is highly expressed in kidney proximal tubule epithelial cells, where it mediates the reabsorption of SELENOP from the primary urine (106). Accordingly, *Lrp2* mutant mice display increased urinary SELENOP excretion and global selenium deficiency (107). Depletion of LRP8 increases ferroptosis vulnerability in neurons, neuroblastomas, and breast cancer cells (108–110), but whether LRP2 plays a role in ferroptosis remains to be determined.

In addition to SELENOP, selenium can be utilized by cells in other organic or inorganic forms, where system x_c^- may play an essential role. For instance, selenocystine ($SeCys_2$) is imported by system x_c^- at the exchange of glutamate (111, 112) (**FIGURE 3**). The rapid utilization of selenite (SeO_3^{2-}) also relies on the cystine/cysteine cycle mediated by system x_c^-, which creates a reducing extracellular milieu facilitating the conversion of SeO_3^{2-} to selenide (HSe^-) (113, 114) (**FIGURE 3**). Thus, system x_c^- not only controls the substrate of GPX4 but also controls its expression by mediating selenium uptake, especially when the SELENOP system is impaired (109, 115). Notably, a recent study indicates that high concentrations

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

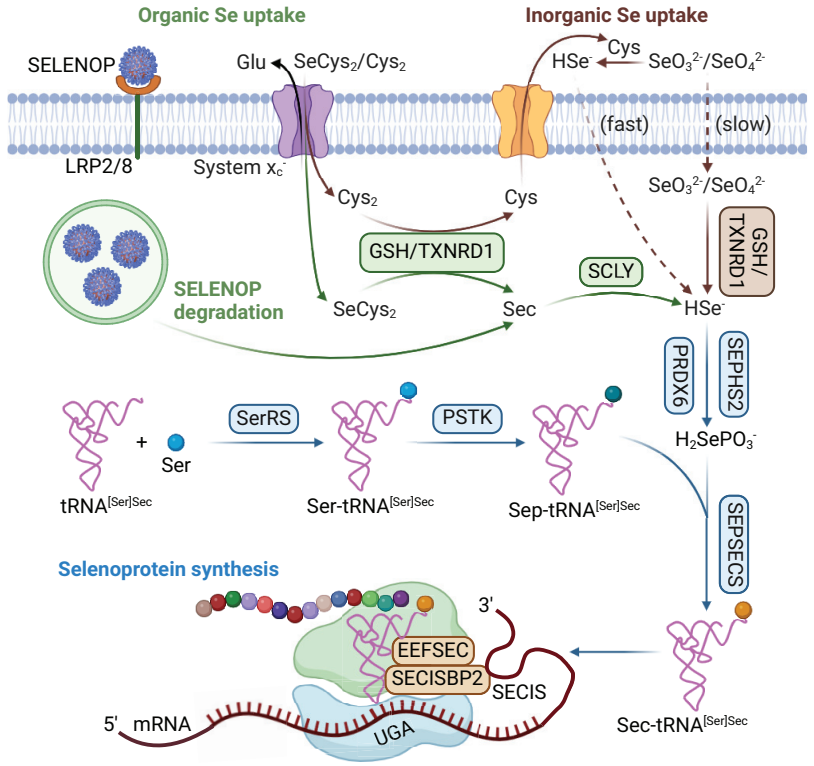


FIGURE 3. Selenium metabolism. Cells utilize selenium (Se) in different forms depending on the context. Selenoprotein P (SELENOP) is a major selenium shuttle in the body, containing up to 10 selenocysteine (Sec) residues, and its uptake is mediated via low-density lipoprotein receptor-related proteins 8 (LRP8) and 2 (LRP2). Selenocystine ($SeCys_2$) is imported by system x_c^- with the exchange of glutamate, then reduced to Sec by glutathione (GSH)- and thioredoxin reductase 1 (TXNRD1)-dependent systems. The rapid utilization of the inorganic selenocompound selenite (SeO_3^{2-}) also relies on system x_c^-, which maintains a reduced extracellular milieu by mediating the cystine/cysteine cycle, thereby facilitating the conversion of SeO_3^{2-} to selenide (HSe^-). Alternatively, SeO_3^{2-} and selenate (SeO_4^{2-}) can be slowly imported into cells, then reduced to HSe^- by GSH- and TXNRD1-dependent systems. For selenoprotein synthesis, both organic and inorganic selenocompounds must first be converted to HSe^-. The conversion of Sec to HSe^- is mainly catalyzed by Sec lyase (SCLY), although SCLY-independent pathways exist. Selenide then binds to one of the Cys residues in peroxiredoxin 6 (PRDX6), which shuttles HSe^- to selenophosphate synthetase 2 (SEPHS2), phosphorylating selenide to yield selenophosphate ($H_2SePO_3^-$). The biosynthesis of Sec-tRNA$^{[Ser]Sec}$ involves 3 sequential steps catalyzed by seryl-tRNA synthetase (SerRS), phosphoseryl-tRNA kinase (PSTK), and Sep-tRNA:Sec-tRNA synthase (SEPSECS), with $H_2SePO_3^-$ as the selenium donor in the final step. Sec-tRNA$^{[Ser]Sec}$ is then used for selenoprotein synthesis requiring a Sec insertion sequence (SECIS) element present in the 3′-untranslated region of selenoprotein mRNAs. SECIS-binding protein 2 (SECISBP2) and associated factors, such as Sec-tRNA-specific eukaryotic elongation factor (EEFSEC), are essential for recognizing the SECIS element and dictating the ribosome to decode the UGA opal codon as Sec instead of translational termination. Figure created with a licensed version of BioRender.com.

of inorganic selenocompounds protect cells from mitochondrial lipid peroxidation in a GPX4-independent manner, i.e., by promoting sulfide quinone oxidoreductase (SQOR)-mediated regeneration of ubiquinol ($CoQH_2$) (116). However, whether this concept applies to pathophysiological conditions remains to be validated.

As a prerequisite for selenoprotein synthesis, both organic and inorganic selenocompounds must first be converted to HSe^- (117). Inorganic selenocompounds like SeO_3^{2-} and selenate (SeO_4^{2-}), once inside the cells, can be reduced to HSe^- by GSH- and thioredoxin reductase 1 (TXNRD1)-dependent reducing systems (117) (**FIGURE 3**). Organic selenocompounds, including SELENOP, are usually catabolized in lysosomes to release Sec, which is then further processed by Sec lyase (SCLY) to yield HSe^- (**FIGURE 3**). In some specific cell lines like neuroblastoma SK-N-DZ, which are highly sensitive to selenium deprivation, loss of SCLY alone is sufficient to trigger ferroptosis (118). Interestingly, SCLY-deficient mice do not show as severe phenotypes as *Selenop* knockout mice, implying the presence of SCLY-independent metabolic pathways (119). This assumption was recently confirmed in human T lymphoma Jurkat cells as well as SK-N-DZ cells, but the underlying mechanism remains unclear (118, 120). To be incorporated into Sec-tRNA$^{[Ser]Sec}$, HSe^- needs to be phosphorylated to selenophosphate ($H_2SePO_3^-$) by selenophosphate synthetase 2 (SEPHS2) (**FIGURE 3**). Ablation of SEPHS2 not only impedes selenoprotein synthesis but also leads to HSe^- toxicity; therefore, cancer cells with a high demand for selenium and selenoproteins are particularly sensitive to SEPHS2 perturbation (114, 121). Furthermore, peroxiredoxin 6 (PRDX6) was found to facilitate efficient utilization of HSe^- by

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

SEPHS2 (**FIGURE 3**), as loss of PRDX6 depletes GPX4 and sensitizes cells to ferroptosis (118, 122, 123).

The biosynthesis of Sec-tRNA$^{[Ser]Sec}$ is unique and requires three steps. First, tRNA$^{[Ser]Sec}$ (encoded by the *Trsp* gene) is aminoacylated with serine by seryl-tRNA synthetase (SerRS), yielding Ser-tRNA$^{[Ser]Sec}$. Subsequently, the seryl moiety is phosphorylated by phosphoseryl-tRNA kinase (PSTK), resulting in *O*-phosphoseryl (Sep)-tRNA$^{[Ser]Sec}$. Finally, $H_2SePO_3^-$ is incorporated into Sep-tRNA$^{[Ser]Sec}$ by Sep-tRNA:Sec-tRNA synthase (SEPSECS), generating Sec-tRNA$^{[Ser]Sec}$ (**FIGURE 3**). Disruption of this finely orchestrated process can lead to impaired selenoprotein synthesis and associated phenotypes. For instance, constitutive deletion of *Trsp* causes embryonic death almost at the same developmental stage as *Gpx4* knockout mice (27, 124). The deletion of *Trsp* in certain neurons leads to neuronal cell death and a neurodegenerative phenotype similar to the isolated deletion of *Gpx4* (125, 126). A missense mutation in *Sepsecs* causes cardiorespiratory failure and perinatal death in mice, which can be rescued by the expression of the GPX4 U46C variant (127). These studies suggest the unique position of GPX4 among other selenoproteins and that in certain contexts ferroptosis is the direct consequence of selenoprotein depletion.

Sec is encoded by the opal stop codon UGA. To decode this stop codon into a Sec residue, a stem-loop-like structure, known as Sec insertion sequence (SECIS) element, is required in the 3′-untranslated region of selenoproteins in vertebrates. The SECIS element is recognized by SECIS-binding protein 2 (SECISBP2), which in turn recruits other factors such as Sec-tRNA specific eukaryotic elongation factor (EEFSEC) to facilitate the insertion of Sec-tRNA$^{[Ser]Sec}$ (**FIGURE 3**). Constitutive loss of SECISBP2 in mouse leads to embryonic death (128), and mice with neuron-specific deletion of SECISBP2 display a phenotype similar to but milder than deletion of *Trsp* (129). Although individuals carrying homozygous or compound heterozygous mutations in the *SECISBP2* gene are viable, they exhibit a multisystem disorder with selenoprotein deprivation (130, 131). Recently, patients with SECISBP2 mutations have been found to have a higher risk of aortic aneurysm formation; studies on patient samples and correlated animal models reveal ferroptosis underlying the pathogenesis (132).

3.2. Amino Acid Metabolism

3.2.1. Cyst(e)ine.

The unique position of cyst(e)ine in the control of ferroptosis is largely attributed to its effects on GPX4. Cysteine is the rate-limiting substrate for GSH synthesis (**FIGURE 4**), and GSH is the preferred substrate of GPX4. In the absence of GSH, cysteine itself may serve as an alternative reducing substrate of GPX4, albeit with lower efficiency (133, 134). Cysteine also promotes protein synthesis of GPX4 by facilitating the uptake of selenium (as outlined in sect. 3.1.2). Furthermore, cysteine is a sulfur donor in ISC biogenesis, which may impact on mitochondrial iron homeostasis and thus ferroptosis (as discussed in sect. 3.1.1) (**FIGURE 4**). Cysteine also provides sulfur for hydropersulfide (RSSH) generation, which is cell-intrinsic RTAs halting lipid peroxidation in a GPX4-independent manner (135, 136) (**FIGURE 4**). Finally, cysteine is a building block of coenzyme A (CoA) (**FIGURE 4**), which plays an antiferroptotic role, although the mechanism remains to be established (137, 138).

Cells acquire cysteine mainly via uptake from the extracellular space and by de novo synthesis (139). In vivo, extracellular cysteine can be imported by neutral amino acid transporters (NAATs) such as alanine serine cysteine transporter 1/2 (ASCT1/2), L-type amino acid transporter 2 (LAT2), and excitatory amino acid carrier type 1 (EAAC1, aka EAAT3, SLC1A1) (**FIGURE 4**). In cell culture, however, the level of extracellular cysteine is negligible because cysteine is rapidly converted to cystine under atmospheric oxygen. Therefore, cultured cells are forced to express system x_c^- for cystine import (139). Intracellular cystine is then quickly reduced to cysteine by GSH- or TXNRD1-dependent reducing systems (140, 141) (**FIGURE 4**). The distinct availability of extracellular cyst(e)ine between in vitro and in vivo conditions thus can easily lead to different outcomes, raising caution for results obtained by in vitro and in vivo investigations.

Besides system x_c^-, system $b^{0,+}$ mediates the uptake of cystine, but its expression is restricted to the kidney. Humans and mice with defects in system $b^{0,+}$ develop cystinuria due to impaired renal reabsorption of cystine (142). Ectopic expression of SLC3A1, the heavy chain of system $b^{0,+}$, has been linked to elevated GSH level and tumorigenesis in breast cancer (143). In addition to cystine, cysteine-enriched proteins and GSH-related peptides can also serve as alternative sources for intracellular cysteine. For instance, albumin is taken up by cancer cells for cysteine supply via cathepsin B (CTSB)-mediated lysosomal degradation (144) (**FIGURE 4**). Extracellular GSH is hydrolyzed to Cys-Gly dipeptide by the ectoenzyme γ-glutamyltransferase 1 (GGT1), followed by import and dipeptidase-mediated digestion to yield cysteine (145) (**FIGURE 4**). Ablation of GGT1 in glioblastomas and inhibition of carnosine dipeptidase II (CNDP2) in HCCs sensitize cells to cystine deprivation-induced ferroptosis (146, 147). Nevertheless, forced expression of GGT1 in chromophobe renal cell carcinomas paradoxically suppresses GSH levels and cell proliferation (148).

Cysteine can be synthesized from serine and homocysteine via the transsulfuration pathway. This is an

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

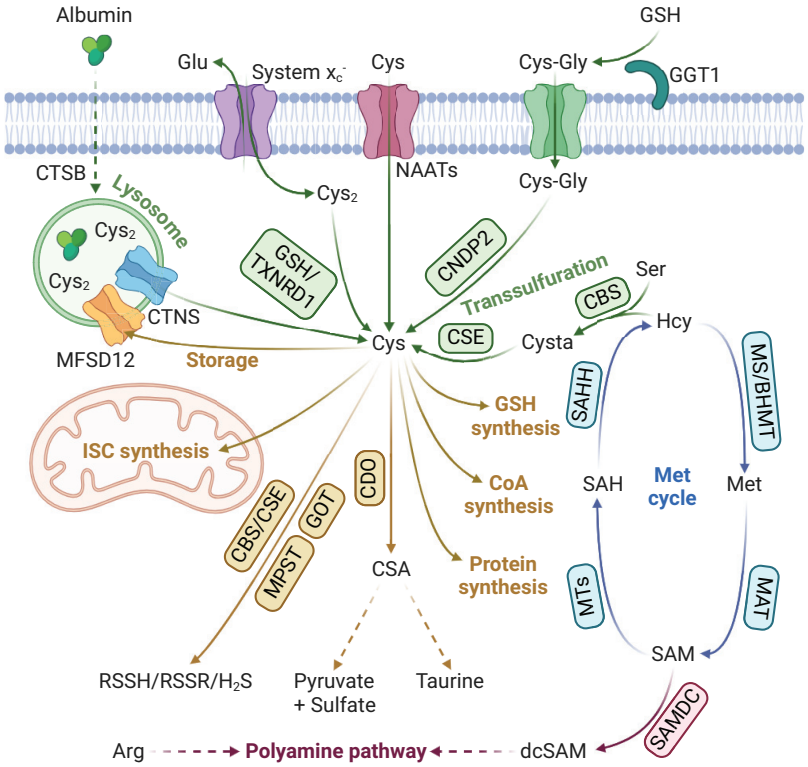


FIGURE 4. Cyst(e)ine and methionine metabolism. In vivo, extracellular cysteine (Cys) can be imported via neutral amino acid transporters (NAATs), whereas in vitro cells rely heavily on system x_c^--mediated cystine (Cys_2) import, which is then reduced to Cys by glutathione (GSH)- and thioredoxin reductase 1 (TXNRD1)-dependent systems. Lysosomal degradation of albumin mediated by cathepsin B (CTSB) and sequential hydrolysis of GSH mediated by γ-glutamyltransferase 1 (GGT1) and carnosine dipeptidase II (CNDP2) can also contribute to the intracellular Cys pool. Additionally, Cys can be synthesized through the transsulfuration pathway, where serine (Ser) and homocysteine (Hcy) are converted to cystathionine (Cysta) by cystathionine β-synthase (CBS) and subsequently hydrolyzed by cystathionine γ-lyase (CSE). Intracellular Cys, possibly stored as Cys_2 in lysosomes, is crucial for the synthesis of GSH, coenzyme A (CoA), and various proteins. Major facilitator superfamily domain containing 12 (MFSD12) and cystinosin (CTNS) mediate the lysosomal influx and efflux of Cys and Cys_2, respectively. Cys also serves as a sulfur donor for iron-sulfur cluster (ISC) biogenesis and the synthesis of persulfide species, including hydropersulfide (RSSH), persulfide (RSSR), and hydrogen sulfide (H_2S). This process can occur sequentially via glutamate oxaloacetate transaminase (GOT) and mercaptopyruvate sulfurtransferase (MPST) or via CBS or CSE using oxidized Cys (i.e., Cys_2) or Cys plus Hcy as substrates. Cys can also be oxidized by cysteine dioxygenase (CDO) to cysteine sulfinic acid (CSA), serving as a carbon donor for pyruvate and taurine. Methionine (Met) is a precursor to Hcy and, subsequently, Cys. The conversion of Met to Hcy involves 3 steps catalyzed by methionine adenosyltransferase (MAT), methyltransferases (MTs), and *S*-adenosyl-L-homocysteine hydrolase (SAHH), producing the intermediates *S*-adenosyl methionine (SAM) and *S*-adenosyl-homocysteine (SAH). Hcy can be remethylated to Met by methionine synthase (MS) or betaine-homocysteine *S*-methyltransferase (BHMT), completing the Met cycle. Notably, SAM can be decarboxylated by *S*-adenosylmethionine decarboxylase (SAMDC) to produce decarboxylated SAM (dcSAM), which, along with arginine (Arg), may drive the polyamine pathway. Figure created with a licensed version of BioRender.com.

irreversible process involving two vitamin B_6-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE, aka cystathionase, CTH) (**FIGURE 4**). CBS catalyzes the condensation of homocysteine and serine to form cystathionine, which is then hydrolyzed by CSE to generate cysteine, α-ketobuyrate (α-KB), and ammonia. CBS and CSE are most strongly expressed in liver tissue (149). Mice with hepatocyte-specific deletion of both TXNRD1 and glutathione-disulfide reductase (GSR) remain viable regardless of their inefficiency in reducing cystine, as transsulfuration maintains the basic supply of cysteine (150). Likewise, primary murine hepatocytes with xCT deletion maintain cysteine levels and proliferate normally (151). On the other hand, mice with global *Cbs* knockout suffer from severe growth retardation and early death associated with homocysteinemia and liver injury (152). Primary murine hepatocytes with CTH loss are highly sensitive to cystine deprivation (153). Although *Cse* knockout mice do not show any obvious phenotype, their viability is highly dependent on dietary cysteine supply (153, 154), and these mice are more susceptible to acetaminophen-induced hepatotoxicity (155). Together, these studies highlight the physiological importance of transsulfuration pathway for cysteine supply. The transsulfuration pathway is also exploited by some cancers. In a subset of cancer cell lines, CBS and CSE are constitutively expressed, whereas in some others CBS and CSE are induced upon cysteine deficiency (156–159). Moreover, the transsulfuration pathway can be activated by oncogenic MYCN in neuroblastoma (160, 161), by PI3KCA in breast cancer cells (162), and by interleukin 1 receptor

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

accessory protein (IL1RAP) in Ewing sarcoma cells to counteract ferroptosis (163).

Intracellular cysteine is presumably stored in the form of cystine in lysosomes (164). Upon cystine deprivation, the decrease of lysosomal cystine instead of cytoplasmic cysteine is responsible for the induction of activating transcription factor 4 (ATF4) (165), which governs the transcription of a set of genes including *xCT* and *CSE* to rescue ferroptosis (166, 167). The lysosomal influx and efflux of cysteine and cystine are controlled by major facilitator superfamily domain containing 12 (MFSD12) and cystinosin (CTNS), respectively (168, 169) (**FIGURE 4**). Breast cancer cells with loss-of-function mutations in MFSD12 are more vulnerable to ROS challenges because of insufficient cysteine storage and GSH synthesis, whereas cells with MFSD12 overexpression are more tolerant (170). Consistent with this, CTNS depletion sensitizes cells to oxidative stress and ferroptosis because of blockade of lysosomal cystine export (165, 170). However, it is noteworthy that the pathogenic mechanism underlying cystinosis, a genetic disorder caused by biallelic mutations in the *CTNS* gene, is unlikely relevant to ferroptosis but rather the accumulation of cystine crystals in lysosomes.

Intracellular cysteine is involved in multiple metabolic pathways (**FIGURE 4**). For example, cysteine can be degraded sequentially by glutamate oxaloacetate transaminase [GOT, also known as cysteine aminotransferase (CAT) or aspartate aminotransferase (AST)] and mercaptopyruvate sulfurtransferase (MPST) to yield persulfide (RSSR)/hydrogen sulfide (H_2S). H_2S can be further converted to RSSH by reacting with an oxidized thiol species (e.g., RSSR) or by mitochondrially localized SQR (171). The transsulfuration enzymes CBS and CSE also facilitate RSSH/H_2S production by using either cystine (oxidized cysteine) or homocysteine plus cysteine as substrates (172, 173) (**FIGURE 4**). Depletion of SQR and CSE sensitizes cells to ferroptosis, whereas depletion of persulfide dioxygenase, which catalyzes persulfide degradation, has the opposite effect (135, 136). These studies clarify the mechanism underlying the previous observation that CBS and CSE protect against ferroptosis independently of cysteine formation (158). Remarkably, H_2S per se is not an effective RTA (135). Upon cystine deprivation, H_2S may even sensitize cancer cells to ferroptosis by inhibiting the enzyme activity of *S*-adenosyl-L-homocysteine hydrolase (SAHH) and thus the transsulfuration pathway (174). Intracellular cysteine is also utilized for protein synthesis and cysteine dioxygenase (CDO)-mediated oxidation (**FIGURE 4**), which do not exert obvious antiferroptotic effects. These metabolic processes may compete with the others for cysteine utilization and thereby promote ferroptosis. For example, the expression of CDO sensitizes cancer cells to ferroptosis (175–177), whereas blocking protein synthesis by targeting mTOR complex 1 (mTORC1) suppresses cystine deprivation-induced ferroptosis (178).

3.2.2. Methionine.

As a precursor of homocysteine, methionine should theoretically protect against ferroptosis by boosting intracellular cysteine level via the transsulfuration pathway. Unexpectedly, two independent groups recently reported opposing results that methionine deprivation inhibits ferroptosis induced by cystine deprivation, which are discussed here (179, 180).

The conversion of methionine to homocysteine involves three steps: first, methionine and adenosine triphosphate (ATP) are condensed by methionine adenosyl transferase (MAT) to generate *S*-adenosyl methionine (SAM); SAM is a universal methyl group donor, and its methyl group can be removed by a variety of methyltransferases (MTs) including glycine *N*-methyltransferase (GNMT) to yield *S*-adenosyl-homocysteine (SAH); SAH is then hydrolyzed by *S*-adenosyl-L-homocysteine hydrolase (SAHH, aka adenosyl homocysteinase, AHCY) to produce homocysteine. Note that homocysteine can be converted back to methionine by either methionine synthase (MS) or betaine-homocysteine *S*-methyltransferase (BHMT), completing the methionine cycle (149) (**FIGURE 4**). The conversion of SAM to SAH is rate limiting for homocysteine synthesis, given that ectopic expression of GNMT, but not MAT or SAHH, restores cell proliferation upon cystine deprivation (156). On the other hand, ablation of GNMT and inactivation of SAHH both sensitize cancer cells to ferroptosis (156, 174, 181). Taken together, these studies suggest that methionine replenishes the intracellular cysteine pool and thereby impedes ferroptosis.

For certain cancer cells, methionine is obligatory in the culture media and cannot be replaced by homocysteine (182–184). Forced substitution leads to cell cycle arrest and eventually apoptosis in methionine-dependent cells (185–187). Interestingly, two recent studies have shown that a number of cancer cells tolerate combined deprivation of methionine and cysteine better than cysteine deprivation alone (179, 180). In particular, both studies suggest that cysteine deprivation alone induces ferroptosis, whereas double deprivation only delays cell proliferation without causing robust cell death induction. Furthermore, both studies agree that SAM and not SAH is the key metabolite that determines cell fate. Nevertheless, Homma et al. (179) proposed that SAM promotes cell cycle progression by providing the methyl group for DNA methylation and that cell cycle progression is required for ferroptosis to occur. By contrast, Zhang et al. (180) suggested that SAM activates

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

the polyamine pathway, which generates hydrogen peroxide (H_2O_2) that sensitizes cells to ferroptosis (**FIGURE 4**). In support of the latter viewpoint, amine oxidase copper-containing 1 (AOC1) and spermidine/spermine N^1-acetyltransferase 1 (SAT1), which mediate polyamine catabolism and thus ROS generation, have been linked to ferroptosis vulnerability (188, 189). Furthermore, depletion of arginine or ornithine decarboxylase 1 (ODC1), which are involved in polyamine synthesis, renders cells resistant to ferroptosis (178, 190). Recently, a third mechanism was proposed to account for the proferroptotic effect of SAM upon cysteine deprivation, i.e., SAM contributes to methylation-dependent coenzyme Q (CoQ) synthesis, thus sustaining the operation of electron transport chain (ETC) and ROS generation (as discussed in sect. 3.3.3) (191). In summary, it appears that methionine can either promote or prevent ferroptosis, with the consequences crucially dependent on the metabolic pathway of SAM (**FIGURE 4**).

3.2.3. Glutamine/glutamate.

Glutamine/glutamate may affect ferroptosis sensitivity in several ways. First, excessive extracellular glutamate may directly inhibit the function of system x_c^-, thereby triggering ferroptosis (4). Second, intracellular glutamate derived from extracellular glutamine is required for the exchange of extracellular cystine by system x_c^-. Since both cysteine and glutamate are building blocks of GSH (**FIGURE 5**), glutamine uptake facilitates GSH biosynthesis and ferroptosis prevention (192, 193). Third, glutamine may drive ferroptosis

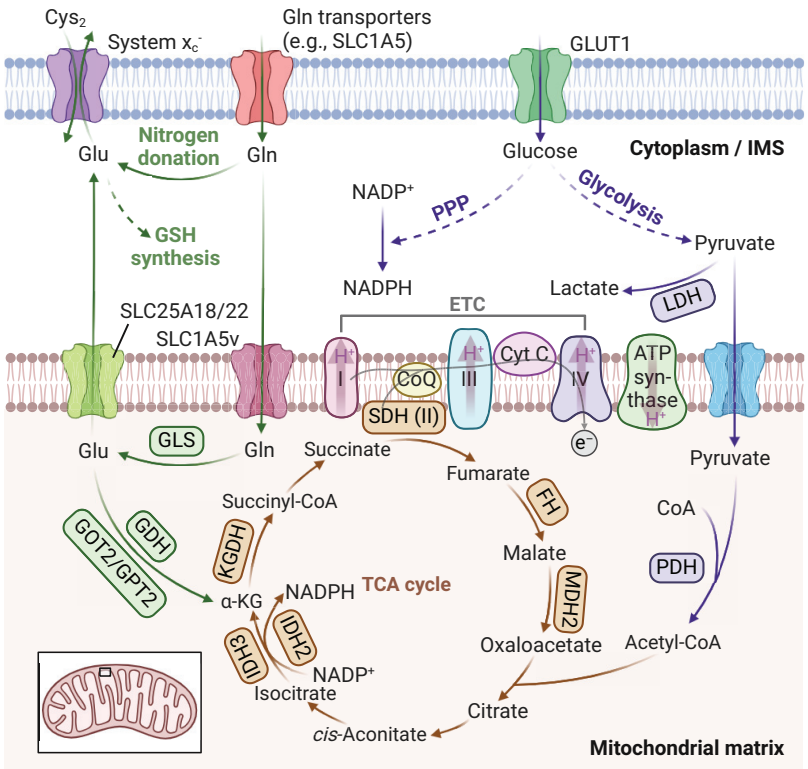


FIGURE 5. Glutamine/glutamate and carbohydrate metabolism. A series of transporters, including SLC1A5, can import glutamine (Gln). Intracellular Gln serves as a primary nitrogen donor for various essential biosynthetic reactions and is converted to glutamate (Glu) upon nitrogen donation. Gln can also enter the mitochondrial matrix via the SLC1A5 variant (SLC1A5v), where it is converted to Glu by glutaminase (GLS). Mitochondrial Glu can be exported to the cytosol by the Glu carriers SLC25A18 and SLC25A22 or be converted to α-ketoglutarate (α-KG) by glutamate dehydrogenase (GDH), glutamic-oxaloacetic transaminase 2 (GOT2), or glutamic-pyruvic transaminase 2 (GPT2), thereby fueling the tricarboxylic acid (TCA) cycle. Cytosolic Glu can be used as a building block to synthesize glutathione (GSH) or exchanged for extracellular Cys_2 via system x_c^-. The cellular uptake of glucose is mediated by glucose transporters such as glucose transporter 1 (GLUT1). Intracellular glucose can be utilized for nicotinamide adenine dinucleotide phosphate (NADPH) production via the pentose phosphate pathway (PPP) or for pyruvate biogenesis via glycolysis. Depending on the context, pyruvate can either be converted to lactate by lactate dehydrogenase (LDH) or be imported into the mitochondrial matrix, where it is oxidized by the pyruvate dehydrogenase (PDH) complex to promote the biogenesis of acetyl-CoA, which then fuels the TCA cycle. The TCA cycle involves a series of enzymes such as α-KG dehydrogenase (KGDH), succinate dehydrogenase (SDH, aka complex II), fumarate hydratase (FH), malate dehydrogenase 2 (MDH2), and mitochondrial $NADP^+/NAD^+$-dependent isocitrate dehydrogenase (IDH2/IDH3), with IDH2 being the only NADPH-producing enzyme in the cycle. Activation of the TCA cycle drives the electron transport chain (ETC), which involves coenzyme Q (CoQ), cytochrome *c* (Cyt C), and complexes I to IV. The ETC creates an electrochemical gradient across the inner mitochondrial membrane by pumping protons into the mitochondrial intermembrane space (IMS), driving the generation of adenosine triphosphate (ATP) by ATP synthase. Figure created with a licensed version of BioRender.com.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

upon cystine depletion because glutamate cannot be used for cystine exchange or GSH synthesis in the absence of cystine. As a result, intracellular glutamate accumulates and glutamine fuels the tricarboxylic acid (TCA) cycle via glutaminolysis, followed by the overactivation of electron transport chain (ETC) driving ferroptosis (as discussed in sect. 3.3.3) (33, 194, 195).

A series of transporters are capable of importing glutamine, including SLC1A5 (aka ASCT2), SLC38A1, and SLC38A5 (196) (**FIGURE 5**). Whereas the expression of these transporters is essential for ferroptosis defense across a range of cancer cell lines (197–199), blocking these transporters protects cells from cystine deprivation-induced ferroptosis (33, 195). Intracellular glutamine can serve as a nitrogen donor for the biosynthesis of nucleotides, nicotinamide adenine dinucleotide (NAD), asparagine, and hexosamines, concurrently generating glutamate as a by-product (**FIGURE 5**). Furthermore, glutamine can be imported into the mitochondrial matrix by the transporter SLC1A5 variant (SLC1A5v) (200), where glutamine is converted to glutamate by glutaminase (GLS) (**FIGURE 5**). Expression of GLS2, but not GLS1, contributes to susceptibility to ferroptosis (33, 201). This might be related to their different subcellular localization, which remains ambiguous so far (202).

Mitochondrial glutamate can be exported to the cytosol by the glutamate carriers SLC25A18 and SLC25A22 (203), where it is used for the biosynthesis of GSH and for the exchange of extracellular cystine (**FIGURE 5**). Blockade of SLC25A22 is associated with reduced GSH levels and vulnerability to ferroptosis in pancreatic ductal adenocarcinoma (PDAC) cells (204). Mitochondrial glutamate can also be converted to α-ketoglutarate (α-KG) by glutamate dehydrogenase (GDH/GLUD), glutamic-oxaloacetic transaminase 2 (GOT2), or glutamic-pyruvic transaminase 2 (GPT2), thereby fueling the TCA cycle (**FIGURE 5**). Knockdown of *GDH* and inhibition of the transaminases protect against cystine deprivation-induced ferroptosis in certain contexts (33, 195), whereas supplementation with α-KG or its downstream metabolites, including succinate, fumarate, and malate, restores cell death upon combined glutamine and cysteine deprivation (33, 194). Therefore, intracellular glutamine/glutamate plays a dual role in ferroptosis: under normal conditions it is required for the prevention of ferroptosis, whereas in cystine deficiency it exacerbates ferroptosis via the TCA cycle.

3.2.4. Tryptophan.

Tryptophan is an essential amino acid that mammals obtain exclusively from food and gut microbes. Recently, several tryptophan metabolites have been found to inhibit ferroptosis by acting as RTAs, including indole-3-pyruvate (I3P), serotonin (5-HT), and 3-hydroxyanthranilic acid (3-HA) (205–208). Interestingly, these metabolites are distributed across the three major pathways of tryptophan metabolism. I3P is generated extracellularly from the indole pathway, in which tryptophan is degraded by the secreted L-amino acid oxidase IL4i1 (205). It is hypothesized that an unknown I3P transporter is present that is required for I3P import and exertion of the antiferroptotic effect (209). 5-HT is synthesized via the serotonin pathway mainly in the central nervous system (CNS) by serotonergic neurons and in the gastrointestinal tract by enterochromaffin cells. The antiferroptotic effect of 5-HT depends on the expression of the serotonin transporter SLC6A4 (207), whereas it is abrogated by the expression of monoamine oxidase A, which degrades 5-HT (206). 3-HA is derived from the kynurenine pathway, in which intracellular tryptophan is first converted to kynurenine, followed by the generation of 3-hydroxy-kynurenine (3-HK) and then 3-HA. Indoleamine 2,3-dioxygenase 1 (IDO1), which catalyzes the rate-limiting step in the kynurenine pathway, and kynureninase (KYNU, aka L-kynurenine hydrolase), which mediates the conversion of 3-HK to 3-HA, protect cancer cells from ferroptosis, whereas 3-HA 3,4-dioxygenase (HAAO), which consumes 3-HA, has an opposite effect (206, 208). In addition to directly scavenging free radicals, many of the tryptophan metabolites are able to activate the nuclear factor E2-related factor 2 (NRF2) pathway, which can counteract ferroptosis by promoting GSH biosynthesis (205, 208). Furthermore, in a recent study, *trans*-3-indoleacrylic acid, a tryptophan metabolite derived from the gut microbe *Peptostreptococcus anaerobius*, was found to protect colorectal cancer from ferroptosis in a ferroptosis suppressor protein 1 (FSP1)-dependent manner by promoting the reduction of NAD^+ to NADH (210).

3.3. Carbohydrate Metabolism

Glucose is the driving force of the TCA cycle, in parallel with glutamate/glutamine. Therefore, it is not surprising that glucose starvation protects MEFs and PDAC cells from cystine deprivation-induced ferroptosis (211–213). On the other hand, glucose impels the regeneration of nicotinamide adenine dinucleotide phosphate (NADPH) via the pentose phosphate pathway (PPP). Although conflicting results have been reported on the effects of PPP on ferroptosis, cells with a metabolic profile that favors PPP over glycolysis, as induced by the P47S variant of tumor protein 53 (p53), are more resistant to ferroptosis (214).

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

3.3.1. Glycolysis.

The cellular uptake of glucose is mediated by glucose transporters such as glucose transporter 1 (GLUT1, aka SLC2A1). Part of the glucose is then used for glycolysis, forming pyruvate (**FIGURE 5**). Impeding pyruvate generation by ablation of GLUT1 or pyruvate kinase M2 (PKM2), a key enzyme in glycolysis, renders PDAC resistant to cysteine depletion-induced ferroptosis (212, 215, 216), whereas supplementation with glycolytic metabolites, including high concentrations of pyruvate, sensitizes cancer cells to ferroptosis (217, 218). Cancer cells preferably convert pyruvate to lactate (**FIGURE 5**), also known as the Warburg effect. Interestingly, lactate has an antiferroptotic effect, as evidenced by the fact that disruption of lactate dehydrogenase (LDH) in HT1080 cells and inhibition of lactate import in HCCs by targeting monocarboxylate transporter 1 (MCT1) both sensitize cells to ferroptosis. Mechanistically, it may be related to the acidic environment induced by lactate and the deactivation of AMP-activated protein kinase (AMPK) (218, 219). Under aerobic conditions, pyruvate is imported into the mitochondria, where it is oxidized by the pyruvate dehydrogenase (PDH) complex. As a consequence, its acetyl group is transferred to CoA, yielding acetyl-CoA, which fuels the TCA cycle (**FIGURE 5**). Inhibition of the E1 subunit of PDH complex by RNA interference or by overexpressing pyruvate dehydrogenase kinase 4 (PDK4) protects cells from cystine deprivation (212, 217). However, inhibition of the E3 subunit (aka dihydrolipoyl dehydrogenase, DLD) may lead to different outcomes depending on the context (217, 220). For example, one study argues that autoxidation of dihydrolipoamide in the absence of DLD generates more superoxide anion radical ($O_2^{\bullet -}$), thereby sensitizing cells to ferroptosis (217). The reason for this contradiction remains unclear, but it should be noted that DLD is the E3 subunit shared by α-KG dehydrogenase (KGDH) and branched-chain ketoacid dehydrogenase complexes.

3.3.2. TCA.

Activation of the TCA cycle is associated with susceptibility to cystine deprivation-induced ferroptosis. Enzymes of the TCA cycle, including KGDH (220), succinate dehydrogenase (SDH) (221, 222), fumarate hydratase (FH, aka fumarase) (194), and malate dehydrogenase 2 (MDH2) (223), contribute to ferroptosis in different cellular contexts (**FIGURE 5**). Supplementation with TCA intermediates such as citrate or α-KG counteracts the protective effect of glucose deprivation on ferroptosis in PDAC (212). Interestingly, mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDH2) protects against ferroptosis despite being a TCA cycle enzyme (224–226), perhaps due to NADPH production (**FIGURE 5**). In line with this, mutant IDH2, which consumes NADPH for the conversion of α-KG to 2-hydroxyglutarate (2-HG), sensitizes acute myeloid leukemia (AML) to ferroptosis (227). In contrast, IDH3, which catalyzes the same reaction as IDH2 in the mitochondria but in an NAD-dependent fashion, has not yet been linked to ferroptosis regulation. In parallel to IDH2, cytosolic $NADP^+$-dependent isocitrate dehydrogenase (IDH1) confers resistance to ferroptosis (212), whereas mutant IDH1 exerts an opposite effect (228, 229).

3.3.3. ETC.

Activation of the TCA cycle can drive the ETC (**FIGURE 5**). Mitochondrial membrane potential hyperpolarization is observed during cystine deprivation-induced ferroptosis, suggesting ETC overactivation, and, accordingly, cell death can be rescued by inhibitors of mitochondrial complexes and the mitochondrial uncoupler CCCP (194). However, the contribution of mitochondrial complexes may vary depending on the context. In one study, activation of complex I instead of complex III was proposed to drive cystine deprivation-induced ferroptosis in human fibroblasts (230), whereas in another study using a mouse hepatoma cell line a completely opposite result was reported (231). Activation of complex IV contributes to cystine deprivation-induced ferroptosis in non-small cell lung carcinoma (NSCLC) cells (232), whereas the activation of SDH (complex II) promotes ischemia-reperfusion-induced neuronal ferroptosis (222). Paradoxically, in certain contexts inhibition of complex I can directly lead to ferroptosis (233, 234), which may be partly due to suppression of $CoQH_2$ production (235, 236). Furthermore, depletion of mitochondrial DNA (leading to a lack of respiratory chain catalytic subunits, namely Rho0 or ρ^0) does not significantly affect or even sensitize cells to ferroptosis induced by GPX4 inhibition (4, 237, 238). As such, it can be concluded that the TCA cycle and the ETC promote cystine deprivation-induced ferroptosis, but their roles in ferroptosis driven by other impacts (e.g., GPX4 inhibition) seem to be context dependent.

3.3.4. PPP.

In parallel to glycolysis, intracellular glucose can be metabolized in the PPP shunt, whereby cytosolic $NADP^+$ is reduced to NADPH (**FIGURE 5**). However, how PPP affects ferroptosis appears to be context dependent, as one study found that silencing of two PPP enzymes, namely glucose-6-phosphate dehydrogenase (G6PD) and phosphoglycerate dehydrogenase (PGD), prevented erastin-induced ferroptosis (4), whereas another study claimed the opposite (239). This may be attributed to the

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

dual role of NADPH in ferroptosis regulation. NADPH is a substrate of NADPH oxidases (NOXs) and cytochrome P450 oxidoreductase (POR), which can generate harmful ROS; at the same time, NADPH is also a universal electron donor that provides reducing power for the regeneration of cellular antioxidants involved in ferroptosis defense, including cysteine, GSH, vitamin E, vitamin K, CoQ, and tetrahydrobiopterin (BH_4) (240). Furthermore, NADPH can directly activate the E3 ubiquitin ligase MARCH6 (aka MARCHF6), which inhibits ferroptosis by mediating the degradation of proferroptotic players such as acyl-CoA synthetase long-chain family member 4 (ACSL4) and p53 (241). Overall, it is believed that cells with a high cellular NADPH level are more resistant to ferroptosis (242).

3.4. The Mevalonate Pathway

The mevalonate pathway (also known as the isoprenoid pathway) is notorious for acting upstream of the de novo synthesis of cholesterol. It starts with acetyl-CoA, which is mainly derived from mitochondrial pyruvate oxidation and subsequently exported to the cytosol via the citrate-malate shuttle (**FIGURE 6**). The rate-limiting enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (HMGCR) is the target of statins, which are widely used in the clinic for the management of hypercholesteremia. Intriguingly, statins sensitize cancer cells to ferroptosis (238, 243). Indeed, several intermediates during the biosynthesis of cholesterol such as dimethylallyl pyrophosphate (DMAPP), farnesyl pyrophosphate

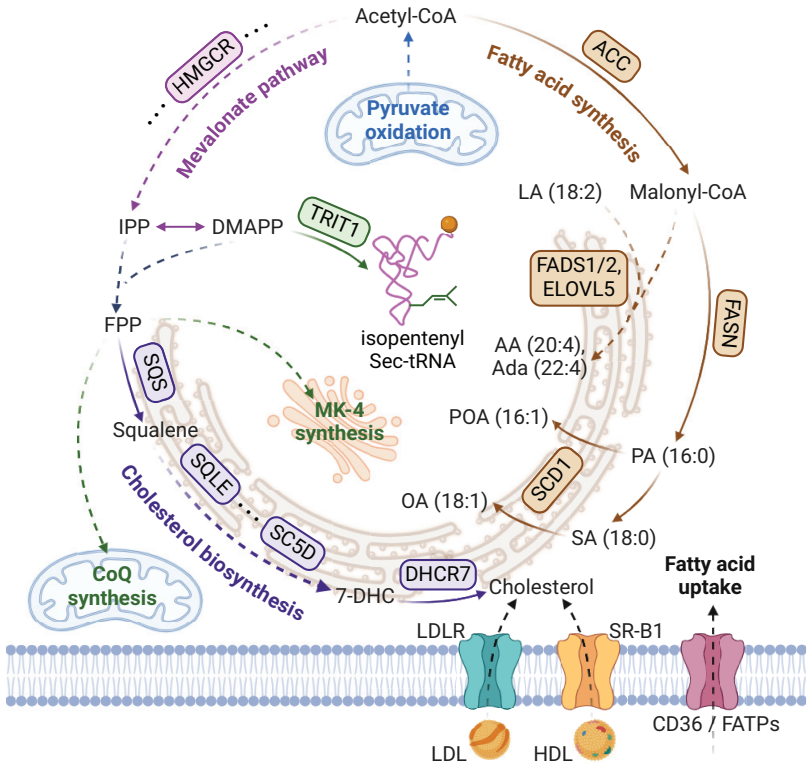


FIGURE 6. The mevalonate pathway and the biosynthesis of fatty acid. The mevalonate pathway begins with acetyl-CoA, mainly derived from mitochondrial pyruvate oxidation and subsequently exported to the cytosol via the citrate-malate shuttle. The rate-limiting enzyme of the mevalonate pathway is 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and the end products are isopentenyl pyrophosphate (IPP) and the isomer dimethylallyl pyrophosphate (DMAPP). DMAPP serves as a substrate for tRNA isopentenyltransferase 1 (TRIT1), which modifies a subset of tRNAs including Sec-$tRNA^{[Ser]Sec}$. IPP and DMAPP are precursors of farnesyl pyrophosphate (FPP), which can be used for synthesizing squalene, coenzyme Q (CoQ), and menaquinone-4 (MK-4) in the endoplasmic reticulum (ER), mitochondria, and Golgi apparatus, respectively. Squalene and 7-dehydrocholesterol (7-DHC) are intermediates in cholesterol biosynthesis. Squalene synthase (SQS) and sterol-C5-desaturase (SC5D) mediate their synthesis, whereas squalene epoxidase (SQLE) and 7-DHC reductase (DHCR7) mediate their metabolism. Notably, DHCR7-mediated metabolism of 7-DHC is the final step in cholesterol biosynthesis. Cholesterol can also be imported in the form of low-density lipoprotein (LDL) or high-density lipoprotein (HDL) via their respective receptors, LDLR and scavenger receptor B1 (SR-B1). Fatty acid biosynthesis starts with cytosolic acetyl-CoA, which is converted to malonyl-CoA by acetyl-CoA carboxylase (ACC). Malonyl-CoA can be utilized by fatty acid synthase (FASN) to produce the long-chain saturated fatty acid palmitic acid (PA, 16:0). PA can be further elongated to stearic acid (SA, 18:0) by elongases, and PA and SA can be desaturated to palmitoleic acid (POA, 16:1, ω-7) and oleic acid (OA, 18:1, ω-9) by stearoyl-CoA desaturase 1 (SCD1), respectively. Malonyl-CoA is also critical for generating long-chain polyunsaturated fatty acids, such as arachidonic acid (AA, 20:4, ω-6) and adrenic acid (AdA, 22:4, ω-6), derived from dietary linoleic acid (LA, 18:2, ω-6) through elongation and desaturation involving fatty acid desaturase 1 (FADS1), FADS2, and very long-chain fatty acid elongase 5 (ELOVL5). Besides de novo synthesis, fatty acids can be imported by membrane-associated proteins such as CD36 and fatty acid transport proteins (FATPs). Figure created with a licensed version of BioRender.com.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

(FPP), squalene, and 7-dehydrocholesterol (7-DHC) all contribute either directly or indirectly to ferroptosis defense.

3.4.1. Sec-tRNA$^{[Ser]Sec}$ modification.

The end products of the mevalonate pathway are isopentenyl pyrophosphate (IPP) and its isomer DMAPP. DMAPP is a substrate of tRNA isopentenyltransferase 1 (TRIT1), which modifies a subset of tRNAs, including Sec-tRNA$^{[Ser]Sec}$, by adding a dimethylallyl group onto the adenine at position 37 (**FIGURE 6**). Remarkably, this modification is required for the efficient translational decoding of the Sec codon UGA and synthesis of selenoproteins (244). Accordingly, inhibition of the mevalonate pathway with statins attenuates the expression of GPX4 (243).

3.4.2. Squalene.

One molecule of DMAPP and two molecules of IPP can be sequentially condensed to obtain farnesyl pyrophosphate (FPP), which is the common precursor of squalene, CoQ, and menaquinone-4 (MK-4, a type of vitamin K_2) (**FIGURE 6**). The synthesis of squalene from FPP is mediated by squalene synthase (SQS, encoded by *Fdft1* gene). Squalene is an essential metabolite that prevents ferroptosis in a subset of ALK$^+$ anaplastic large-cell lymphoma cell lines that lack squalene epoxidase (SQLE, aka squalene monooxygenase), the enzyme acting downstream of squalene (**FIGURE 6**); as such, these cells are enriched with squalene, while being auxotrophic for cholesterol (245). The mechanism underlying the antiferroptotic effect of squalene remains unclear but appears to depend on its accumulation. Ablation of SQLE renders HT1080 cells resistant, whereas ablation of SQS has minimal effect on ferroptosis sensitivity (246). Furthermore, supplementation with exogenous squalene fails to prevent ferroptosis (245, 247). In certain contexts, such as FIN56-induced ferroptosis, inhibition of SQS even exerts a protective effect, probably by increasing CoQ levels (238).

3.4.3. CoQ.

CoQ consists of the functional quinone group and a polyisoprenyl tail derived from FPP (**FIGURE 6**). Depending on the number of the isoprene units in the tail, CoQ includes CoQ_6, CoQ_7, CoQ_8, CoQ_9 (the dominant form in mice), and CoQ_{10} (the prevailing form in humans). CoQ is generally appreciated as an electron carrier in the ETC in the inner mitochondrial membrane (**FIGURE 5**). In addition, CoQ can act as an RTA recycled by FSP1 to prevent lipid peroxidation at the plasma membrane (248, 249). As such, variations in the FPP level may influence CoQ biosynthesis and thus ferroptosis sensitivity. For instance, FIN56 induces ferroptosis in part due to the activation of SQS, which promotes the conversion of FPP to squalene, thereby depleting CoQ (238). On the contrary, excessive cholesterol induces SQLE degradation, leading to ferroptosis resistance not only by accumulating squalene but also by promoting CoQ biosynthesis (246).

3.4.4. Vitamin K.

Vitamin K plays a central role in coagulation but has recently also been identified as a cofactor of FSP1 to prevent ferroptosis, acting in the same way as CoQ (250, 251). According to the structure of the side chain, vitamin K can be divided into vitamin K_1 (phylloquinone) and K_2 (menaquinone-n, MK-n). MK-4, the only form that can be synthesized by animals, is composed of a naphthoquinone head group (menadione) and a geranylgeranyl side chain derived from FPP (**FIGURE 6**). Remarkably, menadione itself can be an alternative substrate of FSP1 to prevent ferroptosis, although it is less efficient and more cytotoxic than MK-4 (250, 251).

3.4.5. 7-DHC.

The final step of cholesterol biosynthesis is mediated by 7-DHC reductase (DHCR7), which converts 7-DHC to cholesterol (**FIGURE 6**). Most recently, 7-DHC has been identified as a potent cell-intrinsic RTA suppressing ferroptosis. Ablation of DHCR7 renders cells resistant to ferroptosis, whereas ablation of sterol-C5-desaturase (SC5D, aka lathosterol oxidase), which catalyzes the synthesis of 7-DHC, has the opposite effect (**FIGURE 6**) (247, 252, 253). Remarkably, 7-DHC appears to be essential for the survival of certain cancer cell lines, suggesting that enzymes acting upstream of 7-DHC are potential therapeutic targets (252). Conversely, targeting DHCR7 may be beneficial for acetaminophen- or ischemia-reperfusion-induced liver injury (253).

3.4.6. Cholesterol.

Whether cholesterol plays a major role in ferroptosis is under debate. Although cholesterol is in principle susceptible to peroxidation and treatment with cholesterol hydroperoxides indeed contributes to ferroptosis (98, 243, 254), it is known that supplementation with cholesterol does not significantly affect ferroptosis sensitivity (252, 255) or even protect against ferroptosis (246). Consistent with the latter, inhibition of cholesterol uptake by targeting the receptors for high-density lipoprotein (i.e., scavenger receptor B1, SR-B1) or low-density lipoprotein (i.e., LDLR) (256, 257) or

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

leukocyte immunoglobulin-like receptor B1 (LILRB1) that facilitates LDLR functioning (258) sensitizes certain cancer cell lines to ferroptosis (**FIGURE 6**). Mechanistically, cholesterol may promote the degradation of SQLE by activating the E3 ubiquitin ligase MARCH6 (258–261). As a consequence, the levels of squalene and CoQ are elevated (246). Cholesterol can also increase the stiffness of the plasma membrane and thereby alleviate the propagation of lipid peroxidation (256). In addition, cholesterol uptake has been found to be correlated with GPX4 expression in cholesterol auxotrophic cells (257), probably because the limited amount of DMAPP cannot support the cholesterol biosynthesis and Sec-tRNA$^{[Ser]Sec}$ modification simultaneously.

3.5. Fatty Acid Metabolism

Fatty acid metabolism essentially affects ferroptosis sensitivity through the control of substrates of lipid peroxidation. Among a variety of lipid species, polyunsaturated fatty acids (PUFAs) are particularly prone to undergo peroxidation because of the presence of bisallylic protons, which are highly susceptible to hydrogen atom abstraction, whereas monosaturated fatty acids (MUFAs) can counteract ferroptosis (255). The accumulation of saturated fatty acids (SFAs) usually leads to "nonferroptotic" lipotoxicity (262), but in certain contexts SFAs may contribute to ferroptosis by promoting the biosynthesis of ether phospholipids (ePLs) (263). To drive the overwhelming lipid peroxidation that culminates in ferroptosis, a radical chain reaction must be incited among the PUFAs esterified in PLs (264). As such, the processes of uptake, biosynthesis, activation, catabolism, storage, and membrane incorporation of fatty acids may all have an impact on ferroptosis sensitivity.

3.5.1. Fatty acid uptake.

Mammalian cells acquire fatty acid via either uptake or de novo synthesis. Membrane-associated proteins such as CD36 (aka scavenger receptor B2, SR-B2) and fatty acid transport proteins (FATPs, encoded by solute carrier family 27, *Slc27*) are critical for the uptake process (**FIGURE 6**). Interestingly, expression of CD36 and FATP2 increases ferroptosis sensitivity in CD8$^+$ tumor infiltrating lymphocytes and polymorphonuclear myeloid-derived suppressor cells, respectively (265–268), whereas other FATP members are proposed to prevent ferroptosis in melanoma cells (269). This discrepancy might be related to the preference of these transporters for different forms of fatty acids. For example, CD36 preferentially imports SFAs, PUFAs, and oxidized lipids (265, 266, 270), whereas FATP4 selectively imports MUFAs (271). Remarkably, CD36 can be palmitoylated upon SFA overdose, changing its preference for MUFA over SFA (272). This may explain how cancer cells with high CD36 expression evade lipotoxicity and ferroptosis. Moreover, the abundance of different fatty acids in the extracellular environment can also be critical, as melanoma cells that metastasize via the lymph are more resistant to GPX4 perturbations because of the higher abundance of MUFAs in the lymph than in the blood (269). In cell culture conditions, limitation of extracellular lipid sensitizes cancer cells to ferroptosis, as lipid starvation induces lipolysis that promotes PUFA trafficking from lipid droplets to the plasma membrane (as discussed in sect. 3.5.5) (273).

3.5.2. Fatty acid biosynthesis.

Mammals are able to synthesize SFAs and certain MUFAs like palmitoleic acid (POA, 16:1, ω-7) and oleic acid (OA, 18:1, ω-9) de novo but not ω-3 or ω-6 MUFAs or PUFAs. As in the mevalonate pathway, de novo synthesis of SFAs begins with cytosolic acetyl-CoA (**FIGURE 6**). Acetyl-CoA carboxylase (ACC) mediates the conversion of acetyl-CoA to malonyl-CoA, which is then utilized by fatty acid synthase (FASN) to generate the long-chain SFA palmitic acid (PA, 16:0). PA can be further elongated to stearic acid (SA, 18:0) by elongases, and PA and SA can be desaturated to POA and OA by stearoyl-CoA desaturase 1 (SCD1), respectively (**FIGURE 6**). Remarkably, malonyl-CoA is also a critical element for the generation of long-chain PUFAs such as arachidonic acid (AA, 20:4, ω-6) and adrenic acid (AdA, 22:4, ω-6), which can be derived from dietary linoleic acid (LA, 18:2, ω-6) through elongation and desaturation wherein fatty acid desaturase 1 (FADS1), FADS2, and very long-chain fatty acid elongase 5 (ELOVL5) are involved (**FIGURE 6**).

As illustrated in **FIGURE 6**, ACC-mediated malonyl-CoA synthesis is a building block of SFAs, MUFAs, and long-chain PUFAs; inhibition of ACC or glycolysis providing acetyl-CoA can lead to a composite outcome of ferroptosis resistance (211–213). Distinct from ACC, FASN is only involved in the de novo synthesis of SFA and MUFA (**FIGURE 6**). Ablation of FASN may therefore elevate the proportion of PUFA in PLs and sensitize cells to ferroptosis (274, 275). However, in certain contexts inhibition of FASN may lead to ferroptosis resistance (212, 213), perhaps because of the decline in NADPH consumption, which occurs in each fatty acid elongation cycle (276). AA and AdA, which possess double bisallylic groups, are preferable substrates for lipid peroxidation compared to LA. Accordingly, the expressions of FADS1, FADS2, and ELOVL5 are positively correlated with

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

ferroptosis sensitivity (277–279). Conversely, MS4A15, which depletes Ca^{2+} from the endoplasmic reticulum (ER) and thereby blocks PUFA elongation and desaturation, drives ferroptosis resistance (280).

SCD1 is the dominant enzyme mediating the conversion from SFAs to MUFAs (**FIGURE 6**). Cancer cells with SCD1 overexpression are resistant to ferroptosis (281–283). Notably, some cancer cells are capable of synthesizing MUFAs like sapienate (16:1, ω-10) and *cis*-8-octadecenoate (18:1, ω-10) via a FADS2-dependent pathway; however, their roles in ferroptosis remain to be clarified (284). ACC, FASN, and SCD1 are all under the control of sterol regulatory element-binding protein 1 (SREBP1), a master regulator of lipogenesis. However, SREBP1 seems to have a stronger influence on SCD1 (285). Interestingly, the SREBP1-SCD1 axis is positively regulated by the oncogenic phosphatidylinositol 3-kinase (PI3K)-AKT-mTOR complex 1 (mTORC1) and FLT3-C/EBPα pathways (285–287) and negatively regulated by the tumor suppressors p53 and AMPK (219, 288, 289), suggesting the SREBP1-SCD1 axis as a potential therapeutic target to sensitize cancer cells to ferroptosis.

3.5.3. Fatty acid activation.

Free fatty acid must first be activated by ligation to CoA to participate in further metabolic processes such as β-oxidation as well as triglyceride (TAG) and PL biosynthesis. Long-chain fatty acyl-CoA ligase (aka acyl-CoA synthetase long-chain or ACSL) is a group of enzymes that catalyze this process. Of the ACSL family, ACSL4 and ACSL3 are most strongly associated with ferroptosis, as they preferentially activate long-chain PUFAs and MUFAs, respectively (**FIGURE 7**). Ablation of ACSL4 confers robust protection against ferroptosis in a number of cell lines (290, 291), whereas ablation of ACSL3 has the opposite effect (262, 269). Furthermore, ACSL1 can also contribute to ferroptosis by activating α-eleostearic acid (18:3) (292), but this seems to be case specific, perhaps because of the scarcity of the substrate.

3.5.4. Fatty acid catabolism.

β-Oxidation is the catabolic process by which fatty acids are broken down in the mitochondrial matrix, though for certain fatty acid species such as very long-chain ($C \geq 22$) fatty acids peroxisomes are also involved in the initial oxidation steps (**FIGURE 7**). β-Oxidation generally suppresses ferroptosis presumably by degrading fatty acids, despite the fact that it generates acetyl-CoA that fuels the TCA cycle. To reach the mitochondrial matrix, long-chain fatty acids must pass through the mitochondrial membranes with the help of the carnitine shuttle system. Interfering with this system by inhibition of carnitine palmitoyltransferase 1A (CPT1A) triggers ferroptosis, which synergizes with immunotherapy in lung cancer stem cells (293), whereas restoration of von Hippel–Lindau (VHL) that positively regulates CPT1A expression protects clear cell renal cell carcinoma (ccRCC) from ferroptosis (294). Consistently, the high level of CPT1A in cytotoxic T lymphocyte subset 9 cells accounts for their resistance to ferroptosis and their long-acting antitumor effects (295). Furthermore, cardiomyocytes with a defect in the carnitine transporter OCTN2 (encoded by *SLC22A5*) exhibit features of ferroptosis, underlying the pathogenesis of an autosomal recessive disorder called primary carnitine deficiency (296). Whereas β-oxidation is usually applicable to all long-chain species including SFA, MUFA, and PUFA, mitochondrial 2,4-dienoyl-CoA reductase (DECR1) is an auxiliary enzyme specific for PUFA catabolism. This enzyme is highly expressed by castration-resistant prostate cancer to counteract ferroptosis (297, 298). Interestingly, under certain conditions β-oxidation selectively decreases MUFA and increases PUFA, thus sensitizing cells to ferroptosis (299), though the underlying mechanism remains unclear.

3.5.5. Fatty acid storage.

Excessive fatty acids are stored in lipid droplets in the form of TAGs (**FIGURE 7**). This process is generally believed to be antiferroptotic because it helps to sequester PUFAs away from the plasma membrane (300, 301). In line with this, glioblastomas lacking CDKN2A, which selectively promotes the storage of PUFAs in TAG, are prone to ferroptosis regardless of unchanged total TAG levels compared to wild-type cells (302). The biosynthesis of TAG requires several steps, of which the transfer of an acyl group from acyl-CoA to diacylglycerol (DAG) mediated by diacylglycerol acyltransferase (DGAT) is the last and only committed step (**FIGURE 7**). Inhibition of DGAT sensitizes cancer cells to ferroptosis (303), whereas activation of DGAT by cell cycle arrest renders cells resistant (304). Recently, an alternative TAG biosynthesis pathway mediated by DGAT1/2-independent enzyme synthesizing storage lipids (DIESL, aka TMEM68) was uncovered. In contrast to DGAT, DIESL transfers fatty acyl chains derived from membrane PLs or their precursors to DAG (305). Whether TMEM68 plays a role in ferroptosis remains to be elucidated. Nevertheless, tumor protein D52 (TPD52), fatty acid binding protein-4 (FABP4), and phospholipid transfer protein (PLTP) have been reported to protect cancer cells from ferroptosis by promoting lipid droplet formation (282, 306, 307).

The breakdown of lipid droplets is mediated by lipolysis and lipophagy (**FIGURE 7**). These processes promote the release of free fatty acids and can therefore increase

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

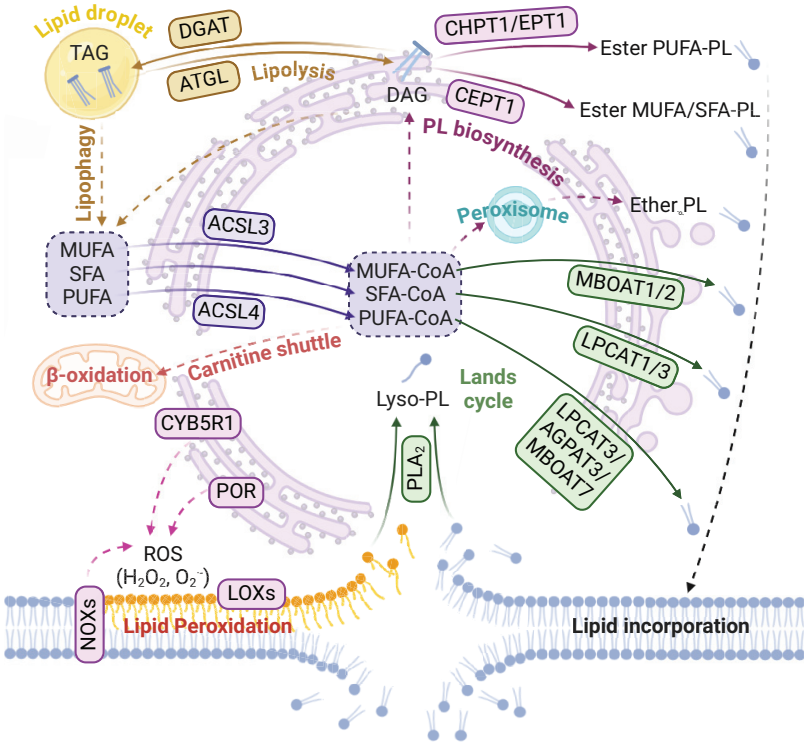


FIGURE 7. Fatty acid metabolism and lipid peroxidation. Free fatty acids, including saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), must first be ligated to CoA by long-chain fatty acyl-CoA ligase (ACSL) to participate in further metabolic processes. Among the ACSL family, ACSL3 and ACSL4 preferentially activate long-chain MUFAs and PUFAs, respectively. Fatty acids are potential energy sources as they can cross mitochondrial membranes with the help of the carnitine shuttle system and undergo β-oxidation in the mitochondrial matrix. Excess fatty acids can be stored in lipid droplets in the form of triglycerides (TAGs). Diacylglycerol acyltransferase (DGAT)-mediated transfer of an acyl group from acyl-CoA to diacylglycerol (DAG) is the last and only committed step in TAG biosynthesis. Lipid droplets are broken down via lipolysis and lipophagy. The first and rate-limiting step of lipolysis is catalyzed by adipose triglyceride lipase (ATGL). Fatty acids can also be utilized for phospholipid (PL) biosynthesis. For example, DAG can be attached to a choline or ethanolamine head group by choline phosphotransferase 1 (CHPT1), ethanolamine phosphotransferase 1 (EPT1), and choline/ethanolamine phosphotransferase 1 (CEPT1), which have different preferences in DAG with PUFA, MUFA, and SFA chains. Ester PLs are synthesized solely in the endoplasmic reticulum (ER), whereas the biosynthesis of ether PLs requires peroxisomes in addition. The remodeling of fatty acid composition in PLs is known as the Lands cycle, which involves generating lysophospholipids (lyso-PLs) from PLs by cleavage of a fatty acyl chain and regenerating PLs from lyso-PLs by incorporating a new fatty acyl chain. In the former process, the phospholipase A_2 (PLA_2) family specifically recognizes the *sn-2* position where PUFAs are usually incorporated. In the latter process, various lipid acyltransferases, including membrane-bound *O*-acyltransferase 1 (MBOAT1), MBOAT2, MBOAT7, lyso-PC acyltransferase 1 (LPCAT1), LPCAT3 (also known as MBOAT5), and 1-acylglycerol-3-phosphate *O*-acyltransferase 3 (AGPAT3), selectively incorporate SFA, MUFA, and PUFA. Lipid peroxidation occurs in PUFAs esterified in PLs. Although the requirement for enzymatic oxygenation remains controversial, cytochrome P450 oxidoreductase (POR), cytochrome *b*5 reductase 1 (CYB5R1), and NADPH oxidases (NOXs), which generate reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$), as well as lipoxygenases (LOXs) that directly catalyze the dioxygenation of PUFAs all contribute to lipid peroxidation in specific contexts. Figure created with a licensed version of BioRender.com.

sensitivity to ferroptosis. Deletion of adipose triglyceride lipase (ATGL, encoded by *PNPLA2*), which catalyzes the first and rate-limiting step of lipolysis, suppresses GPX4 inhibition-induced ferroptosis in certain cancer cells (292). Upon extracellular lipid depletion, ATGL-mediated lipolysis contributes to the ferroptosis susceptibility of cancer cells (273). Likewise, depletion of autophagy protein 5 (ATG5) and RAB7A, which are involved in lipophagy, leads to ferroptosis resistance in HCCs (307). In addition, it is worth noting that Fas-associated factor 1 (FAF1) is a protein that may directly sequester free PUFAs, mimicking the antiferroptotic mechanism of TAG synthesis and lipid droplet formation (308).

3.5.6. (e)PL biosynthesis.

Lipid peroxidation occurs in PUFAs esterified in PLs rather than free PUFAs. Initially, it was believed that PUFA-containing phosphatidylethanolamines (PEs) are specific targets for peroxidation during ferroptosis (291), but subsequent studies identified a wide range of PL species that can all be proximate targets for peroxidation regardless of their head groups, such as phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidic acid (PA), and even cholesteryl ester (82, 274, 309–312). In mammalian cell membranes, PC and PE are the most abundant PLs, which can be synthesized from DAG by

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

attaching the respective head groups. Choline phosphotransferase 1 (CHPT1) and ethanolamine phosphotransferase 1 (EPT1) catalyze the synthesis of PC and PE, respectively, whereas choline/ethanolamine phosphotransferase 1 (CEPT1) is a dual-functional enzyme that can catalyze both processes (**FIGURE 7**). Intriguingly, CHPT1 and EPT1 potently drive ferroptosis, whereas CEPT1 plays the opposite role (313). These results are consistent with previous reports that CHPT1 and EPT1 preferentially generate PC/PE with PUFA side chains, whereas CEPT1 preferentially generates PC/PE with SFA and MUFA chains (314, 315). Beyond de novo synthesis, PE can also be synthesized from PS in the mitochondria. In this regard, ATG2A, which mediates PS import into mitochondria, may boost PE level, thereby promoting ferroptosis (316).

ePLs are a group of PLs with an ether bond instead of a typical ester bond at the *sn*-1 position. Although they are a rather minor fraction in membranes (accounting for ~20% of total PLs), ePLs can significantly influence ferroptosis sensitivity in certain contexts. In contrast to the ester PLs, which are synthesized solely in the ER, the biosynthesis of ePL requires peroxisomes in addition (**FIGURE 7**). Ablation of *PEX* genes involved in peroxisomal biogenesis and depletion of enzymes involved in ePL biosynthesis both prevent ferroptosis in some cancer cells (263, 317). Nevertheless, ePLs are not always essential for ferroptosis (318), and a lack of ePLs even promotes ferroptosis in germ cells of *Caenorhabditis elegans* fed a high-PUFA diet (319). These disparities might be due to the differences in composition of ePLs across different cell types and species. Specifically, ePLs are known to be highly enriched in certain mammalian tissues such as brain and heart, whereas in liver, for instance, ePLs are present in a low amount (320). Furthermore, ePLs in *C. elegans* contain a lower proportion of PUFA compared to mammalian cells. As such, ePL depletion may decrease MUFA rather than PUFA content in *C. elegans* cell membranes (321). Indeed, the presence of the PUFA chain but not the ether bond is critical for ferroptosis sensitization (317). This is supported by the fact that myeloid cells, which have a lower proportion of PUFA-PLs but are enriched in ePLs, are more resistant to ferroptosis than lymphoid cells (322) and that forced enrichment of MUFA-ePLs by MS4A15 overexpression leads to ferroptosis resistance (280). Most recently, ePLs were found to facilitate CD44-mediated iron uptake, which might account for their proferroptotic effect (323). Alternatively, it could also simply be due to the fact that ePLs have a higher probability of harboring a PUFA chain at the *sn*-2 position (317).

Of note, a subgroup of ePLs, namely plasmalogen, has been claimed to prevent ferroptosis, given that targeting *TMEM189*, a professional gene mediating the generation of plasmalogen from alkyl ePLs, sensitized cells to ferroptosis (263). However, other studies failed to corroborate these results (317, 321), suggesting that the statement may be context dependent. Indeed, the antiferroptotic effect of plasmalogen is indirect but relies on its negative feedback regulation on fatty acyl-CoA reductase (FAR1), which mediates ePL biosynthesis (324). As such, preventing the conversion of alkyl ePLs to plasmalogen may boost ePL biosynthesis and sensitize cells to ferroptosis (263).

3.5.7. PL remodeling.

The remodeling of fatty acid composition in PLs is known as the Lands cycle. Briefly, it describes the generation of lysophospholipids (lyso-PLs) from PLs by phospholipase-mediated cleavage of a fatty acyl chain and the regeneration of PLs from lyso-PLs by lipid acyltransferase-mediated incorporation of a new fatty acyl chain (**FIGURE 7**). The Lands cycle has a decisive impact on ferroptosis, not only because it can alter the PUFA content in PLs but also because it helps to replace the oxidatively truncated PUFA chains from PLs, thereby terminating the chain reaction of peroxidation (**FIGURE 7**). In this regard, the phospholipase A_2 (PLA_2) family, which specifically recognizes the *sn*-2 position where PUFAs are usually incorporated, is particularly relevant (**FIGURE 7**). Several PLA_2 members, including PLA2G2F (325), PLA2G4B (326), PLA2G4C (274), PLA2G6 (aka calcium-independent $PLA_2\beta$) (327–329), and PLA2G7 (aka lipoprotein-associated PLA_2 and platelet-activating factor acetylhydrolase, PAFAH) (330, 331), as well as enzymes with PLA_2 activity such as PRDX6 (332, 333), ABHD12 (334), and PAFAH2 (335), have been shown to protect from ferroptosis in various contexts.

The effect of lyso-PL reacylation on ferroptosis is mainly determined by the type of fatty acid chain incorporated. For example, lyso-PC acyltransferase 3 (LPCAT3, aka membrane-bound *O*-acyltransferase 5, MBOAT5), MBOAT7 (aka LPIAT1), and 1-acylglycerol-3-phosphate *O*-acyltransferase 3 (AGPAT3), which preferentially mediate the reacylation of PUFA, sensitize cells to ferroptosis (311, 317, 336) (**FIGURE 7**). TMEM164, which selectively transfers AA acyl chains from PCs to lyso-ePLs, thereby producing PUFA-ePLs, has a similar effect (337). By contrast, MBOAT1 (aka LPEAT1) and MBOAT2 (aka LPCAT4), which preferentially mediate the reacylation of MUFA, and LPCAT1, which preferentially mediates the reacylation of SFA, protect from ferroptosis (325, 338) (**FIGURE 7**). Of note, a recent study suggests that LPCAT3 favors the incorporation of PA-CoA over AA-CoA when they are present at equimolar concentration, which may account for its antiferroptotic effect in *KRAS* mutant lung cancer (274) (**FIGURE 7**). Furthermore, since

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

LPCAT3 does not mediate the incorporation of AdA-CoA (339), an excess of AA caused by LPCAT3 ablation may be redirected and become elongated to AdA and finally incorporated into PLs (340). Regardless of all these inherently complex metabolic processes, the relationship between fatty acids and ferroptosis is clear, i.e., the higher PUFA content in the PLs, the higher the sensitivity to ferroptosis. In support of this, a recent paper proposed that PLs incorporated with two PUFA chains are efficient drivers of ferroptosis, despite their low abundance (341).

3.6. Lipid Peroxidation

The essence of ferroptosis is unrestrained lipid peroxidation, which may occur via two primary mechanisms: a free radical chain reaction (autoxidation) or enzymatic oxygenation. During the last few years this topic has been under intense debate (264, 342) (**FIGURE 7**). Although no conclusion can be drawn so far on which mechanism is more important, it appears that enzymatic oxygenation perhaps contributes to ferroptosis in certain contexts, yet there is no such enzyme universally required for the catalyzation of lipid peroxidation.

An involvement of enzymatic oxygenation was initially suggested by the proferroptotic effects of a number of LOXs in different contexts, including eLOX-3 (255), 5-LOX (343, 344), 12-LOX (345), and 15-LOX alone with its scaffold protein PE binding protein 1 (PEBP1) (28, 291, 346). Theoretically, LOXs catalyze the dioxygenation of PUFAs, resulting in fatty acid hydroperoxides, thereby potentially driving ferroptosis directly (**FIGURE 7**). However, the importance of LOXs has been questioned, not only because the ablation of 12/15-LOX (encoded by the *Alox15* gene), which can act on esterified PUFAs in membranes, fails to prevent GPX4 deficiency-induced ferroptosis either in vitro or in vivo (31, 347, 348) but also because the vast majority of LOX inhibitors that suppress ferroptosis show off-target RTA activity (254). In parallel to LOX, cyclooxygenase (COX, aka prostaglandin-endoperoxide synthase, PTGS) catalyzes the peroxidation of PUFAs. Whereas *PTGS2* upregulation has been observed during ferroptosis, inhibition of COX-1/COX-2 fails to confer protection of cells to ferroptosis (30).

POR is a membrane-bound enzyme transferring electrons from NADPH to cytochrome P450s (CYPs) or other electron acceptors. Remarkably, some CYPs possess the epoxygenase activity that can catalyze the epoxidation of PUFAs (349). However, only POR, but none of the CYPs, was identified in the CRISPR/Cas9 screens as strong drivers of ferroptosis (350). Mechanistically, POR and to some extent cytochrome *b*5 reductase 1 (CYB5R1) were found to generate H_2O_2 independently of CYPs and thus promote ferroptosis (351) (**FIGURE 7**). Nonetheless, neither the knockout of *POR* nor the double knockout of *POR*/*CYB5R1* completely prevents ferroptosis (350, 351). Like POR, NOXs are membrane-bound enzymes that utilize electrons from NADPH to generate $O_2^{\bullet-}$ that further dismutates to H_2O_2 (**FIGURE 7**). NOX1 and NOX4 in particular have been implicated in the induction of ferroptosis (4, 352–354), but their contributions to ferroptosis seem to be context specific. ETC is another major source of intracellular ROS, but as mentioned in sect. 3.3.3 blockade of ETC does not always rescue ferroptosis. In fact, exogenous H_2O_2 does not induce ferroptosis in the presence of wild-type GPX4, distinguishing between ROS-induced cytotoxicity and lipid peroxidation (98). However, when the defense system is compromised, for example, when wild-type GPX4 is replaced by the GPX4 U46C variant, H_2O_2 can induce ferroptosis by irreversibly inactivating the catalytic cysteine in GPX4 (98). In this regard, the consumption of NADPH by POR and NOXs may undermine the antiferroptotic systems to some extent, thereby sensitizing cells to ROS-induced ferroptosis.

4. THE MAIN FERROPTOSIS CONTROLLING SYSTEMS

4.1. The Cyst(e)ine/GSH/GPX4 Axis

The cyst(e)ine/GSH/GPX4 axis is the central antiferroptotic hub that is ubiquitously expressed in cells and tissues (**FIGURE 8**). To effectively trigger ferroptosis, perturbation of this system is generally necessary and, in many contexts, easily sufficient. Accordingly, when ferroptosis is induced by unknown factors, this axis is usually impaired. In vitro, disruption of this axis can be readily achieved by pharmacological or genetic means targeting system x_c^-, GSH biosynthesis, and GPX4, but still only few tools are available for in vivo studies. In addition to direct targeting, manipulating the factors that regulate the expression or activity of this axis can also be effective. On the other hand, when an impairment of the cyst(e)ine/GSH/GPX4 system causes pathological conditions, administration of ferroptosis inhibitors may be of therapeutic value.

4.1.1. System x_c^-.

Since cyst(e)ine metabolism is extensively reviewed in sect. 3.2.1, we focus here on system x_c^-, the main transporter mediating cystine uptake. System x_c^- is a heterodimer consisting of a light chain (i.e., xCT, encoded by *Slc7a11*) and a heavy chain (i.e., 4F2, aka CD98, encoded by *Slc3a2*) (21). Since the heavy chain is shared by

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

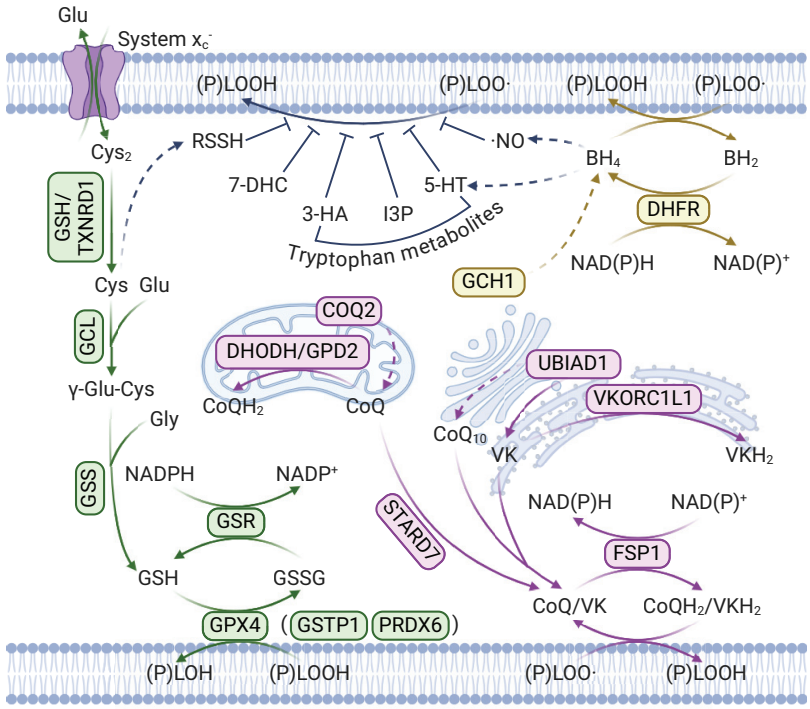


FIGURE 8. The main ferroptosis defending systems. The cyst(e)ine/glutathione (GSH)/glutathione peroxidase 4 (GPX4) axis is the central antiferroptotic hub. In vitro, the major source of Cys is extracellular Cys_2, which is imported by system x_c^- in exchange for Glu and then reduced by GSH and thioredoxin reductase 1 (TXNRD1)-dependent reducing systems. For GSH synthesis, Cys and Glu are ligated by glutamate-cysteine ligase (GCL) to generate γ-glutamylcysteine (γ-Glu-Cys), and then glycine (Gly) is attached by GSH synthetase (GSS). GPX4 uses GSH as a substrate to reduce (phospho)lipid hydroperoxide [(P)LOOH] to (phospho)lipid alcohol [(P)LOH], whereby 2 molecules of GSH are oxidized and condensed to 1 molecule of oxidized glutathione (GSSG). Glutathione-disulfide reductase (GSR) then recycles GSSG back to GSH using NADPH. Besides GPX4, peroxiredoxin 6 (PRDX6) and glutathione *S*-transferase P1 (GSTP1) may possess peroxidase activity. The NAD(P)H/ferroptosis suppressor protein 1 (FSP1)/coenzyme Q (CoQ)/vitamin K (VK) system represents the second critical pillar against ferroptosis. CoQ biosynthesis occurs in mitochondria, initiated by 4-hydroxybenzoate polyprenyltransferase (COQ2). Cytosolic StAR-related lipid transfer domain protein 7 (STARD7) mediates the trafficking of CoQ to the plasma membrane. VK biosynthesis occurs in the endoplasmic reticulum (ER) and is mediated by UbiA prenyltransferase domain-containing protein 1 (UBIAD1). Notably, UBIAD1 also mediates CoQ_{10} biosynthesis in the Golgi membranes of zebrafish and human cells. On the plasma membrane, FSP1 utilizes both NADH and NADPH to reduce CoQ or VK. The reduced forms, $CoQH_2$ and VKH_2, act as radical trapping antioxidants (RTAs), reacting with (phospho)lipid peroxyl radicals [(P)LOO·] to form (P)LOOH. Additionally, it has been suggested that dihydroorotate dehydrogenase (DHODH) and glycerol-3-phosphate dehydrogenase 2 (GPD2) protect mitochondrial membranes from lipid peroxidation by reducing intramitochondrial CoQ and that vitamin K epoxide reductase complex subunit 1-like 1 (VKORC1L1) protects the ER from lipid peroxidation by reducing VK. The guanosine triphosphate cyclohydrolase 1 (GCH1)/tetrahydrobiopterin (BH_4)/dihydrofolate reductase (DHFR) axis is the third pillar in ferroptosis control. GCH1 catalyzes the rate-limiting step in BH_4 biosynthesis, which acts as an endogenous RTA, whereas DHFR regenerates BH_4 using NADPH as the reducing power. Note BH_4 is also a cofactor in the biosynthesis of serotonin (5-HT) and nitric oxide (NO), both of which can act as RTAs. Furthermore, indole-3-pyruvate (I3P), 3-hydroxyanthranilic acid (3-HA), 7-dehydrocholesterol (7-DHC), and hydropersulfide (RSSH) are potent RTAs. Figure created with a licensed version of BioRender.com.

several other amino acid transporters facilitating proper folding of their respective light subunits (355), xCT determines substrate specificity of system x_c^-. The distinct significance of xCT in cell/organoid cultures versus in vivo suggests that caution should be exercised when interpreting in vitro findings. The complex regulation of xCT implies its potential response to different types of stress.

In vitro, most cells cannot survive in the absence of xCT, with the exception of a few cases such as primary hepatocytes (151). Lack of xCT usually induces ferroptosis, but some cells like mouse melanoma B16F10 may undergo nonferroptotic cell death, perhaps because they require high amounts of cysteine for melanin synthesis (356, 357). In vivo, xCT appears to be redundant, as *Slc7a11* knockout mice develop normally and have a normal lifespan (22, 358), but upon oxidative challenge system x_c^- may exert a protective effect (359, 360). The expression of xCT is low in most tissues, except brain, thymus, and spleen (361). Analysis of metabolites from xCT-deficient mice revealed a major decrease in cystathionine (another substrate of system x_c^-) instead of cysteine in thymus and spleen (362), implying that system x_c^- is not important for cysteine uptake even in the tissues where it is highly expressed. Therefore, perturbation of xCT is unlikely to trigger ferroptosis in physiological conditions, making it an appealing target for cancer therapy (as discussed in sect. 5.3).

Mouse xCT consists of 502 amino acids, but its longest transcript spans ~12 kb (21), suggesting complex

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

regulation of its expression. *xCT* is transcriptionally activated by NRF2 and ATF4 in response to oxidative stress and ER stress, respectively (166, 363). Aberrant activation of NRF2 and ATF4 can therefore protect cancer cells from ferroptosis (364–366). Glioma amplified sequence 41 (GAS41) and AT-rich interaction domain 1A (ARID1A), which epigenetically enhance the binding of NRF2 to the *xCT* promoter region (367, 368), and transcription factor ETS-1, which transactivates *xCT* in synergy with ATF4 (369), inhibit ferroptosis in different contexts. Furthermore, xCT is upregulated by ZNF706 downstream of oncogenic MYC in HCCs (370), by transcription factor 7 (TCF7, aka TCF1) downstream of the Wnt signaling pathway in colorectal tumor organoids (371), and by the RNA-binding protein RBMS1 at the translational level in lung cancers to counteract ferroptosis (372). On the other hand, transcription factors that negatively regulate *xCT* expression sensitize cells to ferroptosis, such as p53 (373), c-Fos (374), ATF3 (375), BTB domain and CNC homolog 1 (BACH1) (376), protein arginine methyltransferase 1 (PRMT1) (377), SMAD3 downstream of transforming growth factor β1 (TGF-β1) (378), and STAT1 downstream of interferon-γ (IFN-γ) (367). The tumor suppressor BRCA1-associated protein 1 (BAP1), which epigenetically suppresses *xCT*, also has a proferroptotic effect (379). Posttranscriptionally, *xCT* mRNA is cleaved and degraded by inositol-requiring enzyme 1 (IRE1α), an ER-resident protein with endoribonuclease (RNase) activity (380). In terms of posttranslational modifications, xCT is stabilized by a CD44 variant (CD44v) (381), epidermal growth factor receptor (EGFR) (382), and the deubiquitylases OTUB1 and USP18 (383, 384), whereas it is destabilized by $CRL3^{KCTD10}$ and SOCS2-mediated ubiquitination (384, 385). The activity of xCT is enhanced by IL1RAP (163) but suppressed by the direct interaction of BECN1 downstream of AMPK (386) and by mTOR complex 2 (mTORC2)- and oncogenic PI3KCA-mediated S26 phosphorylation (162, 387). Together, these studies greatly expand our understanding of the regulatory network of xCT, particularly in cancer cells.

4.1.2. GSH.

GSH is the most abundant nonprotein thiol found in mammalian cells. It is a tripeptide composed of glycine, cysteine, and glutamate. The biosynthesis of GSH requires two ATP-consuming steps. First, glutamate and cysteine are ligated by glutamate-cysteine ligase (GCL, aka γ-glutamylcysteine synthetase, γ-GCS) to generate γ-glutamylcysteine; then, glycine is added to the COOH terminus by GSH synthetase (GSS) to yield GSH (**FIGURE 8**). Upon cystine deprivation, GCL ligates glutamate with alternative amino acids, thereby keeping glutamate away from the TCA cycle and partially rescuing ferroptosis (388). GCL is a heterodimeric enzyme composed of the catalytic subunit GCLC and the modifier subunit GCLM. These subunits have been found to be upregulated in certain cancer cells to counteract ferroptosis (389, 390). Interestingly, although ablation of GCLC is lethal to cells, cell death can be fully rescued by the supplementation of *N*-acetyl-L-cysteine (NAC) (391) or by overexpression of xCT (140), suggesting that GSH is not necessarily required in vitro. In these contexts, cysteine is probably used by GPX4 as an electron donor to directly inhibit ferroptosis (133, 134).

In mice, global deletion of *Gclc* or *Gss* leads to embryonic death (391–393), whereas systemic deletion of *Gclm* does not cause any overt phenotype (394). This is because GCLC alone may catalyze the reaction like the holoenzyme GCL, albeit with lower efficiency. Tissue-specific deletion of *Gclc* can cause tissue damage, dysfunction, and even death, e.g., in the liver (395), endocrine pancreas (396), endothelial cell (397), keratinocytes (398), and CNS (399). Ferroptosis is at least partially involved in tissue damage in the liver and keratinocytes, as shown by lipid peroxidation-associated cell death, whereas its role in other contexts remains to be determined. In humans, mutations in the *GCLC* gene and *GSS* gene are rare autosomal recessive diseases. The reason why these patients are born, in contrast to genetic knockout mice that die embryonically, could be that the enzymes are not absent but partially impaired (400). All patients present with hemolytic anemia, whereas the severe cases develop neurological symptoms (400, 401). Vitamin E supplementation has been regularly used to treat GSS deficiency since the 1970s (402), but whether it counteracts ferroptosis remains to be clarified.

GSH inhibits ferroptosis mainly by acting as a substrate of GPX4, whereby two molecules of GSH are oxidized and condensed to one molecule of GSSG. GSR then recycles GSSG back to GSH at the expense of NADPH (**FIGURE 8**). Therefore, it is generally assumed that NADPH protects ferroptosis at least in part by recycling GSH. However, direct evidence supporting a putative antiferroptotic role of GSR is curiously lacking. Moreover, mice in which GSR has been ablated show no obvious phenotype (403). Accordingly, humans with large fragment deletion in the *GSR* gene, which is supposed to be a null mutation, do not develop a severe disease outcome (404). Thus, it appears that GSR is not essential in physiological conditions and its role in ferroptosis remains blurry.

ChaC glutathione-specific γ-glutamylcyclotransferase 1 (CHAC1) and multidrug resistance protein 1 (MRP1, aka ABCC1) mediate the degradation and efflux of GSH, respectively. CHAC1 was originally identified as a

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

pharmacodynamic marker for the inhibition of system x_c^- as it acts downstream of ATF4 in response to cyst(e)ine starvation (405). However, in the last decade, only a few studies have addressed an involvement of CHAC1 in ferroptosis regulation (406, 407). By contrast, MRP1 has been found to promote ferroptosis not only in vitro but also in a rat model of cardiac ischemia-reperfusion (408–410). In aggregate, although GSH is regulated by multiple factors, only its biosynthesis and, to a certain extent, its export have been closely associated with ferroptosis.

4.1.3. GPX4.

GPX4 sits at the center of ferroptosis control, as it is the only enzyme that is ubiquitously present and capable of reducing toxic lipid hydroperoxides (LOOHs), even when embedded in biological membranes. GPX4 comes in three flavors, namely the short form (sGPX4), the mitochondrial matrix form (mGPX4), and the nuclear form (nGPX4), although only sGPX4 plays a major role in ferroptosis prevention. GPX4 is a selenoprotein; thus its expression is directly linked to selenium metabolism. GPX4 is also subject to control by transcriptional and posttranslational mechanisms. In vivo, conditional knockout of *Gpx4* (sometimes in combination with a low-vitamin E diet) is often used to investigate its role in specific tissues. In humans, GPX4 deficiency causes a life-threatening disease called Sedaghatian-type spondylometaphyseal dysplasia (SMDS; OMIM #250220).

The three GPX4 isoforms share the same coding sequence, except that mGPX4 and nGPX4 are additionally furnished with the respective targeting sequences at their NH_2 termini. sGPX4 is known as cytosolic GPX4, but this nomenclature is misleading because sGPX4 also localizes in the mitochondrial intermembrane space and nucleus (411, 412). Ablation of all three isoforms in mice causes early embryonic death (27, 413), which can be rescued by the transgenic reconstitution of sGPX4 but not mGPX4 (411). Indeed, the expression of mGPX4 is predominant only in male germ cells and photoreceptors to some degree, whereas the expression of nGPX4 is more restricted and only present in late spermatids. Selective disruption of mGPX4 or nGPX4 has no effect on mouse survival, although loss of mGPX4 causes male infertility and retinal degeneration (414–416). Together, these results indicate that among the three isoforms only sGPX4 is of vital importance. Consistent with the in vivo observations, reconstitution of sGPX4, but not mGPX4, rescues cells with GPX4 ablation, revealing a key role of sGPX4 in preventing ferroptosis (417, 418). Surprisingly, two recent studies claimed that mGPX4 inhibits ferroptosis in cancer cells (418, 419). However, the major concerns are that mGPX4 is expressed at a rather low level in a range of cancer cell lines (417), as in most somatic cells (420), and that high (artifactual) overexpression of mGPX4 may confer antiferroptotic function due to improper subcellular localization. As such, it remains to be clarified whether the antiferroptotic effect of mGPX4, if any, is (patho)physiologically relevant.

As discussed in sects. 3.1.2 and 3.4.1, the expression of GPX4 is regulated by the availability of selenium, the Sec incorporation machinery, and posttranscriptional modification of Sec-tRNA$^{[Ser]Sec}$. However, there is a hierarchy of selenoproteins during the synthesis of selenoproteins where GPX4 ranks among the top ones (421). For instance, GPX4 levels are less affected by selenium deficiency than GPX1 in mouse and rat tissues (422, 423). In humans, insufficient selenium uptake is associated with a congestive cardiomyopathy known as Keshan disease, whereas studies in mice suggest that the kidney (besides certain types of neurons) is the tissue most vulnerable to GPX4 ablation (31). Thus, it appears that GPX4 synthesis is preferably maintained under (patho)physiological conditions, and it would be difficult to perturb GPX4 by simply limiting selenium. On the other hand, supraphysiological levels of selenium may stimulate *GPX4* expression via the transcription factors AP2-γ (encoded by *TFAP2C*) and stimulating protein 1 (SP1) (424, 425).

In terms of transcriptional regulation, the three GPX4 isoforms appear to be regulated differently. Here we focus on *sGPX4* and refer to it as *GPX4* unless otherwise stated below. *GPX4* is deemed a housekeeping gene with only limited regulation under stress (426). Initially, only a few transcription factors were identified to promote murine *Gpx4* transcription, including SP1, AP2, and nuclear factor Y (NFY) (420, 427). In human *GPX4*, a combined binding site for cAMP response element binding protein (CREB), cAMP response element modulator (CREM), and CCAAT/enhancer binding protein (C/EBP) is located immediately downstream of the NFY site (428), which can be activated by C/EBPα, C/EBPε, and CREB in different contexts to induce *GPX4* expression (429–431). In contrast, abnormal binding of the transcriptional repressor CREMα to this site in neutrophils leads to decreased *GPX4* expression and ferroptosis, which underlies the pathogenesis of systemic lupus erythematosus (432). Several other transcription factors that bind in a distal region ($-$1,000 to $-$2,500 bp) upstream of the *GPX4* transcriptional start site have been recently discovered in the context of cancer cells, including neurogenic differentiation factor 1, androgen receptor, and TCF7L2 (aka TCF4), which promote *GPX4* transcription (433–435), and zinc finger E-box binding homeobox 1 (ZEB1), which suppresses GPX4 transcription (436). In addition, transcriptional inhibition

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

of *GPX4* has been linked to ferroptosis-induced renal tubular injury, regulated by Kruppel-like factor 5 and the interferon regulatory factor-1/zinc finger protein 350 complex (437, 438). Regardless of these findings, further research is needed to carefully validate these mechanisms, as NRF2 is considered to transcriptionally regulate *GPX4*, but this assumption has recently been refuted (439).

Posttranslational regulation may alter the enzyme activity and protein stability of GPX4. For instance, alkylation of GPX4 C66 by itaconate increases GPX4 enzyme activity (440), whereas succination of GPX4 C93 by fumarate has the opposite effect (441). GPX4 degradation is partially mediated by the ubiquitin-proteosome system. Deubiquitinating enzymes such as ubiquitin-specific protease 8 (442), OTUB1 (443), and OTUD5 (444) have been shown to inhibit ferroptosis by stabilizing GPX4 in different contexts. E3 ligases NEDD4 and TRIM25, which mediate the K48-linked ubiquitination on GPX4, promote ferroptosis in dopaminergic neurons and cancer cells, respectively (445, 446). However, ubiquitination can also stabilize the target protein depending on the way ubiquitins are conjugated (447), such as M1- and K63-linked ubiquitination of GPX4 as mediated by the LUBAC complex and the TRIM26 E3 ligase, respectively (448, 449). Besides the ubiquitin system, GPX4 can be degraded by macroautophagy and chaperone-mediated autophagy (450–452), the latter of which is abrogated by creatine kinase B-mediated phosphorylation at GPX4 S104 (453).

GPX4, or more specifically sGPX4, is ubiquitously expressed in vivo. Not only is global *Gpx4* knockout embryonic lethal (27), but also hepatocyte- and neuron-specific *Gpx4* knockout causes perinatal or postnatal death in mice (28, 125, 126, 454). In adult mice, tamoxifen-inducible deletion of *Gpx4* in the whole body (except brain) causes fatal acute kidney failure (31), whereas in neurons it induces paralysis, muscle atrophy, and early death (455). These studies suggest that the importance of GPX4 is not only restricted to a single type of tissue. However, the lethality of hepatocyte-specific *Gpx4* deletion can be compensated by vitamin E supplementation (454). Adult mice with GPX4 ablation in hepatocytes do not present an overt phenotype unless fed a vitamin E-deficient diet (251). Likewise, specific deletion of *Gpx4* in endothelial cells or the hematopoietic system only induces pathologies under vitamin E deficiency (456–458). As such, the antiferroptotic effect of GPX4 can be masked by vitamin E in certain tissues and contexts. In terms of the immune cells, GPX4 is essential for the survival of invariant natural killer T (iNKT) cells, proliferating $CD4^+$ (in particular follicular helper) and $CD8^+$ T cells, as well as B1 and marginal zone B cells but not for the survival of myeloid cells, T regulatory cells, or follicular B2 cells (347, 459–462). These discrepancies may be at least partly due to their different PUFA content (322). In neurons, constitutive *Gpx4* knockout leads to the loss of a specific population, i.e., the parvalbumin-positive, GABAergic, inhibitory neurons; accordingly, the pups are hyperexcitable and suffer from spontaneous and severe seizures (28, 125, 126). It is noteworthy that the loss of parvalbumin-positive neurons is not rescued by the reconstitution of GPX4 U46C variant, suggesting that they are strictly dependent on selenium-containing GPX4 (98). On the other hand, agouti-related protein (AgRP)- and proopiomelanocortin (POMC)-expressing neurons are tolerant to GPX4 ablation, at least in physiological conditions (463). In adult mice, spinal motor neurons are selectively vulnerable to *Gpx4* deletion, which explains the rapid death upon *Gpx4* deletion in neurons (455). Furthermore, inducible deletion of GPX4 in forebrain neurons leads to hippocampal neurodegeneration and cognitive impairment (464).

SMDS is an ultrarare, usually lethal autosomal recessive disorder caused by mutations in the *GPX4* gene. The disease was first reported in 1980 (465), but the link to *GPX4* was not established until 2014 (466). Several *GPX4* variants associated with SMDS have been described, including frameshifting and truncating mutations, but patients with these mutations usually die in utero or shortly after birth (466, 467). Only a few patients with a biallelic homozygous *GPX4 R152H* mutation survive to childhood. Studies on this GPX4 variant are ongoing and indicate decreased enzyme activity and a defect in binding to membrane cardiolipins (468, 469). Recently, a novel mutation, *GPX4 K162E*, has been reported that likely impairs the interactions of GPX4 with its substrates (470). Together, these studies underscore the importance of GPX4 in human life and propel in-depth studies on GPX4 and the development of therapeutic approaches for SMDS.

4.2. The NAD(P)H/FSP1/CoQ/Vitamin K System

The NAD(P)H/FSP1/CoQ/vitamin K system represents the second major pillar that protects against ferroptosis (**FIGURE 8**). In the absence of GPX4, this system alone can fully prevent ferroptosis, at least in vitro. This system differs from the cyst(e)ine/GSH/GPX4 axis in several aspects. First, GPX4 acts by reducing (P)LOOH to (phospho)lipid alcohol [(P)LOH], preventing further autoxidation steps, whereas FSP1 acts by reducing CoQ or vitamin K that react with (phospho)lipid peroxyl radicals [(P)LOO·] to form (P)LOOH, thereby halting the lipid peroxidation chain reaction (248, 249, 251) (**FIGURE 8**). Second, GPX4 uses GSH as the main reducing substrate recycled by GSR and NADPH, whereas FSP1 can utilize both NADH and NADPH as a source of reducing power,

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

with no obvious preference (471) (**FIGURE 8**). In support of this, the antiferroptotic effect of NADH in colorectal cancer is FSP1 dependent (210). Third, under physiological conditions, FSP1 expression is restricted to brown adipose tissue, and mice with global *Fsp1* knockout are generally healthy despite increased body weight (472), in contrast to the ubiquitous expression of GPX4 and the early lethality of *Gpx4* knockout embryos. Finally, although FSP1 is highly expressed in a range of cancer cell lines (249), inhibition of FSP1 alone is usually not sufficient to induce ferroptosis owing to the presence of GPX4.

4.2.1. FSP1.

FSP1 was initially described as a p53-responsive gene (called PRG3) in 1999 (473) and then in 2002 as a mitochondria-associated apoptosis-inducing factor (called AIFM2 or AMID) based on its structural homology with AIFM1 (aka AIF, apoptosis-inducing factor) (474, 475). Meanwhile, the antiferroptotic role of FSP1 was hinted at by an inconspicuous study, where FSP1 appeared as an anonymous gene with putative NADH oxidase function, preventing "apoptosis" (now known as ferroptosis) along with GPX4 in Burkitt lymphoma (476). Almost two decades later, the p53 dependency as well as the "apoptosis-inducing function" of FSP1 were convincingly refuted, whereas its antiferroptotic role has been widely acknowledged (248, 249), leading to an exponential increase in studies on FSP1 in recent years.

Physiologically, FSP1 is a lipid droplet-associated protein enriched in brown adipose tissue. In response to cold or β-adrenergic stimulation, FSP1 translocates to the inner mitochondrial membrane (facing the intermembrane space), where it oxidizes NADH to NAD^+ and replenishes the cytosolic NAD^+ pool for robust glycolysis (472). Furthermore, FSP1 is strongly induced in extensor digitorum longus muscle during exercise, also to elevate NAD^+-to-NADH ratios (477). These functional studies are in line with previous sequence analysis classifying FSP1 as a member of the type II NADH:quinone oxidoreductase (NDH-2) family, which catalyzes the same reaction as complex I in the ETC, but without pumping protons (478). Therefore, the major role of FSP1 under physiological conditions is to support glucose utilization. Intriguingly, sequence analysis also reveals that FSP1 contains the elements required for condensate formation involving phase separation, which can indeed be induced by a small molecule compound called icFSP1 (479). Whether this feature has a physiological role, for example, in mediating FSP1 translocation, requires further investigation. Besides, FSP1 was recently discovered to be the long sought-after warfarin-resistant vitamin K reductase, sustaining the reduction of vitamin K during warfarin overdose (250, 251). Accordingly, high-dose vitamin K treatment fails to rescue *Fsp1* knockout mice upon warfarin poisoning (251).

To date, the antiferroptotic role of FSP1 has mainly been associated with cancer cells: the higher the FSP1 expression in cancer cells, the less dependent they are on the cyst(e)ine/GSH/GPX4 axis (248, 249). For instance, *FSP1* is transcriptionally upregulated by NRF2 in KRAS mutant cancers and KEAP1 (kelch-like ECH associated protein 1) mutant lung cancers (480, 481) and epigenetically upregulated by bromodomain-containing protein 4 (BRD4) in the germinal center B cell-like subtype of diffuse large B cell lymphoma to counteract ferroptosis (482). By contrast, T and B acute lymphoblastic leukemia cells, which have low FSP1 expression due to DNA hypermethylation, are highly susceptible to perturbation of the cyst(e)ine/GSH/GPX4 axis (483). The antiferroptotic activity of FSP1 is based on its plasma membrane localization, mediated by *N*-myristoylation (248, 249). Dimethylation at R316 by PRMT1 dampens FSP1 *N*-myristoylation and thus its enzyme activity (377). The COOH-terminal domain of human FSP1, which facilitates its functional dimerization, may also be crucial (471). Furthermore, the E3 ubiquitin ligases TRIM21 and RNF126 that are responsible for membrane binding of FSP1 by ubiquitination contribute to ferroptosis resistance in gastrointestinal cancers and group 3 medulloblastoma cells, respectively (484, 485). However, another E3 ubiquitin ligase, TRIM69, was found to mediate the degradation of FSP1 in HCCs (486).

4.2.2. CoQ and vitamin K.

Both CoQ and vitamin K can be reduced by FSP1 to prevent ferroptosis (248, 249, 251), and site-directed mutational analysis suggests that they use the same binding pocket of FSP1 (250). CoQ and vitamin K (or more specifically, MK-4) are structurally similar, both containing a (naphtho)quinone group and an FPP-derived polyisoprenyl tail (**FIGURE 6**). Biosynthesis of CoQ occurs in the inner mitochondrial membrane and is initiated by 4-hydroxybenzoate polyprenyltransferase (COQ2) (**FIGURE 8**), which catalyzes the prenylation of 4-hydroxybenzoic acid serving as the precursor of the benzoquinone ring of CoQ. Disruption of COQ2 largely abrogates the antiferroptotic effect of FSP1 (248, 249), suggesting that mitochondrion-derived CoQ is the major source of this cofactor. In line with this, depletion of cytosolic StAR-related lipid transfer domain protein 7 (STARD7), which is responsible for the trafficking of CoQ from mitochondria to the plasma membrane (**FIGURE 8**), sensitizes cells to ferroptosis without affecting mitochondrial CoQ generation (487). Vitamin K is

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

synthesized in the ER, where UbiA prenyltransferase domain-containing protein 1 (UBIAD1) catalyzes the condensation of the isoprenyl side chain to the naphthoquinone group (488) (**FIGURE 8**). Interestingly, UBIAD1 also mediates the biosynthesis of CoQ_{10} in the Golgi membranes of zebrafish and human cells (489) (**FIGURE 8**), though it remains unclear whether it facilitates CoQ_9 synthesis in mice. In human melanoma cells, UBIAD1-mediated CoQ_{10} synthesis is crucial for preventing lipid peroxidation and cell death (490). Overall, despite the structural and functional similarities between CoQ and vitamin K, CoQ is likely the dominant cofactor in FSP1-mediated ferroptosis prevention, considering that it is more abundant than vitamin K in mammals (491).

4.3. The GCH1/BH_4/DHFR Axis

Guanosine triphosphate cyclohydrolase 1 (GCH1) was identified as a ferroptosis suppressor in 2020 in two independent studies using different CRISPR/Cas9 screens (326, 492). Mechanistically, GCH1 catalyzes the rate-limiting step in the biosynthesis of BH_4, which is a potent RTA, and GCH1 may also promote CoQ synthesis in certain contexts (326, 492) (**FIGURE 8**). One of the studies further showed that dihydrofolate reductase (DHFR), the enzyme regenerating BH_4 using NADPH as the reducing power, is crucial for the prevention of ferroptosis (326) (**FIGURE 8**). Remarkably, GCH1 overexpression compensates for the loss of GPX4 in MEFs, indicating the robustness of this antiferroptotic system (492). However, there have been only a very few follow-up studies in recent years. For instance, DHFR was found to protect macrophages from ferroptosis in aged mice (493), and GCH1 was reported to protect neurons from ferroptosis in the context of amyotrophic lateral sclerosis (ALS) and mandibulofacial dysostosis with microcephaly (494, 495). Moreover, it is noteworthy that BH_4 is a cofactor in the biosynthesis of 5-HT and nitric oxide (NO), both of which can act as RTAs (**FIGURE 8**).

4.4. Other Ferroptosis Defense Systems

Antiferroptotic systems that act independently of GPX4, FSP1, and DHFR are categorized and briefly discussed here. The first group of antiferroptotic systems mimics GPX4 using GSH to reduce (P)LOOH, represented by PRDX6 and GSH *S*-transferase P1 (GSTP1) (**FIGURE 8**). It has long been known that PRDX6 possesses peroxidase activity (496), but its activity depends on the presence of GSTP1, which catalyzes the glutathionylation of oxidized C47 in PRDX6, thus regenerating its peroxidase activity using GSH (497, 498). Accordingly, the peroxidase activity of PRDX6 can hardly be detected in the cell-free context (122). Intriguingly, a recent study indicated that GSTP1 per se harbors peroxidase activity that is independent of PRDX6 (499). In addition, PRDX6 harbors PLA_2 activity and is involved in cellular selenium utilization (118, 122, 123, 332, 333), whereas GSTP1 has GSH conjugation activity (499), all of which contribute to ferroptosis prevention. Nevertheless, overexpression of PRDX6 fails to substitute for GPX4 in vitro (118, 123), whereas GSTP1 overexpression compensates for the loss of GPX4 at least within a certain timeframe (499).

The second group of antiferroptotic systems mimics FSP1 reducing CoQ or vitamin K and is represented by dihydroorotate dehydrogenase (DHODH), glycerol-3-phosphate dehydrogenase 2 (GPD2), and vitamin K epoxide reductase complex subunit 1 like 1 (VKORC1L1) (**FIGURE 8**). DHODH and GPD2 are enzymes localized in the outer leaflet of the inner mitochondrial membrane, both mediating the reduction of CoQ. It has been suggested that DHODH and GPD2 together with mGPX4 protect mitochondrial membranes from lipid peroxidation, in parallel with sGPX4 and FSP1 surveilling the plasma membrane (418, 419). However, the validity of this model has recently been questioned, not only because of the improper use of the DHODH inhibitor brequinar leading to off-target effects on FSP1 but also because FSP1 alone completely prevents ferroptosis in cells with compound GPX4 and DHODH deletion (417). VKORC1L1 is an ER-resident enzyme reducing vitamin K. Unlike FSP1, VKORC1L1 cannot reduce CoQ and is sensitive to warfarin (500). In vitro, warfarin treatment synergizes with GPX4 inhibition to induce ferroptosis in pancreatic tumor cells, suggesting the antiferroptotic role of VKORC1L1. However, it remains unsolved how warfarin alone in the presence of GPX4 can induce ferroptosis in vivo (500).

The third group of antiferroptotic systems acts through the direct generation of RTAs, such as RSSH, several tryptophan metabolites, 7-DHC, and NO (**FIGURE 8**), among which the first three have already been introduced in sects. 3.2.1, 3.2.4, and 3.4.5, respectively. The identification of NO as an inhibitor of lipid peroxidation dates back ~30 years. It was initially found to inhibit LOXs (501), but like most LOX inhibitors NO also acts as an endogenous RTA (502, 503). Not surprisingly, M1 macrophages expressing high levels of inducible nitric oxide synthase (iNOS, aka NOS2) are therefore enriched with NO and are more resistant to ferroptosis than M2 macrophages. Consequently, treatment with NO donors renders M2 macrophages as well as HCCs resistant to ferroptosis (504, 505). Remarkably, the iNOS/NO system can substitute for GPX4 to prevent ferroptosis in macrophages and HT1080 cells (325, 505). Furthermore, macrophage-derived NO can cell nonautonomously rescue epithelial cells from ferroptosis induced by

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

Pseudomonas aeruginosa, revealing a novel intercellular defense mechanism against ferroptosis (506).

In addition to the systems mentioned above, ferroptosis is essentially influenced by iron and fatty acid metabolism, which are discussed in detail in sect. 3. In the broadest sense, antiferroptotic systems should also include those that deplete intracellular iron or membrane PUFA content. For instance, overexpression of MBOAT1 or MBOAT2, which remodels the membrane profile to a high-MUFA state, can compensate for the loss of GPX4 (325), again highlighting a major impact of fatty acid metabolism on ferroptosis.

4.5. Proferroptosis System—ACSL4

As discussed in sect. 3.6, there are several enzymes that potentially promote lipid peroxidation, such as LOXs and POR. However, since ACSL4 is the only protein known so far whose loss can fully prevent GPX4 ablation-induced cell death at least in the in vitro context, we focus on ACSL4 in this section, reviewing its role in ferroptosis and its regulation and physiological function.

The first time that *ACSL4* emerged as a proferroptotic gene was in a genetic screen in haploid cells (336). Subsequently, ACSL4 was identified as a requirement for the execution of ferroptosis along with the successful establishment of *Gpx4* and *Acsl4* double-mutant cells (290). Curiously, ablation of ACSL4 does not always fully prevent ferroptosis. For example, *ACSL4* knockout cells are less protected from ferroptosis induced by cystine deprivation than by GPX4 inhibition, whereas cell death can be fully rescued by ferrostatin-1 in both cases (318). Furthermore, photodynamic therapy-induced ferroptosis and, in certain contexts, LOX-mediated ferroptosis may bypass ACSL4 (310, 343, 345, 507, 508). It is noteworthy that in one of these studies a lipidomic analysis was performed and PUFA-PA was identified as the main substrate of lipid peroxidation (310). However, since the biosynthesis of PUFA-PA does not bypass ACSL4, which mediates PUFA activation, it remains unexplained why ACSL4 can be dispensable.

The expression of ACSL4 determines susceptibility to ferroptosis. For instance, *Acsl4* is upregulated during intestinal ischemia-reperfusion injury and acute kidney injury, contributing to ferroptosis-associated tissue damage. Mechanistically, these regulations are controlled by the transcription factors SP1 and hypoxia-inducible factor 1α (HIF-1α), which positively and negatively regulate *Acsl4*, respectively (509, 510). Ferroptosis is known to be inhibited at high cell density (28, 511), which may be partially due to the downregulation of ACSL4 downstream of E-cadherin and the Hippo pathway. Mechanistically, the activation of Hippo pathway increases the phosphorylation of yes-associated protein (YAP), blocking its function as a coactivator for TEAD4-mediated *ACSL4* transcription (512). Prostate cancer cells with loss of the tumor suppressor gene *RB1* are particularly sensitive to ferroptosis due to the upregulation of *ACSL4* by the transcription factor E2F (513). The high sensitivity of mesenchymal cancer cells to ferroptosis is partially due to *ACSL4* upregulation by ZEB1 (514). In antitumor immunity, $CD8^+$ T cell-derived IFN-γ sequentially activates STAT1, interferon regulatory factor 1, and the *ACSL4* gene, sensitizing cancer cells to ferroptosis (515). In terms of posttranslational modifications, ACSL4 can be phosphorylated by the protein kinase C family member PKCβII at T328 and by mitochondrial phosphoenolpyruvate carboxykinase 2 (PCK2) at T697, which are essential for its dimerization/activation and binding to substrate AA, respectively (516, 517). By contrast, methylation of ACSL4 at R339 by protein arginine *N*-methyltransferase 4 (PRMT4, aka CARM1) promotes its degradation by the E3 ubiquitin ligase RNF25 (518). Besides, the E3 ubiquitin ligases MARCH6 and F-box only protein 10 have been reported to mediate the degradation of ACSL4 (241, 519).

In vivo, ACSL4 is not essential, at least for male mice. *Acsl4* is a gonosomal X-linked gene, and *Acsl4* knockout male mice (genotyped $Acsl4^{-/y}$) appear normal and are fertile. When mated with wild-type female, their offspring have a normal genotype distribution ($Acsl4^{+/y}$ and $Acsl4^{+/-}$). In contrast, heterozygous female mice (genotyped $Acsl4^{+/-}$) have reduced fertility when crossed with wild-type male, and the pups carrying the disrupted allele ($Acsl4^{-/y}$ and $Acsl4^{+/-}$) have a high risk of embryonic death (520). Thus, it appears that embryonic death is attributed to the simultaneous deficiency of ACSL4 in pups and mothers. Studies in male *Acsl4* knockout mice revealed that their bone marrow-derived macrophages (BMDMs) and lungs have markedly less PUFA-derived fatty acyl-CoA and thus lower PUFA-PLs (521, 522), which is consistent with the in vitro studies. In addition, these mice are resistant to paraquat-induced lung injury and protected from methotrexate-induced lung fibrosis and acetaminophen-induced hepatotoxicity, because of the attenuated lipid peroxidation (522, 523). Tissue-specific ablation of ACSL4 in adipocytes, myeloid cells, and kidney tubule cells protects mice from diet-induced obesity, LPS-induced peritonitis, and renal ischemia-reperfusion injury, respectively, associated with attenuated lipid peroxidation (510, 524, 525). It therefore can be concluded that ACSL4 does play a proferroptotic role in vivo by enriching PUFA-PLs. However, the deletion of ACSL4 is not always beneficial, as male mice with global *Acsl4* knockout were shown to be more sensitive to LPS-induced septic shock because of overproduction of COX-derived eicosanoids (526), suggesting that care must be taken when considering ACSL4 as a potential therapeutic target.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

5. POTENTIAL PHYSIOLOGICAL ROLE AND DISEASE RELEVANCE OF FERROPTOSIS

5.1. Physiological Role of Ferroptosis

Cell death is an important physiological process by which unwanted cells are removed. Defects in cell death induction may lead to aberrant survival of malignant, senescent, damaged, or infected cells. Mounting evidence suggests that ferroptosis is actively involved in the processes of aging, tumor suppression, and infection control (**FIGURE 9**), perhaps implying ferroptosis as an innate (nonartificially induced) cell death modality.

5.1.1. Aging.

A link between ferroptosis and aging was first demonstrated in *Caenorhabditis elegans*. When ferroptosis is blocked by either an RTA or an iron chelator, their lifespan and health span are markedly increased (527). In line with this, *C. elegans* fed a high-MUFA diet survive longer, associated with lower lipid peroxidation (528). In rodents, ferroptosis has been associated with arterial aging, and inhibition of ferroptosis improves vascular function in aged animals (529, 530). The presence of ferroptosis in various mouse organs during aging as well as in the process of embryonic erythropoiesis has been shown by immunohistochemical staining of 4-hydroxy-2-nonenal (HNE)-modified proteins (531). However, since HNE is not exclusively specific to ferroptosis and may also mark other lipid peroxidation events, further studies are required to critically evaluate the presence of cell death.

5.1.2. Tumor suppression.

To eliminate cancer or precancerous cells, ferroptosis can be triggered by intrinsic signals derived from certain tumor suppressor genes such as p53 or by extrinsic signals represented by $CD8^+$ T cell-derived IFN-γ (**FIGURE 9**). As the best-studied tumor suppressor, p53 is known for its key roles to induce cell cycle arrest, senescence, and apoptosis. Interestingly, mutant $p53^{3KR}$, which is defective in carrying out these processes, retains the ability to suppress tumors (532). Further studies reveal that $p53^{3KR}$ promotes tumor ferroptosis by negatively regulating xCT, indicating that ferroptosis may be an additional protective shield in tumor suppression besides apoptosis (373). Furthermore, p53 may facilitate ferroptosis by downregulating VKORC1L1 and SCD1 (288, 500), suppressing the mevalonate pathway (533) and activating SAT1-mediated polyamine catabolism (188). Nevertheless, p21, the major target gene of p53 responsible for its cell

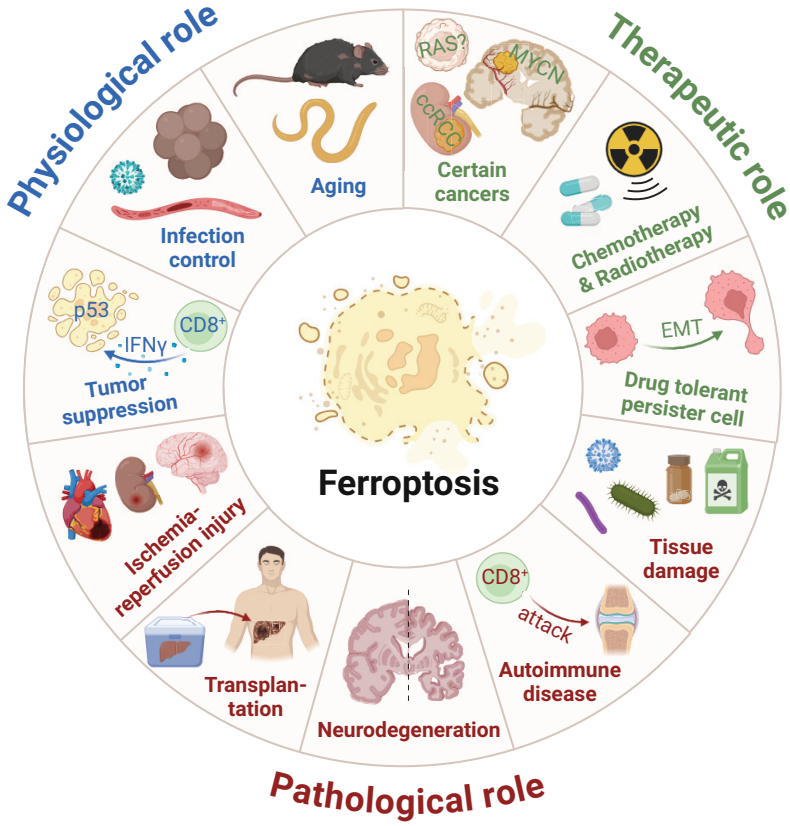


FIGURE 9. The physiological, pathological, and therapeutic roles of ferroptosis. Ferroptosis has been implicated in the aging process of rodent tissues, and inhibition of ferroptosis has been shown to increase the lifespan and health span of *Caenorhabditis elegans*. Ferroptosis can be activated by specific tumor suppressor genes, such as p53, or by $CD8^+$ T cell-derived interferon-γ (IFN-γ), suggesting its role as an innate tumor suppression mechanism. Furthermore, in some contexts, ferroptosis occurring in infected cells may contribute to infection control, indicating a physiological role of ferroptosis. Ferroptosis has been implicated in a variety of diseases, including ischemia-reperfusion injury, neurodegeneration, and autoimmune diseases. Organ damage during organ transplantation, where ischemia-reperfusion is unavoidable, may also involve ferroptosis. Additionally, ferroptosis may underlie tissue damage caused by certain pathogens and drugs, suggesting a pathological role. Certain cancer cells, such as clear cell renal cell carcinoma (ccRCC) and *MYCN*-amplified neuroblastoma, are inherently sensitive to ferroptosis. In certain contexts, cancer cells with mutant RAS are also susceptible. Drug-tolerant persister cells, which usually display characteristics of epithelial-mesenchymal transition (EMT) or dedifferentiation, are more vulnerable to ferroptosis. Irradiation and some existing drugs have been found to eradicate tumor cells by inducing ferroptosis, suggesting a therapeutic role for ferroptosis induction. Figure created with a licensed version of BioRender.com.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

cycle arrest function, plays an antiferroptotic role (534, 535). Therefore, it could be hypothesized that ferroptosis induction is a backup option of p53, especially in case it loses control over p21. In addition to p53, tumor suppressors that exert a proferroptotic effect include BAP1, which epigenetically silences *xCT* (379), FH, which drives the TCA cycle and thus mitochondrial ROS (194), and phosphatase and tensin homolog deleted from chromosome 10 (PTEN), which suppresses NRF2-mediated xCT expression and GSH synthesis (536). In terms of extrinsic signals, $CD8^+$ T cell-derived IFN-γ may trigger ferroptosis in cancer cells by mediating upregulation of ACSL4 and downregulation of 4F2 and xCT (515, 537).

5.1.3. Infection control.

Ferroptosis is not restricted to mammals but also occurs in other kingdoms of life (538). For example, *Staphylococcus aureus* is highly sensitive to PUFA-induced lipid peroxidation (539), and mice fed a high-PUFA diet are more tolerant to septic *S. aureus* infection (540). *Leishmania infantum* is susceptible to lipid peroxidation, and it exploits the antiferroptotic effects of NRF2 in macrophages to escape cell death (541). Although it remains to be determined whether the immune system can kill pathogens by inducing ferroptosis, the available evidence indicates that ferroptosis occurring in infected cells may afford infection control. For example, replication of hepatitis C virus and neuroinvasive viruses is restrained by lipid peroxidation (542, 543). Enhancing hepatocyte ferroptosis during liver-stage malaria infection is beneficial to the host (544). Microsporidia and *Brucella* even facilitate their host cells to avoid ferroptosis, thereby promoting their own proliferation (545, 546). The p53 P47S variant, which predominates in humans of African descent, is known to be defective in ferroptosis induction (547). Mice carrying this mutation are more susceptible to bacterial infection as their macrophages tolerate a higher iron level that supports bacterial growth (548).

5.2. Pathological Role of Ferroptosis

Ferroptosis has been implicated in a variety of diseases such as ischemia-reperfusion injury and neurodegeneration (**FIGURE 9**). The emerging role of ferroptosis in autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and psoriasis) has already been acknowledged and reviewed in detail elsewhere (549, 550) (**FIGURE 9**). Despite the beneficial role in infection control (as discussed in the foregoing), ferroptosis may be exploited by certain pathogens to promote host tissue damage, e.g., *Mycobacterium tuberculosis* (551), *Pseudomonas aeruginosa* (552), and SARS-CoV-2 (553, 554). Furthermore, ferroptosis has been associated with doxorubicin-induced cardiotoxicity (81), paraquat-induced lung injury (522), folic acid-induced nephrotoxicity (555), and acetaminophen-induced hepatotoxicity (523), although the latter is still controversial (556, 557) (**FIGURE 9**).

5.2.1. Tissue ischemia-reperfusion injury.

The link between ferroptosis and tissue ischemia-reperfusion injury was established largely based on the rescue effect of synthetic RTAs (e.g., liproxstatin-1, ferrostatin-1) in rodent models with heart (81), liver (31), kidney (558), lung (559), and intestinal (509) ischemia-reperfusion and ischemic stroke (560). Ischemia-reperfusion injury describes a pathological condition in tissue that occurs when the blood supply is restored after a transient period of ischemia. Mechanistically, tissue damage is attributed, at least in part, to mitochondrial dysregulation, i.e., succinate that accumulates during the ischemic phase rapidly fuels complex II during the reperfusion phase, resulting in reverse electron transport in complex I associated with overwhelming ROS generation (561). Moreover, dysregulation of key systems controlling ferroptosis was found in various models of ischemia-reperfusion. For instance, GPX4 degradation is induced by kidney ischemia-reperfusion (444). ACSL4 is upregulated during ischemia-reperfusion in the lung and intestine (509, 559). MRP1-mediated GSH efflux and 15-LOX are activated during heart ischemia-reperfusion (408, 562, 563). Remarkably, inhibition of mitochondrial complex II alone fails to restore the GSH pool, whereas inhibition of MRP1 and complex II synergistically protect cardiomyocytes in an in vitro anoxia-reoxygenation model (408), implying that the dysregulation of ferroptosis controlling systems may not be a downstream event of mitochondrial ROS but is triggered independently by ischemia-reperfusion. However, further studies are required to validate this statement in vivo and to shed light on the underlying mechanisms.

The knowledge gained from ischemia-reperfusion studies has been applied to organ transplantation, where ischemia-reperfusion is unavoidable (**FIGURE 9**). Treatment with ferroptosis inhibitors during organ preservation alleviates the damage to donor lungs and livers (564, 565), and administration of ferroptosis inhibitor to heart transplant recipients reduces cardiomyocyte cell death and blocks neutrophil recruitment (566). Interestingly, reports regarding the therapeutic effect of ferroptosis inhibition on kidney transplantation are oddly lacking, although it is well known that the kidney is one of the most susceptible organs to ferroptosis and ischemia-reperfusion. On the contrary, it was reported that treatment with a low dose of RSL3 on aged donor kidneys helps to remove the

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

senescent tubular cells by ferroptosis, thereby reducing tissue damage and inflammation following transplantation (567). Nevertheless, whether this counterintuitive therapeutic strategy is valid and applies to other organs requires further investigations.

5.2.2. Neurodegeneration.

Brain iron accumulation has long been acknowledged as a feature of aging, and abnormal iron deposition in specific brain regions is linked to certain neurodegenerative diseases (568). As unchaperoned iron is redox-active and can generate ROS via the Fenton reaction, it is generally believed that excessive iron may be toxic and may drive neuron damage and dysfunction. With the establishment of the concept "ferroptosis," iron-induced neuronal death has attracted attention. Recent studies suggest that ferroptosis may be actively involved in the development of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and ALS.

AD is the most common form of dementia, characterized with a progressive decline in memory, thinking, and behavior. The first hint that ferroptosis may contribute to AD is that conditional deletion of *Gpx4* in mouse forebrain neurons leads to an AD-like phenotype that is ameliorated by treatment with liproxstatin-1 (464). Correspondingly, GPX4 overexpression rescues neurodegeneration and cognitive impairment in a genetic AD mouse model (569). Furthermore, presenilin and apolipoprotein E (*APOE*), the two most studied genes associated with early-onset and late-onset AD, respectively, were found to protect against ferroptosis. Mechanistically, presenilin activates Notch-1 signaling, which maintains LRP8-mediated selenium uptake and thus GPX4 expression. Mutations in presenilin that lead to abnormal generation of amyloid-β simultaneously suppress Notch-1 signaling (108). APOE activates the PI3K/AKT pathway, which inhibits the degradation of ferritin. APOE ε4 variant carriers with a low expression of APOE may therefore have a high ferritin degradation rate (570). In addition, APOE ε4 blocks intracellular handling of LRP8 back to the cell surface (571), which may negatively impact on selenium uptake.

PD is the second most common neurodegenerative disease after AD, characterized by involuntary or uncontrollable movements. The gradual loss of midbrain dopaminergic neurons and aggregation of α-synuclein are the major pathological features of PD. Evidence supporting the link between ferroptosis and PD includes evidence that the metabolite of 15-LOX accumulates in the midbrains of PD rodent models and patient-derived fibroblasts (329) and that iron-induced dopamine oxidation modifies GPX4 for subsequent degradation (445). Furthermore, α-synuclein may drive ferroptosis in neurons by ROS generation and ePL biosynthesis (572, 573), and overexpression of human α-synuclein in the primary motor cortex of mice leads to ferroptosis-associated parvalbumin interneuron loss and motor learning impairment (574). Nevertheless, conditional deletion of *Gpx4* in mouse dopaminergic neurons does not lead to a significant consequence, suggesting that these neurons are somewhat resistant to ferroptosis (463). Analysis of postmortem human brain tissue reveals that GPX4 is upregulated in the surviving nigral cells of PD patients (575). Whether ferroptosis underlies the loss of dopaminergic neurons in PD therefore remains to be investigated.

ALS is a fatal neurodegenerative disease that affects motor neurons and results in weakness, atrophy of voluntary skeletal muscles, paralysis, and eventually respiratory failure. There are several signs suggesting a strong relationship between ALS and ferroptosis. First, a longitudinal study on 512 ALS patients identified four prognostic biomarkers for ALS, including neuronal integrity, DNA oxidation, lipid peroxidation, and iron accumulation, all related to ferroptosis (576). Second, a multiomics analysis of human induced pluripotent stem cell-derived spinal motor neurons revealed dysregulation in lipid metabolism with elevated AA levels, whereas treatment with 5-LOX inhibitors reversed ALS-related phenotypes both in vitro and in *Drosophila* and mouse models (577). Third, GPX4 depletion was found in postmortem spinal cords of both sporadic and familial ALS patients, as well as the spinal cord and brain of different ALS mouse models (578). Fourth, conditional deletion of *Gpx4* in neurons of adult mice led to rapid degeneration of spinal motor neurons and paralysis followed by death, whereas neurons in other regions such as cerebral cortex do not display overt degeneration at the early stage despite the loss of GPX4, suggesting that motor neurons are particularly vulnerable to ferroptosis (455). Finally, motor neuron degeneration and disease onset were significantly delayed in an ALS mouse model by forced expression of GPX4, suggesting that targeting ferroptosis might be a viable therapeutic approach to mitigate ALS (578, 579).

5.3. Therapeutic Role of Ferroptosis

Induction of ferroptosis emerges as a novel therapeutic strategy for cancer. Certain cancer cells such as ccRCC and *MYCN*-amplified neuroblastoma are inherently sensitive to ferroptosis (**FIGURE 9**). ccRCC is the most common form of kidney cancer, which is mainly caused by the loss of *VHL* gene. These cells are sensitive to cyst(e)ine deprivation or GPX4 inhibition, as loss of the *VHL* gene leads to selective accumulation of PUFA-PLs and impaired fatty acid degradation (294, 350). *MYCN*-

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

amplified neuroblastoma is highly aggressive because *MYCN* controls a variety of cellular processes such as cell growth, proliferation, and TFR1-mediated iron uptake. The high iron content therefore renders the cells sensitive to ferroptosis induced by cyst(e)ine deprivation and GPX4 inhibition (39, 580). Furthermore, these cells exhibit low expression of xCT, and forced xCT expression negatively affects their survival for unknown reasons (109). Thus, the cells rely on transsulfuration-mediated cysteine supply and LRP8-mediated uptake of organic selenium to counteract ferroptosis (109, 160, 161).

Ferroptosis was originally introduced as a cell death modality that selectively eradicates cancer cells with mutant RAS, but subsequent studies showed that multiple cell lines with wild-type RAS can actually undergo ferroptosis (30). Indeed, the effect of mutant RAS on ferroptosis is complex. For instance, HRAS V12G mutant cells display increased MUFA uptake and resistance to SCD1 inhibition (581), whereas KRAS G12D mutant cells display increased de novo lipogenesis and vulnerability to FASN and ACSL3 perturbation (274, 582). The KRAS G12D mutant cells also display increased GSH synthesis and FSP1 expression driven by NRF2 signaling, making the cells tolerant to GPX4 depletion while sensitive to FSP1 inhibition and xCT ablation (138, 481, 583). Therefore, it is difficult to state whether mutant RAS promotes or inhibits ferroptosis without specifying the details, e.g., what type of mutant RAS is present and how ferroptosis is induced (**FIGURE 9**). More importantly, these studies imply that cancer cells that are resistant to ferroptosis induced in one way may remain susceptible to ferroptosis induced in another way.

The occurrence of drug-tolerant persister cells represents a major challenge in cancer therapy, as they can lead to cancer relapse. In contrast to antibiotic-resistant bacteria, the persister cells do not exhibit any genomic alterations but become transiently tolerant to the drugs by metabolic rewiring and epigenetic modifications. Interestingly, persister cells are highly dependent on GPX4 to prevent ferroptosis (584) (**FIGURE 9**). Consistent with this, cells undergoing epithelial-mesenchymal transition (EMT) or dedifferentiation, both of which have been associated with drug resistance, become more vulnerable to ferroptosis (243, 585). Mechanistically, the EMT regulator ZEB1, which regulates lipid homeostasis and suppresses *GPX4* transcription, is at least partially involved (243, 436, 514). Iron accumulation induced by CD44-mediated iron uptake and decreased FPN expression may also make some contribution (46, 586). On the other hand, epithelial cells are resistant to ferroptosis due to intracellular interactions mediated by E-cadherin and $\alpha6\beta4$ integrin. E-cadherin activates the Hippo pathway to downregulate ACSL4 and TFR1 (512), whereas $\alpha6\beta4$ integrin activates the SCR signaling to downregulate ACSL4 (587). Furthermore, activation of the Hippo pathway sustains the expression of laminin 332, which is the ligand of $\alpha6\beta4$ integrin, whereas the binding of laminin 332 and $\alpha6\beta4$ integrin suppresses ZEB1-mediated EMT (588).

Although ferroptosis induction appears to have high potential for tumor eradication, the difficulty remains as to how to target tumor cells. For instance, a systemic inhibition of GPX4 may inevitably impair other healthy cells including those of the adaptive and innate immune systems. In this regard, a compound called N6F11 has been developed to induce tumor-specific GPX4 degradation by activating the tumor-specific E3 ligase TRIM25 (446). Unlike GPX4, xCT and FSP1 are considered safer targets given their dispensability for mouse survival. Indeed, xCT ablation significantly delays tumor progression and tumor metastasis in vivo, as evidenced by several xenograft models and a genetically engineered mouse model of PDAC (138, 357, 589, 590). In contrast, targeting FSP1 alone is usually not sufficient to trigger ferroptosis due to the presence of GPX4. Nevertheless, the combination of FSP1 inhibition and irradiation may be a promising strategy (480), as irradiation also has some proferroptotic effects (591–593) (**FIGURE 9**). To date, inhibitors of xCT and FSP1 for in vivo application are still under development, including imidazole ketone erastin (IKE) and icFSP1 (479, 594), but existing drugs like sulfasalazine and brequinar have been found to have off-target effects on xCT and FSP1, respectively (23, 417) (**FIGURE 10**). Other drugs, which have been implicated in ferroptosis induction, include altretamine that inhibits GPX4 activity (595), withaferin A that promotes GPX4 degradation and iron release from heme (82), methotrexate that targets DHFR (326), statins that interfere with the mevalonate pathway (238), APR-246 (aka eprenetapopt) that induces GSH depletion and NFS1 inhibition (596), and imetelstat that enriches PUFA-PLs (597) (**FIGURE 9**, **FIGURE 10**). The diversity of their proferroptotic mechanisms thus provides a toolbox that may be harnessed to selectively target the Achilles heel of certain types of cancer.

6. FUTURE PERSPECTIVE AND CONCLUDING REMARKS

When we compiled our previous review article on the relation between cell metabolism and ferroptosis published in 2020 (2), there were fewer than 1,000 publications on “ferroptosis” available on PubMed. Today, this number has risen to over 15,000 and continues to grow,

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

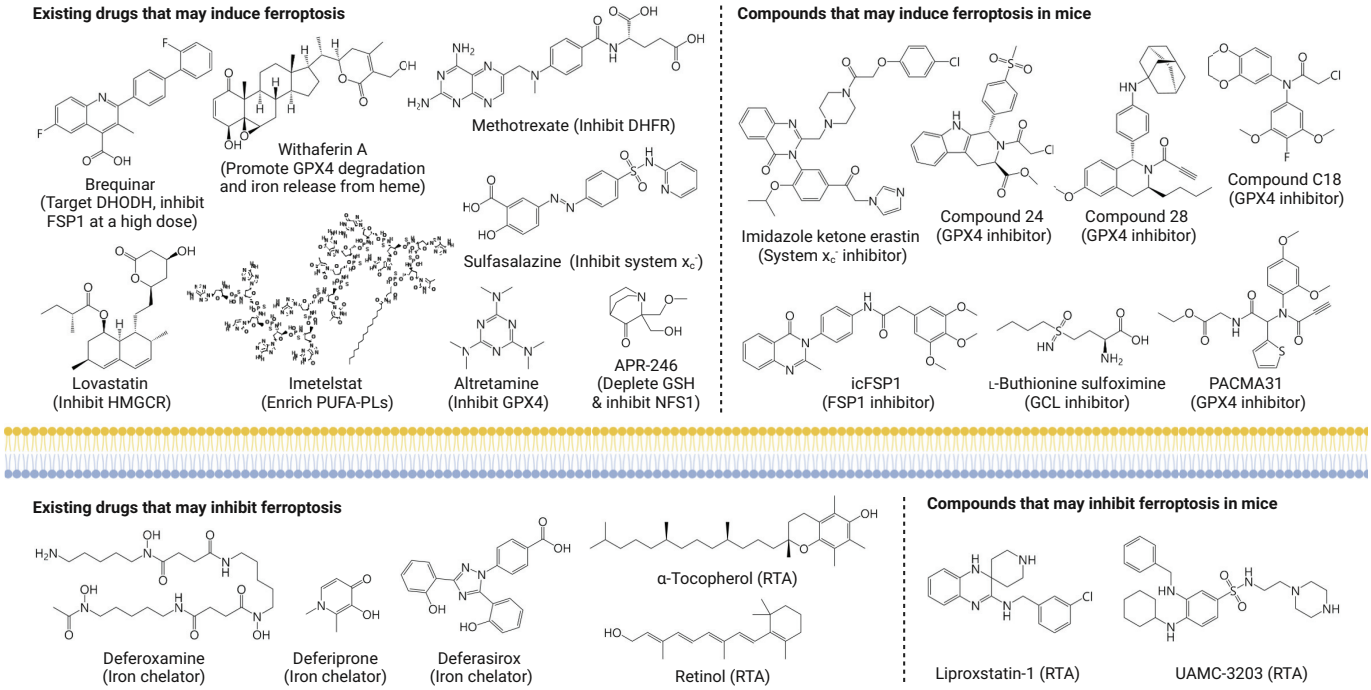


FIGURE 10. Existing drugs and compounds that may induce or inhibit ferroptosis in vivo. Existing drugs that may exert a proferroptotic effect include brequinar that targets dihydroorotate dehydrogenase (DHODH) but inhibits ferroptosis suppressor protein 1 (FSP1) at a high dose, statins (e.g., lovastatin) that block the mevalonate pathway by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), withaferin A that promotes glutathione peroxidase 4 (GPX4) degradation and iron release from heme, imetelstat that enriches polyunsaturated fatty acid (PUFA)-phospholipids (PLs), methotrexate that inhibits dihydrofolate reductase (DHFR), sulfasalazine that inhibits system x_c^-, altretamine that inhibits GPX4, and APR-246 that depletes glutathione (GSH) and blocks iron-sulfur cluster biogenesis by inhibiting NFS1. Compounds that have been reported to induce ferroptosis in mice include imidazole ketone erastin that inhibits system x_c^-, icFSP1 that inhibits FSP1, L-buthionine sulfoximine that blocks GSH synthesis by inhibiting glutamate-cysteine ligase (GCL), and compound 24, compound 28, compound C18, and PACMA31, which all inhibit GPX4. Existing drugs that may exert an antiferroptotic effect include deferoxamine, deferiprone, and deferasirox, which are iron chelators, and α-tocopherol and retinol, which act as radical trapping antioxidants (RTAs). Compounds that suitable for ferroptosis inhibition in mice include liproxstatin-1 and UAMC-3203, which are RTAs. Figure created with a licensed version of BioRender.com.

with dozens of new papers appearing daily. The significant efforts in this area have undoubtedly contributed to the rapid development of the field. However, several major challenges remain to be overcome.

First, we have not yet identified a readily available protein biomarker for ferroptosis that is as specific as the executioner proteins in apoptosis, necroptosis, and pyroptosis. Although an elevation in TFR1 may help distinguish ferroptotic cells from apoptotic ones (41, 42), it is important to note that TFR1 is ubiquitously expressed and regulated by various factors, including intracellular iron status. Recently, hyperoxidized peroxiredoxin 3 (PRDX3) was proposed as a potential marker for ferroptosis applicable to both in vitro and in vivo conditions (598). Nevertheless, cells lacking PRDX3 can still undergo ferroptosis (598). Moreover, its specificity to ferroptosis needs to be examined under a broader range of oxidative conditions and validated in typical ferroptotic mouse models with tissue-specific Gpx4 knockout. At present, lipid peroxidation is the most widely used biomarker for ferroptosis, but lipid peroxidation does not necessarily drive cell death, as a critical threshold of LOOH must be reached to trigger ferroptosis (254). Furthermore, lipid peroxidation can also occur in other oxidative stress conditions unrelated to ferroptosis. For instance, sorafenib induces cell death alongside lipid peroxidation, but this cell death is not rescued by liproxstatin-1 or the iron chelator deferiprone (356). Therefore, the current standard for identifying ferroptosis requires the simultaneous fulfillment of three conditions: cell death, lipid peroxidation, and rescue by ferroptosis inhibitors.

Second, we still have limited models to study ferroptosis in vivo. To date, the only widely acknowledged animal models for ferroptosis are tissue-specific *Gpx4* knockout mice, but these models have several shortcomings. One common criticism is the artificial nature of these models, as *Gpx4* is a housekeeping gene, and the loss of GPX4 is unlikely the primary driving force of ferroptosis under (patho)physiological conditions. Another significant limitation is the irreversibility of *Gpx4* deletion. Consequently, even if cell death can be temporarily inhibited by antiferroptotic agents, ferroptosis may still occur once these agents are withdrawn. Furthermore,

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

the extent of lipid peroxidation induced is not scalable, at the level of individual cells. Although administering a lower dose of tamoxifen can reduce the activation of Cre recombinase, thereby decreasing the number of ferroptotic cells and mitigating the phenotype in a given tissue/organ (599), the induction of lipid peroxidation in individual cells is an all-or-nothing decision. In addition to GPX4 perturbation, ischemia-reperfusion might be an alternative way to trigger ferroptosis in vivo. However, the major concern with this approach is that it may induce a mixture of cell death modalities.

Third, we have limited tools to manipulate ferroptosis in the human body. Although a few drugs have been found to promote ferroptosis as an off-target effect (as mentioned in sect. 5.2), no drug has been specifically developed to trigger ferroptosis hitherto. For in vivo experiments, several agents have been developed: engineered human cyst(e)inase to deplete serum cyst(e)ine (600), IKE to inhibit system x_c^- (594), L-buthionine sulfoximine (BSO) to target GCL (601), icFSP1 to target human FSP1 (479), and several compounds (e.g., PACMA31, compound C18, compound 24, and compound 28) to target GPX4 (351, 602–604) (**FIGURE 10**). Whether these developments can be eventually translated into clinical practice remains to be seen.

As for ferroptosis inhibitors, iron chelators such as deferoxamine, deferiprone, and deferasirox are clinically approved but are typically used to treat systemic iron overload (**FIGURE 10**). Two recent clinical trials investigating the use of deferiprone to alleviate symptoms in patients with amyloid-confirmed early Alzheimer's disease and in newly diagnosed Parkinson's disease patients not receiving dopaminergic treatment resulted in even worsened outcomes (605, 606). Another clinical trial is currently underway to assess deferiprone in the treatment of early amyotrophic lateral sclerosis (NCT03293069). However, whether iron chelators can prevent ferroptosis in vivo remains unclear.

As a representative of RTAs, α-tocopherol is clinically available and has been used worldwide as a dietary supplement (**FIGURE 10**). Despite numerous positive results from cell culture and animal studies including models of genetic *Gpx4* deficiency, α-tocopherol supplementation does not appear to significantly affect all-cause mortality or provide benefits for cardiovascular diseases in humans (607). Conversely, daily dietary supplementation with 400 IU of all-rac-α-tocopheryl acetate significantly increases the risk of prostate cancer among healthy men (608). Recently, retinol (vitamin A) and its metabolites were found to halt lipid peroxidation by acting as RTAs (609, 610) (**FIGURE 10**). Additionally, all-*trans* retinoic acid also orchestrates the expression of antiferroptotic genes via the retinoic acid receptor (611). Nevertheless, whether α-tocopherol and retinol can inhibit ferroptosis in the human body remains uncertain, similar to the case with iron chelators. Compared to α-tocopherol and retinol, ferrostatin-1 and liproxstatin-1 are much more effective at inhibiting ferroptosis (612). However, only liproxstatin-1 is suitable for in vivo conditions because of its superior pharmacokinetic properties (31) (**FIGURE 10**). In addition, UAMC-3203, a metabolically more stable ferrostatin-1 analog, showed improved in vivo efficacy (613) (**FIGURE 10**). The (preclinical) development of next-generation liproxstatins is currently in progress, and we are eagerly looking forward to its potential clinical translation.

In conclusion, given the active involvement of ferroptosis in various pathological conditions and its substantial potential in cancer therapy, we believe that the field of ferroptosis is poised for a promising and impactful future.

CORRESPONDENCE

M. Conrad (marcus.conrad@helmholtz-munich.de).

ACKNOWLEDGMENTS

Graphical abstract created with a licensed version of BioRender.com.

GRANTS

This work was supported by Deutsche Forschungsgemeinschaft (DFG) (CO 291/7-1), the DFG Priority Program SPP 2306 (CO 291/9-1, No. 461385412; CO 291/10-1, No. 461507177), and the CRC TRR 353 (CO 291/11-1; No. 471011418); the German Federal Ministry of Education and Research (BMBF) FERROPATH (01EJ2205B); and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (Grant Agreement No. GA 884754).

DISCLOSURES

M.C. is a cofounder and shareholder of ROSCUE Therapeutics GmbH and holds patents for some of the compounds described here. J.Z. has no conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

J.Z. prepared figures; J.Z. drafted manuscript; M.C. edited and revised manuscript; J.Z. and M.C. approved final version of manuscript.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

REFERENCES

1. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. **Cell Death Differ** 25: 486–541, 2018. doi:10.1038/s41418-017-0012-4.

2. Zheng J, Conrad M. The metabolic underpinnings of ferroptosis. **Cell Metab** 32: 920–937, 2020. doi:10.1016/j.cmet.2020.10.011.

3. Dixon SJ. Ferroptosis: bug or feature? **Immunol Rev** 277: 150–157, 2017. doi:10.1111/imr.12533.

4. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison B 3rd, Stockwell BR. Ferroptosis: an iron-dependent form of nonapoptotic cell death. **Cell** 149: 1060–1072, 2012. doi:10.1016/j.cell.2012.03.042.

5. Weichselbaum TE. Cystine deficiency in the albino rat. **Exp Physiol** 25: 363–367, 1935. doi:10.1113/expphysiol.1935.sp000693.

6. Gyorgy P. Relation of dietary factors in liver injury. **Trans Conf Liver Inj** 41: 67–72, 1947.

7. Gyorgy P, Goldblatt H. Further observations on the production and prevention of dietary hepatic injury in rats. **J Exp Med** 89: 245–268, 1949. doi:10.1084/jem.89.2.245.

8. Schwarz K. Über einen tödlichen, ernährungsbedingten Leberschaden und das Vorkommen von Leberschutzstoffen. **Hoppe Seylers Z Physiol Chem** 281: 101–108, 1944. doi:10.1515/bchm2.1944.281.3-4.101.

9. Schwarz K. Tocopherol als Leberschutzstoff. **Hoppe Seylers Z Physiol Chem** 281: 109–116, 1944. doi:10.1515/bchm2.1944.281.3-4.109.

10. Schwarz K, Foltz CM. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. **J Am Chem Soc** 79: 3292–3293, 1957. doi:10.1021/ja01569a087.

11. Corwin LM. Studies on peroxidation in vitamin E-deficient rat liver homogenates. **Arch Biochem Biophys** 97: 51–58, 1962. doi:10.1016/0003-9861(62)90043-7.

12. Eagle H. The specific amino acid requirements of a human carcinoma cell (Stain HeLa) in tissue culture. **J Exp Med** 102: 37–48, 1955. doi:10.1084/jem.102.1.37.

13. Eagle H. The specific amino acid requirements of a mammalian cell (strain L) in tissue culture. **J Biol Chem** 214: 839–852, 1955. doi:10.1016/S0021-9258(18)70932-0.

14. Ishii T, Hishinuma I, Bannai S, Sugita Y. Mechanism of growth promotion of mouse lymphoma L1210 cells in vitro by feeder layer or 2-mercaptoethanol. **J Cell Physiol** 107: 283–293, 1981. doi:10.1002/jcp.1041070215.

15. Hishinuma I, Ishii T, Watanabe H, Bannai S. Mouse lymphoma L1210 cells acquire a new cystine transport activity upon adaptation in vitro. **In Vitro Cell Dev Biol** 22: 127–134, 1986. doi:10.1007/BF02623499.

16. Bannai S. Exchange of cystine and glutamate across plasma membrane of human fibroblasts. **J Biol Chem** 261: 2256–2263, 1986. doi:10.1016/S0021-9258(17)35926-4.

17. Bannai S, Kitamura E. Transport interaction of L-cystine and L-glutamate in human diploid fibroblasts in culture. **J Biol Chem** 255: 2372–2376, 1980. doi:10.1016/S0021-9258(19)85901-X.

18. May PC, Gray PN. The mechanism of glutamate-induced degeneration of cultured Huntington's disease and control fibroblasts. **J Neurol Sci** 70: 101–112, 1985. doi:10.1016/0022-510x(85)90192-3.

19. Choi DW, Maulucci-Gedde M, Kriegstein AR. Glutamate neurotoxicity in cortical cell culture. **J Neurosci** 7: 357–368, 1987. doi:10.1523/JNEUROSCI.07-02-00357.1987.

20. Tan S, Schubert D, Maher P. Oxytosis: a novel form of programmed cell death. **Curr Top Med Chem** 1: 497–506, 2001. doi:10.2174/1568026013394741.

21. Sato H, Tamba M, Ishii T, Bannai S. Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. **J Biol Chem** 274: 11455–11458, 1999. doi:10.1074/jbc.274.17.11455.

22. Sato H, Shiiya A, Kimata M, Maebara K, Tamba M, Sakakura Y, Makino N, Sugiyama F, Yagami K, Moriguchi T, Takahashi S, Bannai S. Redox imbalance in cystine/glutamate transporter-deficient mice. **J Biol Chem** 280: 37423–37429, 2005. doi:10.1074/jbc.M506439200.

23. Gout PW, Buckley AR, Simms CR, Bruchovsky N. Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the x_c^- cystine transporter: a new action for an old drug. **Leukemia** 15: 1633–1640, 2001. doi:10.1038/sj.leu.2402238.

24. Dolma S, Lessnick SL, Hahn WC, Stockwell BR. Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. **Cancer Cell** 3: 285–296, 2003. doi:10.1016/s1535-6108(03)00050-3.

25. Ursini F, Maiorino M, Valente M, Ferri L, Gregolin C. Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. **Biochim Biophys Acta** 710: 197–211, 1982. doi:10.1016/0005-2760(82)90150-3.

26. Ursini F, Maiorino M, Gregolin C. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. **Biochim Biophys Acta** 839: 62–70, 1985. doi:10.1016/0304-4165(85)90182-5.

27. Yant LJ, Ran Q, Rao L, Van Remmen H, Shibatani T, Belter JG, Motta L, Richardson A, Prolla TA. The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. **Free Radic Biol Med** 34: 496–502, 2003. doi:10.1016/s0891-5849(02)01360-6.

28. Seiler A, Schneider M, Förster H, Roth S, Wirth EK, Culmsee C, Plesnila N, Kremmer E, Rådmark O, Wurst W, Bornkamm GW, Schweizer U, Conrad M. Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. **Cell Metab** 8: 237–248, 2008. doi:10.1016/j.cmet.2008.07.005.

29. Yang WS, Stockwell BR. Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. **Chem Biol** 15: 234–245, 2008. doi:10.1016/j.chembiol.2008.02.010.

30. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, Brown LM, Girotti AW, Cornish VW, Schreiber SL, Stockwell BR. Regulation of ferroptotic cancer cell death by GPX4. **Cell** 156: 317–331, 2014. doi:10.1016/j.cell.2013.12.010.

31. Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, Herbach N, Aichler M, Walch A, Eggenhofer E, Basavarajappa D, Rådmark O, Kobayashi S, Seibt T, Beck H, Neff F, Esposito I, Wanke R, Förster H, Yefremova O, Heinrichmeyer M, Bornkamm GW, Geissler EK, Thomas SB, Stockwell BR, O'Donnell

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

VB, Kagan VE, Schick JA, Conrad M. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. **Nat Cell Biol** 16: 1180–1191, 2014. doi:10.1038/ncb3064.

32. Galy B, Conrad M, Muckenthaler M. Mechanisms controlling cellular and systemic iron homeostasis. **Nat Rev Mol Cell Biol** 25: 133–155, 2024. doi:10.1038/s41580-023-00648-1.

33. Gao M, Monian P, Quadri N, Ramasamy R, Jiang X. Glutaminolysis and transferrin regulate ferroptosis. **Mol Cell** 59: 298–308, 2015. doi:10.1016/j.molcel.2015.06.011.

34. Yu Y, Jiang L, Wang H, Shen Z, Cheng Q, Zhang P, Wang J, Wu Q, Fang X, Duan L, Wang S, Wang K, An P, Shao T, Chung RT, Zheng S, Min J, Wang F. Hepatic transferrin plays a role in systemic iron homeostasis and liver ferroptosis. **Blood** 136: 726–739, 2020. doi:10.1182/blood.2019002907.

35. Torii S, Shintoku R, Kubota C, Yaegashi M, Torii R, Sasaki M, Suzuki T, Mori M, Yoshimoto Y, Takeuchi T, Yamada K. An essential role for functional lysosomes in ferroptosis of cancer cells. **Biochem J** 473: 769–777, 2016. doi:10.1042/BJ20150658.

36. Wu Y, Jiao H, Yue Y, He K, Jin Y, Zhang J, Zhang J, Wei Y, Luo H, Hao Z, Zhao X, Xia Q, Zhong Q, Zhang J. Ubiquitin ligase E3 HUWE1/MULE targets transferrin receptor for degradation and suppresses ferroptosis in acute liver injury. **Cell Death Differ** 29: 1705–1718, 2022. doi:10.1038/s41418-022-00957-6.

37. Liu L, Zhang C, Qu S, Liu R, Chen H, Liang Z, Tian Z, Li L, Ma S, Liu X. ESR1 inhibits ionizing radiation-induced ferroptosis in breast cancer cells via the NEDD4L/CD71 pathway. **Arch Biochem Biophys** 725: 109299, 2022. doi:10.1016/j.abb.2022.109299.

38. Shao M, Qi K, Wang L, Yu X, Zhang Q, Yu L, Wang L, Yang C, Fan L. E3 ubiquitin ligase CHIP interacts with transferrin receptor 1 for degradation and promotes cell proliferation through inhibiting ferroptosis in hepatocellular carcinoma. **Cell Signal** 118: 111148, 2024. doi:10.1016/j.cellsig.2024.111148.

39. Lu Y, Yang Q, Su Y, Ji Y, Li G, Yang X, Xu L, Lu Z, Dong J, Wu Y, Bei JX, Pan C, Gu X, Li B. MYCN mediates TFRC-dependent ferroptosis and reveals vulnerabilities in neuroblastoma. **Cell Death Dis** 12: 511, 2021. doi:10.1038/s41419-021-03790-w.

40. Song J, Liu T, Yin Y, Zhao W, Lin Z, Yin Y, Lu D, You F. The deubiquitinase OTUD1 enhances iron transport and potentiates host antitumor immunity. **EMBO Rep** 22: e51162, 2021. doi:10.15252/embr.202051162.

41. Feng H, Schorpp K, Jin J, Yozwiak CE, Hoffstrom BG, Decker AM, Rajbhandari P, Stokes ME, Bender HG, Csuka JM, Upadhyayula PS, Canoll P, Uchida K, Soni RK, Hadian K, Stockwell BR. Transferrin receptor is a specific ferroptosis marker. **Cell Rep** 30: 3411–3423.e7, 2020. doi:10.1016/j.celrep.2020.02.049.

42. Jin J, Schorpp K, Samaga D, Unger K, Hadian K, Stockwell BR. Machine learning classifies ferroptosis and apoptosis cell death modalities with TfR1 immunostaining. **ACS Chem Biol** 17: 654–660, 2022. doi:10.1021/acschembio.1c00953.

43. Jiang W, Liu J, Cui J, Su J, Xu W, Zhang F, Ding Y. Ferroptosis plays a crucial role in lung cell damage caused by ventilation stretch. **Free Radic Biol Med** 209: 84–95, 2023. doi:10.1016/j.freeradbiomed.2023.10.381.

44. Zhang T, Wang S, Hua D, Shi X, Deng H, Jin S, Lv X. Identification of ZIP8-induced ferroptosis as a major type of cell death in monocytes under sepsis conditions. **Redox Biol** 69: 102985, 2024. doi:10.1016/j.redox.2023.102985.

45. Liu Z, Huang J, Li D, Zhang C, Wan H, Zeng B, Tan Y, Zhong F, Liao H, Liu M, Chen ZS, Zou C, Liu D, Qin B. Targeting ZIP8 mediated ferroptosis as a novel strategy to protect against the retinal pigment epithelial degeneration. **Free Radic Biol Med** 214: 42–53, 2024. doi:10.1016/j.freeradbiomed.2024.01.053.

46. Müller S, Sindikubwabo F, Cañeque T, Lafon A, Versini A, Lombard B, Loew D, Wu TD, Ginestier C, Charafe-Jauffret E, Durand A, Vallot C, Baulande S, Servant N, Rodriguez R. CD44 regulates epigenetic plasticity by mediating iron endocytosis. **Nat Chem** 12: 929–938, 2020. doi:10.1038/s41557-020-0513-5.

47. Lee J, Roh JL. Promotion of ferroptosis in head and neck cancer with divalent metal transporter 1 inhibition or salinomycin. **Hum Cell** 36: 1090–1098, 2023. doi:10.1007/s13577-023-00890-x.

48. Turcu AL, Versini A, Khene N, Gaillet C, Cañeque T, Müller S, Rodriguez R. DMT1 inhibitors kill cancer stem cells by blocking lysosomal iron translocation. **Chemistry** 26: 7369–7373, 2020. doi:10.1002/chem.202000159.

49. Protchenko O, Baratz E, Jadhav S, Li F, Shakoury-Elizeh M, Gavrilova O, Ghosh MC, Cox JE, Maschek JA, Tyurin VA, Tyurina YY, Bayir H, Aron AT, Chang CJ, Kagan VE, Philpott CC. Iron chaperone Poly rC Binding Protein 1 protects mouse liver from lipid peroxidation and steatosis. **Hepatology** 73: 1176–1193, 2021. doi:10.1002/hep.31328.

50. Lee J, You JH, Roh JL. Poly(rC)-binding protein 1 represses ferritinophagy-mediated ferroptosis in head and neck cancer. **Redox Biol** 51: 102276, 2022. doi:10.1016/j.redox.2022.102276.

51. Yue L, Luo Y, Jiang L, Sekido Y, Toyokuni S. PCBP2 knockdown promotes ferroptosis in malignant mesothelioma. **Pathol Int** 72: 242–251, 2022. doi:10.1111/pin.13209.

52. Tan Q, Zhang X, Li S, Liu W, Yan J, Wang S, Cui F, Li D, Li J. DMT1 differentially regulates mitochondrial complex activities to reduce glutathione loss and mitigate ferroptosis. **Free Radic Biol Med** 207: 32–44, 2023. doi:10.1016/j.freeradbiomed.2023.06.023.

53. Zhang T, Sun L, Hao Y, Suo C, Shen S, Wei H, Ma W, Zhang P, Wang T, Gu X, Li ST, Chen Z, Yan R, Zhang Y, Cai Y, Zhou R, Jia W, Huang F, Gao P, Zhang H. ENO1 suppresses cancer cell ferroptosis by degrading the mRNA of iron regulatory protein 1. **Nat Cancer** 3: 75–89, 2022. doi:10.1038/s43018-021-00299-1.

54. Tao H, Dar HY, Tian C, Banerjee S, Glazer ES, Srinivasan S, Zhu L, Pacifici R, He P. Differences in hepatocellular iron metabolism underlie sexual dimorphism in hepatocyte ferroptosis. **Redox Biol** 67: 102892, 2023. doi:10.1016/j.redox.2023.102892.

55. Zhang Z, Guo M, Shen M, Kong D, Zhang F, Shao J, Tan S, Wang S, Chen A, Cao P, Zheng S. The BRD7-P53-SLC25A28 axis regulates ferroptosis in hepatic stellate cells. **Redox Biol** 36: 101619, 2020. doi:10.1016/j.redox.2020.101619.

56. Alvarez SW, Sviderskiy VO, Terzi EM, Papagiannakopoulos T, Moreira AL, Adams S, Sabatini DM, Birsoy K, Possemato R. NFS1 undergoes positive selection in lung tumours and protects cells from ferroptosis. **Nature** 551: 639–643, 2017. doi:10.1038/nature24637.

57. Green YS, Ferreira Dos Santos MC, Fuja DG, Reichert EC, Campos AR, Cowman SJ, Acuña Pilarte K, Kohan J, Tripp SR, Leibold EA, Sirohi D, Agarwal N, Liu X, Koh MY. ISCA2 inhibition decreases HIF and induces ferroptosis in clear cell renal carcinoma. **Oncogene** 41: 4709–4723, 2022. doi:10.1038/s41388-022-02460-1.

58. Yuan H, Li X, Zhang X, Kang R, Tang D. CISD1 inhibits ferroptosis by protection against mitochondrial lipid peroxidation. **Biochem**

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

Biophys Res Commun 478: 838–844, 2016. doi:10.1016/j.bbrc.2016.08.034.

59. Kim EH, Shin D, Lee J, Jung AR, Roh JL. CISD2 inhibition overcomes resistance to sulfasalazine-induced ferroptotic cell death in head and neck cancer. **Cancer Lett** 432: 180–190, 2018. doi:10.1016/j.canlet.2018.06.018.

60. Homma T, Kobayashi S, Fujii J. Cysteine preservation confers resistance to glutathione-depleted cells against ferroptosis via CDGSH iron sulphur domain-containing proteins (CISDs). **Free Radic Res** 54: 397–407, 2020. doi:10.1080/10715762.2020.1780229.

61. Li Y, Wang X, Huang Z, Zhou Y, Xia J, Hu W, Wang X, Du J, Tong X, Wang Y. CISD3 inhibition drives cystine-deprivation induced ferroptosis. **Cell Death Dis** 12: 839, 2021. doi:10.1038/s41419-021-04128-2.

62. Du J, Zhou Y, Li Y, Xia J, Chen Y, Chen S, Wang X, Sun W, Wang T, Ren X, Wang X, An Y, Lu K, Hu W, Huang S, Li J, Tong X, Wang Y. Identification of Frataxin as a regulator of ferroptosis. **Redox Biol** 32: 101483, 2020. doi:10.1016/j.redox.2020.101483.

63. Zhang Z, Jiang W, Zhang C, Yin Y, Mu N, Wang Y, Yu L, Ma H. Frataxin inhibits the sensitivity of the myocardium to ferroptosis by regulating iron homeostasis. **Free Radic Biol Med** 205: 305–317, 2023. doi:10.1016/j.freeradbiomed.2023.06.016.

64. Lee J, You JH, Shin D, Roh JL. Inhibition of glutaredoxin 5 predisposes cisplatin-resistant head and neck cancer cells to ferroptosis. **Theranostics** 10: 7775–7786, 2020. doi:10.7150/thno.46903.

65. Wang T, Yang C, Li Z, Li T, Zhang R, Zhao Y, Cheng T, Zong Z, Ma Y, Zhang D, Deng H. Flavonoid 4,4'-dimethoxychalcone selectively eliminates senescent cells via activating ferritinophagy. **Redox Biol** 69: 103017, 2024. doi:10.1016/j.redox.2023.103017.

66. Yang C, Wang T, Zhao Y, Meng X, Ding W, Wang Q, Liu C, Deng H. Flavonoid 4,4'-dimethoxychalcone induced ferroptosis in cancer cells by synergistically activating Keap1/Nrf2/HMOX1 pathway and inhibiting FECH. **Free Radic Biol Med** 188: 14–23, 2022. doi:10.1016/j.freeradbiomed.2022.06.010.

67. Yang X, Ding Y, Sun L, Shi M, Zhang P, Huang Z, Wang J, He A, Wang J, Wei J, Liu M, Liu J, Wang G, Yang X, Li R. Ferritin light chain deficiency-induced ferroptosis is involved in preeclampsia pathophysiology by disturbing uterine spiral artery remodelling. **Redox Biol** 58: 102555, 2022. doi:10.1016/j.redox.2022.102555.

68. Fang X, Cai Z, Wang H, Han D, Cheng Q, Zhang P, Gao F, Yu Y, Song Z, Wu Q, An P, Huang S, Pan J, Chen HZ, Chen J, Linkermann A, Min J, Wang F. Loss of cardiac Ferritin H facilitates cardiomyopathy via Slc7a11-mediated ferroptosis. **Circ Res** 127: 486–501, 2020. doi:10.1161/CIRCRESAHA.120.316509.

69. Gao M, Monian P, Pan Q, Zhang W, Xiang J, Jiang X. Ferroptosis is an autophagic cell death process. **Cell Res** 26: 1021–1032, 2016. doi:10.1038/cr.2016.95.

70. Hou W, Xie Y, Song X, Sun X, Lotze MT, Zeh HJ 3rd, Kang R, Tang D. Autophagy promotes ferroptosis by degradation of ferritin. **Autophagy** 12: 1425–1428, 2016. doi:10.1080/15548627.2016.1187366.

71. Bellelli R, Federico G, Matte A, Colecchia D, Iolascon A, Chiariello M, Santoro M, De Franceschi L, Carlomagno F. NCOA4 deficiency impairs systemic iron homeostasis. **Cell Rep** 14: 411–421, 2016. doi:10.1016/j.celrep.2015.12.065.

72. Yao F, Peng J, Zhang E, Ji D, Gao Z, Tang Y, Yao X, Xia X. Pathologically high intraocular pressure disturbs normal iron homeostasis and leads to retinal ganglion cell ferroptosis in glaucoma. **Cell Death Differ** 30: 69–81, 2023. doi:10.1038/s41418-022-01046-4.

73. Fang Y, Chen X, Tan Q, Zhou H, Xu J, Gu Q. Inhibiting ferroptosis through disrupting the NCOA4-FTH1 Interaction: a new mechanism of action. **ACS Cent Sci** 7: 980–989, 2021. doi:10.1021/acscentsci.0c01592.

74. Zhou H, Zhou YL, Mao JA, Tang LF, Xu J, Wang ZX, He Y, Li M. NCOA4-mediated ferritinophagy is involved in ionizing radiation-induced ferroptosis of intestinal epithelial cells. **Redox Biol** 55: 102413, 2022. doi:10.1016/j.redox.2022.102413.

75. Bartnikas TB, Campagna DR, Antiochos B, Mulhern H, Pondarré C, Fleming MD. Characterization of mitochondrial ferritin-deficient mice. **Am J Hematol** 85: 958–960, 2010. doi:10.1002/ajh.21872.

76. Wang YQ, Chang SY, Wu Q, Gou YJ, Jia L, Cui YM, Yu P, Shi ZH, Wu WS, Gao G, Chang YZ. The protective role of mitochondrial ferritin on erastin-induced ferroptosis. **Front Aging Neurosci** 8: 308, 2016. doi:10.3389/fnagi.2016.00308.

77. Fuhrmann DC, Mondorf A, Beifuß J, Jung M, Brüne B. Hypoxia inhibits ferritinophagy, increases mitochondrial ferritin, and protects from ferroptosis. **Redox Biol** 36: 101670, 2020. doi:10.1016/j.redox.2020.101670.

78. Hong X, Roh W, Sullivan RJ, Wong KH, Wittner BS, Guo H, Dubash TD, Sade-Feldman M, Wesley B, Horwitz E, Boland GM, Marvin DL, Bonesteel T, Lu C, Aguet F, Burr R, Freeman SS, Parida L, Calhoun K, Jewett MK, Nieman LT, Hacohen N, Näär AM, Ting DT, Toner M, Stott SL, Getz G, Maheswaran S, Haber DA. The lipogenic regulator SREBP2 induces transferrin in circulating melanoma cells and suppresses ferroptosis. **Cancer Discov** 11: 678–695, 2021. doi:10.1158/2159-8290.CD-19-1500.

79. Yao F, Deng Y, Zhao Y, Mei Y, Zhang Y, Liu X, Martinez C, Su X, Rosato RR, Teng H, Hang Q, Yap S, Chen D, Wang Y, Chen MM, Zhang M, Liang H, Xie D, Chen X, Zhu H, Chang JC, You MJ, Sun Y, Gan B, Ma L. A targetable LIFR-NF-κB-LCN2 axis controls liver tumorigenesis and vulnerability to ferroptosis. **Nat Commun** 12: 7333, 2021. doi:10.1038/s41467-021-27452-9.

80. Liu J, Song X, Kuang F, Zhang Q, Xie Y, Kang R, Kroemer G, Tang D. NUPR1 is a critical repressor of ferroptosis. **Nat Commun** 12: 647, 2021. doi:10.1038/s41467-021-20904-2.

81. Fang X, Wang H, Han D, Xie E, Yang X, Wei J, Gu S, Gao F, Zhu N, Yin X, Cheng Q, Zhang P, Dai W, Chen J, Yang F, Yang HT, Linkermann A, Gu W, Min J, Wang F. Ferroptosis as a target for protection against cardiomyopathy. **Proc Natl Acad Sci USA** 116: 2672–2680, 2019. doi:10.1073/pnas.1821022116.

82. Hassannia B, Wiernicki B, Ingold I, Qu F, Van Herck S, Tyurina YY, Bayır H, Abhari BA, Angeli JP, Choi SM, Meul E, Heyninck K, Declerck K, Chirumamilla CS, Lahtela-Kakkonen M, Van Camp G, Krysko DV, Ekert PG, Fulda S, De Geest BG, Conrad M, Kagan VE, Vanden Berghe W, Vandenabeele P, Vanden Berghe T. Nano-targeted induction of dual ferroptotic mechanisms eradicates high-risk neuroblastoma. **J Clin Invest** 128: 3341–3355, 2018. doi:10.1172/JCI99032.

83. Menon AV, Liu J, Tsai HP, Zeng L, Yang S, Asnani A, Kim J. Excess heme upregulates heme oxygenase 1 and promotes cardiac ferroptosis in mice with sickle cell disease. **Blood** 139: 936–941, 2022. doi:10.1182/blood.2020008455.

84. Tang Z, Ju Y, Dai X, Ni N, Liu Y, Zhang D, Gao H, Sun H, Zhang J, Gu P. HO-1-mediated ferroptosis as a target for protection against retinal pigment epithelium degeneration. **Redox Biol** 43: 101971, 2021. doi:10.1016/j.redox.2021.101971.

85. Adedoyin O, Boddu R, Traylor A, Lever JM, Bolisetty S, George JF, Agarwal A. Heme oxygenase-1 mitigates ferroptosis in renal

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

proximal tubule cells. **Am J Physiol Renal Physiol** 314: F702–F714, 2018. doi:10.1152/ajprenal.00044.2017.

86. Roh JL, Kim EH, Jang H, Shin D**.** Nrf2 inhibition reverses the resistance of cisplatin-resistant head and neck cancer cells to artesunate-induced ferroptosis. **Redox Biol** 11: 254–262, 2017. doi:10.1016/j.redox.2016.12.010.

87. Bao WD, Pang P, Zhou XT, Hu F, Xiong W, Chen K, Wang J, Wang F, Xie D, Hu YZ, Han ZT, Zhang HH, Wang WX, Nelson PT, Chen JG, Lu Y, Man HY, Liu D, Zhu LQ**.** Loss of ferroportin induces memory impairment by promoting ferroptosis in Alzheimer's disease. **Cell Death Differ** 28: 1548–1562, 2021. doi:10.1038/s41418-020-00685-9.

88. Soofi A, Li V, Beamish JA, Abdrabh S, Hamad M, Das NK, Shah YM, Dressler GR**.** Renal-specific loss of ferroportin disrupts iron homeostasis and attenuates recovery from acute kidney injury. **Am J Physiol Renal Physiol** 326: F178–F188, 2024. doi:10.1152/ajprenal.00184.2023.

89. Shang Y, Luo M, Yao F, Wang S, Yuan Z, Yang Y**.** Ceruloplasmin suppresses ferroptosis by regulating iron homeostasis in hepatocellular carcinoma cells. **Cell Signal** 72: 109633, 2020. doi:10.1016/j.cellsig.2020.109633.

90. Brown CW, Amante JJ, Chhoy P, Elaimy AL, Liu H, Zhu LJ, Baer CE, Dixon SJ, Mercurio AM**.** Prominin2 drives ferroptosis resistance by stimulating iron export. **Dev Cell** 51: 575–586.e4, 2019. doi:10.1016/j.devcel.2019.10.007.

91. Kumar A, Ye C, Nkansah A, Decoville T, Fogo GM, Sajjakulnukit P, Reynolds MB, Zhang L, Quaye O, Seo YA, Sanderson TH, Lyssiotis CA, Chang CH**.** Iron regulates the quiescence of naive CD4 T cells by controlling mitochondria and cellular metabolism. **Proc Natl Acad Sci USA** 121: e2318420121, 2024. doi:10.1073/pnas.2318420121.

92. Zhang HL, Hu BX, Ye ZP, Li ZL, Liu S, Zhong WQ, Du T, Yang D, Mai J, Li LC, Chen YH, Zhu XY, Li X, Feng GK, Zhu XF, Deng R**.** TRPML1 triggers ferroptosis defense and is a potential therapeutic target in AKT-hyperactivated cancer. **Sci Transl Med** 16: eadk0330, 2024. doi:10.1126/scitranslmed.adk0330.

93. Conrad M, Proneth B**.** Selenium: tracing another essential element of ferroptotic cell death. **Cell Chem Biol** 27: 409–419, 2020. doi:10.1016/j.chembiol.2020.03.012.

94. Schweizer U, Streckfuss F, Pelt P, Carlson BA, Hatfield DL, Köhrle J, Schomburg L**.** Hepatically derived selenoprotein P is a key factor for kidney but not for brain selenium supply. **Biochem J** 386: 221–226, 2005. doi:10.1042/BJ20041973.

95. Renko K, Werner M, Renner-Müller I, Cooper TG, Yeung CH, Hollenbach B, Scharpf M, Köhrle J, Schomburg L, Schweizer U**.** Hepatic selenoprotein P (SePP) expression restores selenium transport and prevents infertility and motor-incoordination in Sepp-knockout mice. **Biochem J** 409: 741–749, 2008. doi:10.1042/BJ20071172.

96. Schomburg L, Schweizer U, Holtmann B, Flohé L, Sendtner M, Köhrle J**.** Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues. **Biochem J** 370: 397–402, 2003. doi:10.1042/BJ20021853.

97. Hill KE, Zhou J, McMahan WJ, Motley AK, Atkins JF, Gesteland RF, Burk RF**.** Deletion of selenoprotein P alters distribution of selenium in the mouse. **J Biol Chem** 278: 13640–13646, 2003. doi:10.1074/jbc.M300755200.

98. Ingold I, Berndt C, Schmitt S, Doll S, Poschmann G, Buday K, Roveri A, Peng X, Porto Freitas F, Seibt T, Mehr L, Aichler M, Walch A, Lamp D, Jastroch M, Miyamoto S, Wurst W, Ursini F, Arnér ES, Fradejas-Villar N, Schweizer U, Zischka H, Friedmann Angeli JP, Conrad M**.** Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. **Cell** 172: 409–422.e21, 2018. doi:10.1016/j.cell.2017.11.048.

99. Imai H, Hakkaku N, Iwamoto R, Suzuki J, Suzuki T, Tajima Y, Konishi K, Minami S, Ichinose S, Ishizaka K, Shioda S, Arata S, Nishimura M, Naito S, Nakagawa Y**.** Depletion of selenoprotein GPx4 in spermatocytes causes male infertility in mice. **J Biol Chem** 284: 32522–32532, 2009. doi:10.1074/jbc.M109.016139.

100. Kitabayashi N, Nakao S, Mita Y, Arisawa K, Hoshi T, Toyama T, Ishii KA, Takamura T, Noguchi N, Saito Y**.** Role of selenoprotein P expression in the function of pancreatic beta cells: prevention of ferroptosis-like cell death and stress-induced nascent granule degradation. **Free Radic Biol Med** 183: 89–103, 2022. doi:10.1016/j.freeradbiomed.2022.03.009.

101. Zheng X, Toyama T, Siu S, Kaneko T, Sugiura H, Yamashita S, Shimoda Y, Kanamori M, Arisawa K, Endo H, Saito Y**.** Selenoprotein P expression in glioblastoma as a regulator of ferroptosis sensitivity: preservation of GPX4 via the cycling-selenium storage. **Sci Rep** 14: 682, 2024. doi:10.1038/s41598-024-51259-5.

102. Schomburg L**.** Selenoprotein P—selenium transport protein, enzyme and biomarker of selenium status. **Free Radic Biol Med** 191: 150–163, 2022. doi:10.1016/j.freeradbiomed.2022.08.022.

103. Trommsdorff M, Gotthardt M, Hiesberger T, Shelton J, Stockinger W, Nimpf J, Hammer RE, Richardson JA, Herz J**.** Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. **Cell** 97: 689–701, 1999. doi:10.1016/s0092-8674(00)80782-5.

104. Andersen OM, Yeung CH, Vorum H, Wellner M, Andreassen TK, Erdmann B, Mueller EC, Herz J, Otto A, Cooper TG, Willnow TE**.** Essential role of the apolipoprotein E receptor-2 in sperm development. **J Biol Chem** 278: 23989–23995, 2003. doi:10.1074/jbc.M302157200.

105. Masiulis I, Quill TA, Burk RF, Herz J**.** Differential functions of the Apoer2 intracellular domain in selenium uptake and cell signaling. **Biol Chem** 390: 67–73, 2009. doi:10.1515/BC.2009.011.

106. Olson GE, Winfrey VP, Hill KE, Burk RF**.** Megalin mediates selenoprotein P uptake by kidney proximal tubule epithelial cells. **J Biol Chem** 283: 6854–6860, 2008. doi:10.1074/jbc.M709945200.

107. Chiu-Ugalde J, Theilig F, Behrends T, Drebes J, Sieland C, Subbarayal P, Köhrle J, Hammes A, Schomburg L, Schweizer U**.** Mutation of megalin leads to urinary loss of selenoprotein P and selenium deficiency in serum, liver, kidneys and brain. **Biochem J** 431: 103–111, 2010. doi:10.1042/BJ20100779.

108. Greenough MA, Lane DJ, Balez R, Anastacio HT, Zeng Z, Ganio K, McDevitt CA, Acevedo K, Belaidi AA, Koistinaho J, Ooi L, Ayton S, Bush AI**.** Selective ferroptosis vulnerability due to familial Alzheimer's disease presenilin mutations. **Cell Death Differ** 29: 2123–2136, 2022. doi:10.1038/s41418-022-01003-1.

109. Alborzinia H, Chen Z, Yildiz U, Freitas FP, Vogel FC, Varga JP, et al**.** LRP8-mediated selenocysteine uptake is a targetable vulnerability in MYCN-amplified neuroblastoma. **EMBO Mol Med** 15: e18014, 2023. doi:10.15252/emmm.202318014.

110. Li Z, Ferguson L, Deol KK, Roberts MA, Magtanong L, Hendricks JM, Mousa GA, Kilinc S, Schaefer K, Wells JA, Bassik MC, Goga A, Dixon SJ, Ingolia NT, Olzmann JA**.** Ribosome stalling during selenoprotein translation exposes a ferroptosis vulnerability. **Nat Chem Biol** 18: 751–761, 2022. doi:10.1038/s41589-022-01033-3.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

111. Shimomura T, Hirakawa N, Ohuchi Y, Ishiyama M, Shiga M, Ueno Y. Simple fluorescence assay for cystine uptake via the xCT in cells using selenocystine and a fluorescent probe. **ACS Sens** 6: 2125–2128, 2021. doi:10.1021/acssensors.1c00496.

112. Tobe T, Ueda K, Aoki A, Okamoto Y, Kojima N, Jinno H. Selenium uptake through cystine transporter mediated by glutathione conjugation. **J Toxicol Sci** 42: 85–91, 2017. doi:10.2131/jts.42.85.

113. Olm E, Fernandes AP, Hebert C, Rundlöf AK, Larsen EH, Danielsson O, Björnstedt M. Extracellular thiol-assisted selenium uptake dependent on the x_c^- cystine transporter explains the cancer-specific cytotoxicity of selenite. **Proc Natl Acad Sci USA** 106: 11400–11405, 2009. doi:10.1073/pnas.0902204106.

114. Carlisle AE, Lee N, Matthew-Onabanjo AN, Spears ME, Park SJ, Youkana D, Doshi MB, Peppers A, Li R, Joseph AB, Smith M, Simin K, Zhu LJ, Greer PL, Shaw LM, Kim D. Selenium detoxification is required for cancer-cell survival. **Nat Metab** 2: 603–611, 2020. doi:10.1038/s42255-020-0224-7.

115. Lee N, Carlisle AE, Peppers A, Park SJ, Doshi MB, Spears ME, Kim D. xCT-driven expression of GPX4 determines sensitivity of breast cancer cells to ferroptosis inducers. **Antioxidants (Basel)** 10: 317, 2021. doi:10.3390/antiox10020317.

116. Lee N, Park SJ, Lange M, Tseyang T, Doshi MB, Kim TY, Song Y, Kim DI, Greer PL, Olzmann JA, Spinelli JB, Kim D. Selenium reduction of ubiquinone via SQOR suppresses ferroptosis. **Nat Metab** 6: 343–358, 2024. doi:10.1038/s42255-024-00974-4.

117. Vindry C, Ohlmann T, Chavatte L. Selenium metabolism, regulation, and sex differences in mammals. In: *Selenium*, edited by Michalke B. Cham, Switzerland: Springer International Publishing, 2018, p. 8.

118. Chen Z, Inague A, Kaushal K, Fazeli G, Schilling D, Xavier da Silva TN, et al. PRDX6 contributes to selenocysteine metabolism and ferroptosis resistance. **Mol Cell** 84: 4645–4659.e9, 2024. doi:10.1016/j.molcel.2024.10.027.

119. Raman AV, Pitts MW, Seyedali A, Hashimoto AC, Seale LA, Bellinger FP, Berry MJ. Absence of selenoprotein P but not selenocysteine lyase results in severe neurological dysfunction. **Genes Brain Behav** 11: 601–613, 2012. doi:10.1111/j.1601-183X.2012.00794.x.

120. Mizuno A, Toyama T, Ichikawa A, Sakai N, Yoshioka Y, Nishito Y, Toga R, Amesaka H, Kaneko T, Arisawa K, Tsutsumi R, Mita Y, Tanaka SI, Noguchi N, Saito Y. An efficient selenium transport pathway of selenoprotein P utilizing a high-affinity ApoER2 receptor variant and being independent of selenocysteine lyase. **J Biol Chem** 299: 105009, 2023. doi:10.1016/j.jbc.2023.105009.

121. Eagle K, Jiang Y, Shi X, Li M, Obholzer NP, Hu T, Perez MW, Koren JV, Kitano A, Yi JS, Lin CY, Nakada D. An oncogenic enhancer encodes selective selenium dependency in AML. **Cell Stem Cell** 29: 386–399.e7, 2022. doi:10.1016/j.stem.2022.01.003.

122. Fujita H, Tanaka YK, Ogata S, Suzuki N, Kuno S, Barayeu U, Akaike T, Ogra Y, Iwai K. PRDX6 augments selenium utilization to limit iron toxicity and ferroptosis. **Nat Struct Mol Biol** 31: 1277–1285, 2024. doi:10.1038/s41594-024-01329-z.

123. Ito J, Nakamura T, Toyama T, Chen D, Berndt C, Poschmann G, Mourão AS, Doll S, Suzuki M, Zhang W, Zheng J, Trümbach D, Yamada N, Ono K, Yazaki M, Kawai Y, Arisawa M, Ohsaki Y, Shirakawa H, Wahida A, Proneth B, Saito Y, Nakagawa K, Mishima E, Conrad M. PRDX6 dictates ferroptosis sensitivity by directing cellular selenium utilization. **Mol Cell** 84: 4629–4644.e9, 2024. doi:10.1016/j.molcel.2024.10.028.

124. Bösl MR, Takaku K, Oshima M, Nishimura S, Taketo MM. Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp). **Proc Natl Acad Sci USA** 94: 5531–5534, 1997. doi:10.1073/pnas.94.11.5531.

125. Wirth EK, Conrad M, Winterer J, Wozny C, Carlson BA, Roth S, Schmitz D, Bornkamm GW, Coppola V, Tessarollo L, Schomburg L, Köhrle J, Hatfield DL, Schweizer U. Neuronal selenoprotein expression is required for interneuron development and prevents seizures and neurodegeneration. **FASEB J** 24: 844–852, 2010. doi:10.1096/fj.09-143974.

126. Wirth EK, Bharathi BS, Hatfield D, Conrad M, Brielmeier M, Schweizer U. Cerebellar hypoplasia in mice lacking selenoprotein biosynthesis in neurons. **Biol Trace Elem Res** 158: 203–210, 2014. doi:10.1007/s12011-014-9920-z.

127. Fradejas-Villar N, Zhao W, Reuter U, Doengi M, Ingold I, Bohleber S, Conrad M, Schweizer U. Missense mutation in selenocysteine synthase causes cardio-respiratory failure and perinatal death in mice which can be compensated by selenium-independent GPX4. **Redox Biol** 48: 102188, 2021. doi:10.1016/j.redox.2021.102188.

128. Seeher S, Atassi T, Mahdi Y, Carlson BA, Braun D, Wirth EK, Klein MO, Reix N, Miniard AC, Schomburg L, Hatfield DL, Driscoll DM, Schweizer U. Secisbp2 is essential for embryonic development and enhances selenoprotein expression. **Antioxid Redox Signal** 21: 835–849, 2014. doi:10.1089/ars.2013.5358.

129. Seeher S, Carlson BA, Miniard AC, Wirth EK, Mahdi Y, Hatfield DL, Driscoll DM, Schweizer U. Impaired selenoprotein expression in brain triggers striatal neuronal loss leading to co-ordination defects in mice. **Biochem J** 462: 67–75, 2014. doi:10.1042/BJ20140423.

130. Dumitrescu AM, Liao XH, Abdullah MS, Lado-Abeal J, Majed FA, Moeller LC, Boran G, Schomburg L, Weiss RE, Refetoff S. Mutations in SECISBP2 result in abnormal thyroid hormone metabolism. **Nat Genet** 37: 1247–1252, 2005. doi:10.1038/ng1654.

131. Schoenmakers E, Agostini M, Mitchell C, Schoenmakers N, Papp L, Rajanayagam O, et al. Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. **J Clin Invest** 120: 4220–4235, 2010. doi:10.1172/JCI43653.

132. Schoenmakers E, Marelli F, Jørgensen HF, Visser WE, Moran C, Groeneweg S, et al. Selenoprotein deficiency disorder predisposes to aortic aneurysm formation. **Nat Commun** 14: 7994, 2023. doi:10.1038/s41467-023-43851-6.

133. Roveri A, Maiorino M, Nisii C, Ursini F. Purification and characterization of phospholipid hydroperoxide glutathione peroxidase from rat testis mitochondrial membranes. **Biochim Biophys Acta** 1208: 211–221, 1994. doi:10.1016/0167-4838(94)90106-6.

134. Xia C, Xing X, Zhang W, Wang Y, Jin X, Wang Y, Tian M, Ba X, Hao F. Cysteine and homocysteine can be exploited by GPX4 in ferroptosis inhibition independent of GSH synthesis. **Redox Biol** 69: 102999, 2024. doi:10.1016/j.redox.2023.102999.

135. Wu Z, Khodade VS, Chauvin JR, Rodriguez D, Toscano JP, Pratt DA. Hydropersulfides inhibit lipid peroxidation and protect cells from ferroptosis. **J Am Chem Soc** 144: 15825–15837, 2022. doi:10.1021/jacs.2c06804.

136. Barayeu U, Schilling D, Eid M, Xavier da Silva TN, Schlicker L, Mitreska N, Zapp C, Gräter F, Miller AK, Kappl R, Schulze A, Friedmann Angeli JP, Dick TP. Hydropersulfides inhibit lipid peroxidation and ferroptosis by scavenging radicals. **Nat Chem Biol** 19: 28–37, 2023. doi:10.1038/s41589-022-01145-w.

137. Leu JI, Murphy ME, George DL. Mechanistic basis for impaired ferroptosis in cells expressing the African-centric S47 variant of p53.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

Proc Natl Acad Sci USA 116: 8390–8396, 2019. doi:10.1073/pnas.1821277116.

138. Badgley MA, Kremer DM, Maurer HC, DelGiorno KE, Lee HJ, Purohit V, Sagalovskiy IR, Ma A, Kapilian J, Firl CE, Decker AR, Sastra SA, Palermo CF, Andrade LR, Sajjakulnukit P, Zhang L, Tolstyka ZP, Hirschhorn T, Lamb C, Liu T, Gu W, Seeley ES, Stone E, Georgiou G, Manor U, Iuga A, Wahl GM, Stockwell BR, Lyssiotis CA, Olive KP**.** Cysteine depletion induces pancreatic tumor ferroptosis in mice. **Science** 368: 85–89, 2020. doi:10.1126/science.aaw9872.

139. Conrad M, Sato H**.** The oxidative stress-inducible cystine/glutamate antiporter, system x_c^-: cystine supplier and beyond. **Amino Acids** 42: 231–246, 2012. doi:10.1007/s00726-011-0867-5.

140. Mandal PK, Seiler A, Perisic T, Kölle P, Banjac Canak A, Förster H, Weiss N, Kremmer E, Lieberman MW, Bannai S, Kuhlencordt P, Sato H, Bornkamm GW, Conrad M**.** System x_c^- and thioredoxin reductase 1 cooperatively rescue glutathione deficiency. **J Biol Chem** 285: 22244–22253, 2010. doi:10.1074/jbc.M110.121327.

141. Pader I, Sengupta R, Cebula M, Xu J, Lundberg JO, Holmgren A, Johansson K, Arnér ES**.** Thioredoxin-related protein of 14 kDa is an efficient L-cystine reductase and S-denitrosylase. **Proc Natl Acad Sci USA** 111: 6964–6969, 2014. doi:10.1073/pnas.1317320111.

142. Chillarón J, Font-Llitjós M, Fort J, Zorzano A, Goldfarb DS, Nunes V, Palacín M**.** Pathophysiology and treatment of cystinuria. **Nat Rev Nephrol** 6: 424–434, 2010. doi:10.1038/nrneph.2010.69.

143. Jiang Y, Cao Y, Wang Y, Li W, Liu X, Lv Y, Li X, Mi J**.** Cysteine transporter SLC3A1 promotes breast cancer tumorigenesis. **Theranostics** 7: 1036–1046, 2017. doi:10.7150/thno.18005.

144. Armenta DA, Laqtom NN, Alchemy G, Dong W, Morrow D, Poltorack CD, Nathanson DA, Abu-Remaileh M, Dixon SJ**.** Ferroptosis inhibition by lysosome-dependent catabolism of extracellular protein. **Cell Chem Biol** 29: 1588–1600.e7, 2022. doi:10.1016/j.chembiol.2022.10.006.

145. Ikeda Y, Fujii J**.** The emerging roles of γ-glutamyl peptides produced by γ-glutamyltransferase and the glutathione synthesis system. **Cells** 12: 2831, 2023. doi:10.3390/cells12242831.

146. Hayashima K, Katoh H**.** Expression of gamma-glutamyltransferase 1 in glioblastoma cells confers resistance to cystine deprivation-induced ferroptosis. **J Biol Chem** 298: 101703, 2022. doi:10.1016/j.jbc.2022.101703.

147. Kobayashi S, Homma T, Okumura N, Han J, Nagaoka K, Sato H, Konno H, Yamada S, Takao T, Fujii J**.** Carnosine dipeptidase II (CNDP2) protects cells under cysteine insufficiency by hydrolyzing glutathione-related peptides. **Free Radic Biol Med** 174: 12–27, 2021. doi:10.1016/j.freeradbiomed.2021.07.036.

148. Zhang L, Hobeika CS, Khabibullin D, Yu D, Filippakis H, Alchoueiry M, Tang Y, Lam HC, Tsvetkov P, Georgiou G, Lamb C, Stone E, Puigserver P, Priolo C, Henske EP**.** Hypersensitivity to ferroptosis in chromophobe RCC is mediated by a glutathione metabolic dependency and cystine import via solute carrier family 7 member 11. **Proc Natl Acad Sci USA** 119: e2122840119, 2022. doi:10.1073/pnas.2122840119.

149. Werge MP, McCann A, Galsgaard ED, Holst D, Bugge A, Albrechtsen NJ, Gluud LL**.** The role of the transsulfuration pathway in non-alcoholic fatty liver disease. **J Clin Med** 10: 1081, 2021. doi:10.3390/jcm10051081.

150. Eriksson S, Prigge JR, Talago EA, Arnér ES, Schmidt EE**.** Dietary methionine can sustain cytosolic redox homeostasis in the mouse liver. **Nat Commun** 6: 6479, 2015. doi:10.1038/ncomms7479.

151. Lee J, Kang ES, Kobayashi S, Homma T, Sato H, Seo HG, Fujii J**.** The viability of primary hepatocytes is maintained under a low cysteine-glutathione redox state with a marked elevation in ophthalmic acid production. **Exp Cell Res** 361: 178–191, 2017. doi:10.1016/j.yexcr.2017.10.017.

152. Watanabe M, Osada J, Aratani Y, Kluckman K, Reddick R, Malinow MR, Maeda N**.** Mice deficient in cystathionine beta-synthase: animal models for mild and severe homocyst(e)inemia. **Proc Natl Acad Sci USA** 92: 1585–1589, 1995. doi:10.1073/pnas.92.5.1585.

153. Ishii I, Akahoshi N, Yamada H, Nakano S, Izumi T, Suematsu M**.** Cystathionine gamma-lyase-deficient mice require dietary cysteine to protect against acute lethal myopathy and oxidative injury. **J Biol Chem** 285: 26358–26368, 2010. doi:10.1074/jbc.M110.147439.

154. Mani S, Yang G, Wang R**.** A critical life-supporting role for cystathionine gamma-lyase in the absence of dietary cysteine supply. **Free Radic Biol Med** 50: 1280–1287, 2011. doi:10.1016/j.freeradbiomed.2011.01.038.

155. Hagiya Y, Kamata S, Mitsuoka S, Okada N, Yoshida S, Yamamoto J, Ohkubo R, Abiko Y, Yamada H, Akahoshi N, Kasahara T, Kumagai Y, Ishii I**.** Hemizygosity of transsulfuration genes confers increased vulnerability against acetaminophen-induced hepatotoxicity in mice. **Toxicol Appl Pharmacol** 282: 195–206, 2015. doi:10.1016/j.taap.2014.11.015.

156. Zhu J, Berisa M, Schwörer S, Qin W, Cross JR, Thompson CB**.** Transsulfuration activity can support cell growth upon extracellular cysteine limitation. **Cell Metab** 30: 865–876.e5, 2019. doi:10.1016/j.cmet.2019.09.009.

157. Liu N, Lin X, Huang C**.** Activation of the reverse transsulfuration pathway through NRF2/CBS confers erastin-induced ferroptosis resistance. **Br J Cancer** 122: 279–292, 2020. doi:10.1038/s41416-019-0660-x.

158. Erdélyi K, Ditrói T, Johansson HJ, Czikora A, Balog N, Silwal-Pandit L, Ida T, Olasz J, Hajdú D, Mátrai Z, Csuka O, Uchida K, Tóvári J, Engebraten O, Akaike T, Børresen Dale AL, Kásler M, Lehtiö J, Nagy P**.** Reprogrammed transsulfuration promotes basal-like breast tumor progression via realigning cellular cysteine persulfidation. **Proc Natl Acad Sci USA** 118: e2100050118, 2021. doi:10.1073/pnas.2100050118.

159. Liu J, Lu X, Zeng S, Fu R, Wang X, Luo L, Huang T, Deng X, Zheng H, Ma S, Ning D, Zong L, Lin SH, Zhang Y**.** ATF3-CBS signaling axis coordinates ferroptosis and tumorigenesis in colorectal cancer. **Redox Biol** 71: 103118, 2024. doi:10.1016/j.redox.2024.103118.

160. Floros KV, Chawla AT, Johnson-Berro MO, Khatri R, Stamatouli AM, Boikos SA, Dozmorov MG, Cowart LA, Faber AC**.** MYCN upregulates the transsulfuration pathway to suppress the ferroptotic vulnerability in MYCN-amplified neuroblastoma. **Cell Stress** 6: 21–29, 2022. doi:10.15698/cst2022.02.264.

161. Alborzinia H, Flórez AF, Kreth S, Brückner LM, Yildiz U, Gartlgruber M, et al**.** MYCN mediates cysteine addiction and sensitizes neuroblastoma to ferroptosis. **Nat Cancer** 3: 471–485, 2022. doi:10.1038/s43018-022-00355-4.

162. Lien EC, Ghisolfi L, Geck RC, Asara JM, Toker A**.** Oncogenic PI3K promotes methionine dependency in breast cancer cells through the cystine-glutamate antiporter xCT. **Sci Signal** 10: eaao6604, 2017. doi:10.1126/scisignal.aao6604.

163. Zhang HF, Hughes CS, Li W, He JZ, Surdez D, El-Naggar AM, et al**.** Proteomic screens for suppressors of anoikis identify IL1RAP as a promising surface target in Ewing sarcoma. **Cancer Discov** 11: 2884–2903, 2021. doi:10.1158/2159-8290.CD-20-1690.

164. Abu-Remaileh M, Wyant GA, Kim C, Laqtom NN, Abbasi M, Chan SH, Freinkman E, Sabatini DM**.** Lysosomal metabolomics reveals V-ATPase- and mTOR-dependent regulation of amino acid efflux

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

from lysosomes. **Science** 358: 807–813, 2017. doi:10.1126/science.aan6298.

165. Swanda RV, Ji Q, Wu X, Yan J, Dong L, Mao Y, Uematsu S, Dong Y, Qian SB**.** Lysosomal cystine governs ferroptosis sensitivity in cancer via cysteine stress response. **Mol Cell** 83: 3347–3359.e9, 2023. doi:10.1016/j.molcel.2023.08.004.

166. Sato H, Nomura S, Maebara K, Sato K, Tamba M, Bannai S**.** Transcriptional control of cystine/glutamate transporter gene by amino acid deprivation. **Biochem Biophys Res Commun** 325: 109–116, 2004. doi:10.1016/j.bbrc.2004.10.009.

167. Dickhout JG, Carlisle RE, Jerome DE, Mohammed-Ali Z, Jiang H, Yang G, Mani S, Garg SK, Banerjee R, Kaufman RJ, Maclean KN, Wang R, Austin RC**.** Integrated stress response modulates cellular redox state via induction of cystathionine γ-lyase: cross-talk between integrated stress response and thiol metabolism. **J Biol Chem** 287: 7603–7614, 2012. doi:10.1074/jbc.M111.304576.

168. Adelmann CH, Traunbauer AK, Chen B, Condon KJ, Chan SH, Kunchok T, Lewis CA, Sabatini DM**.** MFSD12 mediates the import of cysteine into melanosomes and lysosomes. **Nature** 588: 699–704, 2020. doi:10.1038/s41586-020-2937-x.

169. Kalatzis V, Cherqui S, Antignac C, Gasnier B**.** Cystinosin, the protein defective in cystinosis, is a H^+-driven lysosomal cystine transporter. **EMBO J** 20: 5940–5949, 2001. doi:10.1093/emboj/20.21.5940.

170. He L, Chen J, Deng P, Huang S, Liu P, Wang C, Huang X, Li Y, Chen B, Shi D, Xiao Y, Chen X, Ouyang Y, Song L, Lin C**.** Lysosomal cyst(e)ine storage potentiates tolerance to oxidative stress in cancer cells. **Mol Cell** 83: 3502–3519.e11, 2023. doi:10.1016/j.molcel.2023.08.032.

171. Ono K, Akaike T, Sawa T, Kumagai Y, Wink DA, Tantillo DJ, Hobbs AJ, Nagy P, Xian M, Lin J, Fukuto JM**.** Redox chemistry and chemical biology of H2S, hydropersulfides, and derived species: implications of their possible biological activity and utility. **Free Radic Biol Med** 77: 82–94, 2014. doi:10.1016/j.freeradbiomed.2014.09.007.

172. Ida T, Sawa T, Ihara H, Tsuchiya Y, Watanabe Y, Kumagai Y, Suematsu M, Motohashi H, Fujii S, Matsunaga T, Yamamoto M, Ono K, Devarie-Baez NO, Xian M, Fukuto JM, Akaike T**.** Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling. **Proc Natl Acad Sci USA** 111: 7606–7611, 2014. doi:10.1073/pnas.1321232111.

173. Singh S, Padovani D, Leslie RA, Chiku T, Banerjee R**.** Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H2S biogenesis via alternative trans-sulfuration reactions. **J Biol Chem** 284: 22457–22466, 2009. doi:10.1074/jbc.M109.010868.

174. Zheng H, Chen H, Cai Y, Shen M, Li X, Han Y, Deng X, Cao H, Liu J, Li H, Liu B, Li G, Wang X, Chen H, Hou J, Lin SH, Zong L, Zhang Y**.** Hydrogen sulfide-mediated persulfidation regulates homocysteine metabolism and enhances ferroptosis in non-small cell lung cancer. **Mol Cell** 84: 4016–4030.e6, 2024. doi:10.1016/j.molcel.2024.08.035.

175. Zhang J, Sun W, Yan W, Kong X, Shen T, Laubach K, Chen M, Chen X**.** TP73 isoform-specific disruption reveals a critical role of TAp73beta in growth suppression and inflammatory response. **Cell Death Dis** 14: 14, 2023. doi:10.1038/s41419-022-05529-7.

176. Yang J, Sun L, Liu XY, Huang C, Peng J, Zeng X, Zheng H, Cen W, Xu YX, Zhu W, Wu XY, Ling D, Zhang LL, Wei M, Liu Y, Wang D, Wang FH, Li YH, Li Q, Du Z**.** Targeted demethylation of the CDO1 promoter based on CRISPR system inhibits the malignant potential of breast cancer cells. **Clin Transl Med** 13: e1423, 2023. doi:10.1002/ctm2.1423.

177. Zhang J, Yimamu M, Cheng Z, Ji J, Wu L, Feng J, Xu X, Wu J, Guo C**.** TRIM47-CDO1 axis dictates hepatocellular carcinoma progression by modulating ferroptotic cell death through the ubiquitin–proteasome system. **Free Radic Biol Med** 219: 31–48, 2024. doi:10.1016/j.freeradbiomed.2024.04.222.

178. Conlon M, Poltorack CD, Forcina GC, Armenta DA, Mallais M, Perez MA, Wells A, Kahanu A, Magtanong L, Watts JL, Pratt DA, Dixon SJ**.** A compendium of kinetic modulatory profiles identifies ferroptosis regulators. **Nat Chem Biol** 17: 665–674, 2021. doi:10.1038/s41589-021-00751-4.

179. Homma T, Kobayashi S, Fujii J**.** Methionine deprivation reveals the pivotal roles of cell cycle progression in ferroptosis that is induced by cysteine starvation. **Cells** 11: 1603, 2022. doi:10.3390/cells11101603.

180. Zhang T, Bauer C, Newman AC, Uribe AH, Athineos D, Blyth K, Maddocks OD**.** Polyamine pathway activity promotes cysteine essentiality in cancer cells. **Nat Metab** 2: 1062–1076, 2020. doi:10.1038/s42255-020-0253-2.

181. Cao J, Chen X, Jiang L, Lu B, Yuan M, Zhu D, Zhu H, He Q, Yang B, Ying M**.** DJ-1 suppresses ferroptosis through preserving the activity of S-adenosyl homocysteine hydrolase. **Nat Commun** 11: 1251, 2020. doi:10.1038/s41467-020-15109-y.

182. Mecham JO, Rowitch D, Wallace CD, Stern PH, Hoffman RM**.** The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. **Biochem Biophys Res Commun** 117: 429–434, 1983. doi:10.1016/0006-291x(83)91218-4.

183. Kreis W, Goodenow M**.** Methionine requirement and replacement by homocysteine in tissue cultures of selected rodent and human malignant and normal cells. **Cancer Res** 38: 2259–2262, 1978.

184. Halpern BC, Clark BR, Hardy DN, Halpern RM, Smith RA**.** The effect of replacement of methionine by homocystine on survival of malignant and normal adult mammalian cells in culture. **Proc Natl Acad Sci USA** 71: 1133–1136, 1974. doi:10.1073/pnas.71.4.1133.

185. Guo H, Lishko VK, Herrera H, Groce A, Kubota T, Hoffman RM**.** Therapeutic tumor-specific cell cycle block induced by methionine starvation in vivo. **Cancer Res** 53: 5676–5679, 1993.

186. Poirson-Bichat F, Gonfalone G, Bras-Gonçalves RA, Dutrillaux B, Poupon MF**.** Growth of methionine-dependent human prostate cancer (PC-3) is inhibited by ethionine combined with methionine starvation. **Br J Cancer** 75: 1605–1612, 1997. doi:10.1038/bjc.1997.274.

187. Hoffman RM, Jacobsen SJ**.** Reversible growth arrest in simian virus 40-transformed human fibroblasts. **Proc Natl Acad Sci USA** 77: 7306–7310, 1980. doi:10.1073/pnas.77.12.7306.

188. Ou Y, Wang SJ, Li D, Chu B, Gu W**.** Activation of SAT1 engages polyamine metabolism with p53-mediated ferroptotic responses. **Proc Natl Acad Sci USA** 113: E6806–E6812, 2016. doi:10.1073/pnas.1607152113.

189. Ding Y, Feng Y, Huang Z, Zhang Y, Li X, Liu R, Li H, Wang T, Ding Y, Jia Z, Yang J**.** SOX15 transcriptionally increases the function of AOC1 to modulate ferroptosis and progression in prostate cancer. **Cell Death Dis** 13: 673, 2022. doi:10.1038/s41419-022-05108-w.

190. Bi G, Liang J, Bian Y, Shan G, Huang Y, Lu T, Zhang H, Jin X, Chen Z, Zhao M, Fan H, Wang Q, Gan B, Zhan C**.** Polyamine-mediated ferroptosis amplification acts as a targetable vulnerability in cancer. **Nat Commun** 15: 2461, 2024. doi:10.1038/s41467-024-46776-w.

191. Xia C, Peng P, Zhang W, Xing X, Jin X, Du J, Peng W, Hao F, Zhao Z, Dong K, Tian M, Feng Y, Ba X, Wei M, Wang Y**.** Methionine-SAM metabolism-dependent ubiquinone synthesis is crucial for ROS accumulation in ferroptosis induction. **Nat Commun** 15: 8971, 2024. doi:10.1038/s41467-024-53380-5.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

192. Xiao Z, Deng S, Liu H, Wang R, Liu Y, Dai Z, Gu W, Ni Q, Yu X, Liu C, Luo G. Glutamine deprivation induces ferroptosis in pancreatic cancer cells. **Acta Biochim Biophys Sin (Shanghai)** 55: 1288–1300, 2023. doi:10.3724/abbs.2023029.

193. Zhao Y, Wang Y, Miao Z, Liu Y, Yang Q. c-Myc protects hepatocellular carcinoma cell from ferroptosis induced by glutamine deprivation via upregulating GOT1 and Nrf2. **Mol Biol Rep** 50: 6627–6641, 2023. doi:10.1007/s11033-023-08495-1.

194. Gao M, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB, Jiang X. Role of mitochondria in ferroptosis. **Mol Cell** 73: 354–363.e3, 2019. doi:10.1016/j.molcel.2018.10.042.

195. Okazaki S, Umene K, Yamasaki J, Suina K, Otsuki Y, Yoshikawa M, Minami Y, Masuko T, Kawaguchi S, Nakayama H, Banno K, Aoki D, Saya H, Nagano O. Glutaminolysis-related genes determine sensitivity to xCT-targeted therapy in head and neck squamous cell carcinoma. **Cancer Sci** 110: 3453–3463, 2019. doi:10.1111/cas.14182.

196. Bhutia YD, Ganapathy V. Glutamine transporters in mammalian cells and their functions in physiology and cancer. **Biochim Biophys Acta** 1863: 2531–2539, 2016. doi:10.1016/j.bbamcr.2015.12.017.

197. Luo M, Wu L, Zhang K, Wang H, Zhang T, Gutierrez L, O'Connell D, Zhang P, Li Y, Gao T, Ren W, Yang Y. miR-137 regulates ferroptosis by targeting glutamine transporter SLC1A5 in melanoma. **Cell Death Differ** 25: 1457–1472, 2018. doi:10.1038/s41418-017-0053-8.

198. Venkateswaran G, McDonald PC, Chafe SC, Brown WS, Gerbec ZJ, Awrey SJ, Parker SJ, Dedhar S. A carbonic anhydrase IX/SLC1A5 axis regulates glutamine metabolism dependent ferroptosis in hypoxic tumor cells. **Mol Cancer Ther** 22: 1228–1242, 2023. doi:10.1158/1535-7163.MCT-23-0041.

199. Kim MJ, Kim HS, Kang HW, Lee DE, Hong WC, Kim JH, Kim M, Cheong JH, Kim HJ, Park JS. SLC38A5 modulates ferroptosis to overcome gemcitabine resistance in pancreatic cancer. **Cells** 12: 2509, 2023. doi:10.3390/cells12202509.

200. Yoo HC, Park SJ, Nam M, Kang J, Kim K, Yeo JH, Kim JK, Heo Y, Lee HS, Lee MY, Lee CW, Kang JS, Kim YH, Lee J, Choi J, Hwang GS, Bang S, Han JM. A variant of SLC1A5 is a mitochondrial glutamine transporter for metabolic reprogramming in cancer cells. **Cell Metab** 31: 267–283.e12, 2020. doi:10.1016/j.cmet.2019.11.020.

201. Suzuki S, Venkatesh D, Kanda H, Nakayama A, Hosokawa H, Lee E, Miki T, Stockwell BR, Yokote K, Tanaka T, Prives C. GLS2 is a tumor suppressor and a regulator of ferroptosis in hepatocellular carcinoma. **Cancer Res** 82: 3209–3222, 2022. doi:10.1158/0008-5472.CAN-21-3914.

202. Katt WP, Lukey MJ, Cerione RA. A tale of two glutaminases: homologous enzymes with distinct roles in tumorigenesis. **Future Med Chem** 9: 223–243, 2017. doi:10.4155/fmc-2016-0190.

203. Fiermonte G, Palmieri L, Todisco S, Agrimi G, Palmieri F, Walker JE. Identification of the mitochondrial glutamate transporter. Bacterial expression, reconstitution, functional characterization, and tissue distribution of two human isoforms. **J Biol Chem** 277: 19289–19294, 2002. doi:10.1074/jbc.M201572200.

204. Liu Y, Wang Y, Lin Z, Kang R, Tang D, Liu J. SLC25A22 as a key mitochondrial transporter against ferroptosis by producing glutathione and monounsaturated fatty acids. **Antioxid Redox Signal** 39: 166–185, 2023. doi:10.1089/ars.2022.0203.

205. Zeitler L, Fiore A, Meyer C, Russier M, Zanella G, Suppmann S, Gargaro M, Sidhu SS, Seshagiri S, Ohnmacht C, Köcher T, Fallarino F, Linkermann A, Murray PJ. Anti-ferroptotic mechanism of IL4i1-mediated amino acid metabolism. **ELife** 10: e64806, 2021. doi:10.7554/eLife.64806.

206. Liu D, Liang CH, Huang B, Zhuang X, Cui W, Yang L, Yang Y, Zhang Y, Fu X, Zhang X, Du L, Gu W, Wang X, Yin C, Chai R, Chu B. Tryptophan metabolism acts as a new anti-ferroptotic pathway to mediate tumor growth. **Adv Sci (Weinh)** 10: e2204006, 2023. doi:10.1002/advs.202204006.

207. Chidley C, Darnell AM, Gaudio BL, Lien EC, Barbeau AM, Vander Heiden MG, Sorger PK. A CRISPRi/a screening platform to study cellular nutrient transport in diverse microenvironments. **Nat Cell Biol** 26: 825–838, 2024. doi:10.1038/s41556-024-01402-1.

208. Fiore A, Zeitler L, Russier M, Groß A, Hiller M-K, Parker JL, Stier L, Köcher T, Newstead S, Murray PJ. Kynurenine importation by SLC7A11 propagates anti-ferroptotic signaling. **Mol Cell** 82: 920–932.e7, 2022. doi:10.1016/j.molcel.2022.02.007.

209. Zeitler L, Murray PJ. IL4i1 and IDO1: Oxidases that control a tryptophan metabolic nexus in cancer. **J Biol Chem** 299: 104827, 2023. doi:10.1016/j.jbc.2023.104827.

210. Cui W, Guo M, Liu D, Xiao P, Yang C, Huang H, Liang C, Yang Y, Fu X, Zhang Y, Liu J, Shi S, Cong J, Han Z, Xu Y, Du L, Yin C, Zhang Y, Sun J, Gu W, Chai R, Zhu S, Chu B. Gut microbial metabolite facilitates colorectal cancer development via ferroptosis inhibition. **Nat Cell Biol** 26: 124–137, 2024. doi:10.1038/s41556-023-01314-6.

211. Lee H, Zandkarimi F, Zhang Y, Meena JK, Kim J, Zhuang L, Tyagi S, Ma L, Westbrook TF, Steinberg GR, Nakada D, Stockwell BR, Gan B. Energy-stress-mediated AMPK activation inhibits ferroptosis. **Nat Cell Biol** 22: 225–234, 2020. doi:10.1038/s41556-020-0461-8.

212. Song X, Liu J, Kuang F, Chen X, Zeh HJ 3rd, Kang R, Kroemer G, Xie Y, Tang D. PDK4 dictates metabolic resistance to ferroptosis by suppressing pyruvate oxidation and fatty acid synthesis. **Cell Rep** 34: 108767, 2021. doi:10.1016/j.celrep.2021.108767.

213. Li C, Dong X, Du W, Shi X, Chen K, Zhang W, Gao M. LKB1-AMPK axis negatively regulates ferroptosis by inhibiting fatty acid synthesis. **Signal Transduct Target Ther** 5: 187, 2020. doi:10.1038/s41392-020-00297-2.

214. Leu JI, Murphy ME, George DL. Functional interplay among thiol-based redox signaling, metabolism, and ferroptosis unveiled by a genetic variant of TP53. **Proc Natl Acad Sci USA** 117: 26804–26811, 2020. doi:10.1073/pnas.2009943117.

215. Chen M, Li X, Du B, Chen S, Li Y. Upstream stimulatory factor 2 inhibits erastin-induced ferroptosis in pancreatic cancer through transcriptional regulation of pyruvate kinase M2. **Biochem Pharmacol** 205: 115255, 2022. doi:10.1016/j.bcp.2022.115255.

216. Ensink E, Jordan T, Medeiros HCD, Thurston G, Pardal A, Yu L, Lunt SY. Pyruvate kinase activity regulates cystine starvation induced ferroptosis through malic enzyme 1 in pancreatic cancer cells (Preprint). **bioRxiv** 2023.09.15.557984, 2023. doi:10.1101/2023.09.15.557984.

217. Vučković AM, Venerando R, Tibaldi E, Bosello Travain V, Roveri A, Bordin L, Miotto G, Cozza G, Toppo S, Maiorino M, Ursini F. Aerobic pyruvate metabolism sensitizes cells to ferroptosis primed by GSH depletion. **Free Radic Biol Med** 167: 45–53, 2021. doi:10.1016/j.freeradbiomed.2021.02.045.

218. Yang Z, Su W, Wei X, Qu S, Zhao D, Zhou J, Wang Y, Guan Q, Qin C, Xiang J, Zen K, Yao B. HIF-1alpha drives resistance to ferroptosis in solid tumors by promoting lactate production and activating SLC1A1. **Cell Rep** 42: 112945, 2023. doi:10.1016/j.celrep.2023.112945.

219. Zhao Y, Li M, Yao X, Fei Y, Lin Z, Li Z, Cai K, Zhao Y, Luo Z. HCAR1/MCT1 regulates tumor ferroptosis through the lactate-mediated AMPK-SCD1 activity and its therapeutic implications. **Cell Rep** 33: 108487, 2020. doi:10.1016/j.celrep.2020.108487.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

220. Shin D, Lee J, You JH, Kim D, Roh JL. Dihydrolipoamide dehydrogenase regulates cystine deprivation-induced ferroptosis in head and neck cancer. **Redox Biol** 30: 101418, 2020. doi:10.1016/j.redox.2019.101418.

221. Yang J, Zhou Y, Li Y, Hu W, Yuan C, Chen S, Ye G, Chen Y, Wu Y, Liu J, Wang Y, Du J, Tong X. Functional deficiency of succinate dehydrogenase promotes tumorigenesis and development of clear cell renal cell carcinoma through weakening of ferroptosis. **Bioengineered** 13: 11187–11207, 2022. doi:10.1080/21655979.2022.2062537.

222. Wang X, Li M, Wang F, Mao G, Wu J, Han R, Sheng R, Qin Z, Ni H. TIGAR reduces neuronal ferroptosis by inhibiting succinate dehydrogenase activity in cerebral ischemia. **Free Radic Biol Med** 216: 89–105, 2024. doi:10.1016/j.freeradbiomed.2024.03.011.

223. Eleftheriadis T, Pissas G, Golfinopoulos S, Efthymiadi M, Liakopoulos V, Stefanidis I. Inhibition of malate dehydrogenase-2 protects renal tubular epithelial cells from anoxia-reoxygenation-induced death or senescence. **Biomolecules** 12: 1415, 2022. doi:10.3390/biom12101415.

224. Kim H, Lee JH, Park JW. Down-regulation of IDH2 sensitizes cancer cells to erastin-induced ferroptosis. **Biochem Biophys Res Commun** 525: 366–371, 2020. doi:10.1016/j.bbrc.2020.02.093.

225. Zhang C, Zhou Y, Chen T, Bhushan S, Sun S, Zhang P, Yang Y. Isocitrate dehydrogenase 2 regulates the proliferation of triple-negative breast cancer through the ferroptosis pathway. **Sci Rep** 14: 4732, 2024. doi:10.1038/s41598-024-55561-0.

226. Murari A, Goparaju NS, Rhooms S-K, Hossain KF, Liang FG, Garcia CJ, Osei C, Liu T, Li H, Kitsis RN, Patel R, Owusu-Ansah E. IDH2-mediated regulation of the biogenesis of the oxidative phosphorylation system. **Sci Adv** 8: eabl8716, 2022. doi:10.1126/sciadv.abl8716.

227. Sternadt D, Pereira-Martins D, Silveira DR, Weinhaeuser I, Yang M, Chatzikyriakou P, Casares Alaez P, Ammatuna E, Oudejans L, Huls GA, Frezza C, Quek L, Schuringa JJ. Metformin-induced ferroptosis is a therapeutic vulnerability in IDH2-mutant AML linked to metabolic rewiring towards fatty acid oxidation. **Blood** 142: 1586–1586, 2023. doi:10.1182/blood-2023-188524.

228. Yu D, Liu Y, Zhou Y, Ruiz-Rodado V, Larion M, Xu G, Yang C. Triptolide suppresses IDH1-mutated malignancy via Nrf2-driven glutathione metabolism. **Proc Natl Acad Sci USA** 117: 9964–9972, 2020. doi:10.1073/pnas.1913633117.

229. Wang TX, Liang JY, Zhang C, Xiong Y, Guan KL, Yuan HX. The oncometabolite 2-hydroxyglutarate produced by mutant IDH1 sensitizes cells to ferroptosis. **Cell Death Dis** 10: 755, 2019. doi:10.1038/s41419-019-1984-4.

230. Lyamzaev KG, Panteleeva AA, Simonyan RA, Avetisyan AV, Chernyak BV. Mitochondrial lipid peroxidation is responsible for ferroptosis. **Cells** 12: 611, 2023. doi:10.3390/cells12040611.

231. Homma T, Kobayashi S, Sato H, Fujii J. Superoxide produced by mitochondrial complex III plays a pivotal role in the execution of ferroptosis induced by cysteine starvation. **Arch Biochem Biophys** 700: 108775, 2021. doi:10.1016/j.abb.2021.108775.

232. Feng Y, Xu J, Shi M, Liu R, Zhao L, Chen X, Li M, Zhao Y, Chen J, Du W, Liu P. COX7A1 enhances the sensitivity of human NSCLC cells to cystine deprivation-induced ferroptosis via regulating mitochondrial metabolism. **Cell Death Dis** 13: 988, 2022. doi:10.1038/s41419-022-05430-3.

233. Basit F, van Oppen LM, Schöckel L, Bossenbroek HM, van Emst-de Vries SE, Hermeling JC, Grefte S, Kopitz C, Heroult M, Willems PH, Koopman WJ. Mitochondrial complex I inhibition triggers a mitophagy-dependent ROS increase leading to necroptosis and ferroptosis in melanoma cells. **Cell Death Dis** 8: e2716, 2017. doi:10.1038/cddis.2017.133.

234. Linjacki S, Wang Y, Baath N, Mantle D, Yang G. H_2S protects from rotenone-induced ferroptosis by stabilizing Fe-S clusters in rat cardiac cells. **Cells** 13: e371, 2024. doi:10.3390/cells13050371.

235. Mao C, Lei G, Horbath A, Wang M, Lu Z, Yan Y, Liu X, Kondiparthi L, Chen X, Cheng J, Li Q, Xu Z, Zhuang L, Fang B, Marszalek JR, Poyurovsky MV, Olszewski K, Gan B. Unraveling ETC complex I function in ferroptosis reveals a potential ferroptosis-inducing therapeutic strategy for LKB1-deficient cancers. **Mol Cell** 84: 1964–1979. e6, 2024. doi:10.1016/j.molcel.2024.04.009.

236. Gopal RK, Vantaku VR, Panda A, Reimer B, Rath S, To TL, Fisch AS, Cetinbas M, Livneh M, Calcaterra MJ, Gigliotti BJ, Pierce KA, Clish CB, Dias-Santagata D, Sadow PM, Wirth LJ, Daniels GH, Sadreyev RI, Calvo SE, Parangi S, Mootha VK. Effectors enabling adaptation to mitochondrial complex I loss in Hürthle cell carcinoma. **Cancer Discov** 13: 1904–1921, 2023. doi:10.1158/2159-8290.CD-22-0976.

237. Akiyama H, Zhao R, Ostermann LB, Li Z, Tcheng M, Yazdani SJ, Moayed A, Pryor ML 2nd, Slngh S, Baran N, Ayoub E, Nishida Y, Mak PY, Ruvolo VR, Carter BZ, Schimmer AD, Andreeff M, Ishizawa J. Mitochondrial regulation of GPX4 inhibition-mediated ferroptosis in acute myeloid leukemia. **Leukemia** 38: 729–740, 2024. doi:10.1038/s41375-023-02117-2.

238. Shimada K, Skouta R, Kaplan A, Yang WS, Hayano M, Dixon SJ, Brown LM, Valenzuela CA, Wolpaw AJ, Stockwell BR. Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. **Nat Chem Biol** 12: 497–503, 2016. doi:10.1038/nchembio.2079.

239. Cao F, Luo A, Yang C. G6PD inhibits ferroptosis in hepatocellular carcinoma by targeting cytochrome P450 oxidoreductase. **Cell Signal** 87: 110098, 2021. doi:10.1016/j.cellsig.2021.110098.

240. Yang J, Lee Y, Hwang CS. The ubiquitin-proteasome system links NADPH metabolism to ferroptosis. **Trends Cell Biol** 33: 1088–1103, 2023. doi:10.1016/j.tcb.2023.07.003.

241. Nguyen KT, Mun SH, Yang J, Lee J, Seok OH, Kim E, Kim D, An SY, Seo DY, Suh JY, Lee Y, Hwang CS. The MARCHF6 E3 ubiquitin ligase acts as an NADPH sensor for the regulation of ferroptosis. **Nat Cell Biol** 24: 1239–1251, 2022. doi:10.1038/s41556-022-00973-1.

242. Shimada K, Hayano M, Pagano NC, Stockwell BR. Cell-line selectivity improves the predictive power of pharmacogenomic analyses and helps identify NADPH as biomarker for ferroptosis sensitivity. **Cell Chem Biol** 23: 225–235, 2016. doi:10.1016/j.chembiol.2015.11.016.

243. Viswanathan VS, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, Seashore-Ludlow B, et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. **Nature** 547: 453–457, 2017. doi:10.1038/nature23007.

244. Warner GJ, Berry MJ, Moustafa ME, Carlson BA, Hatfield DL, Faust JR. Inhibition of selenoprotein synthesis by selenocysteine tRNA [Ser]Sec lacking isopentenyladenosine. **J Biol Chem** 275: 28110–28119, 2000. doi:10.1074/jbc.M001280200.

245. Garcia-Bermudez J, Baudrier L, Bayraktar EC, Shen Y, La K, Guarecuco R, Yucel B, Fiore D, Tavora B, Freinkman E, Chan SH, Lewis C, Min W, Inghirami G, Sabatini DM, Birsoy K. Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death. **Nature** 567: 118–122, 2019. doi:10.1038/s41586-019-0945-5.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

246. Sun Q, Liu D, Cui W, Cheng H, Huang L, Zhang R, Gu J, Liu S, Zhuang X, Lu Y, Chu B, Li J. Cholesterol mediated ferroptosis suppression reveals essential roles of Coenzyme Q and squalene. **Commun Biol** 6: 1108, 2023. doi:10.1038/s42003-023-05477-8.

247. Freitas FP, Alborzinia H, Dos Santos AF, Nepachalovich P, Pedrera L, Zilka O, et al. 7-Dehydrocholesterol is an endogenous suppressor of ferroptosis. **Nature** 626: 401–410, 2024. doi:10.1038/s41586-023-06878-9.

248. Bersuker K, Hendricks JM, Li Z, Magtanong L, Ford B, Tang PH, Roberts MA, Tong B, Maimone TJ, Zoncu R, Bassik MC, Nomura DK, Dixon SJ, Olzmann JA. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. **Nature** 575: 688–692, 2019. doi:10.1038/s41586-019-1705-2.

249. Doll S, Freitas FP, Shah R, Aldrovandi M, da Silva MC, Ingold I, et al. FSP1 is a glutathione-independent ferroptosis suppressor. **Nature** 575: 693–698, 2019. doi:10.1038/s41586-019-1707-0.

250. Jin DY, Chen X, Liu Y, Williams CM, Pedersen LC, Stafford DW, Tie JK. A genome-wide CRISPR-Cas9 knockout screen identifies FSP1 as the warfarin-resistant vitamin K reductase. **Nat Commun** 14: 828, 2023. doi:10.1038/s41467-023-36446-8.

251. Mishima E, Ito J, Wu Z, Nakamura T, Wahida A, Doll S, Tonnus W, Nepachalovich P, Eggenhofer E, Aldrovandi M, Henkelmann B, Yamada KI, Wanninger J, Zilka O, Sato E, Feederle R, Hass D, Maida A, Mourao AS, Linkermann A, Geissler EK, Nakagawa K, Abe T, Fedorova M, Proneth B, Pratt DA, Conrad M. A non-canonical vitamin K cycle is a potent ferroptosis suppressor. **Nature** 608: 778–783, 2022. doi:10.1038/s41586-022-05022-3.

252. Li Y, Ran Q, Duan Q, Jin J, Wang Y, Yu L, Wang C, Zhu Z, Chen X, Weng L, Li Z, Wang J, Wu Q, Wang H, Tian H, Song S, Shan Z, Zhai Q, Qin H, Chen S, Fang L, Yin H, Zhou H, Jiang X, Wang P. 7-Dehydrocholesterol dictates ferroptosis sensitivity. **Nature** 626: 411–418, 2024. doi:10.1038/s41586-023-06983-9.

253. Yamada N, Karasawa T, Ito J, Yamamuro D, Morimoto K, Nakamura T, Komada T, Baatarjav C, Saimoto Y, Jinnouchi Y, Watanabe K, Miura K, Yahagi N, Nakagawa K, Matsumura T, Yamada KI, Ishibashi S, Sata N, Conrad M, Takahashi M. Inhibition of 7-dehydrocholesterol reductase prevents hepatic ferroptosis under an active state of sterol synthesis. **Nat Commun** 15: 2195, 2024. doi:10.1038/s41467-024-46386-6.

254. Shah R, Shchepinov MS, Pratt DA. Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. **ACS Cent Sci** 4: 387–396, 2018. doi:10.1021/acscentsci.7b00589.

255. Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS, Stockwell BR. Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. **Proc Natl Acad Sci USA** 113: E4966–E4975, 2016. doi:10.1073/pnas.1603244113.

256. Zhao X, Lian X, Xie J, Liu G. Accumulated cholesterol protects tumours from elevated lipid peroxidation in the microenvironment. **Redox Biol** 62: 102678, 2023. doi:10.1016/j.redox.2023.102678.

257. Rink JS, Lin AY, McMahon KM, Calvert AE, Yang S, Taxter T, Moreira J, Chadburn A, Behdad A, Karmali R, Thaxton CS, Gordon LI. Targeted reduction of cholesterol uptake in cholesterol-addicted lymphoma cells blocks turnover of oxidized lipids to cause ferroptosis. **J Biol Chem** 296: 100100, 2021. doi:10.1074/jbc.RA120.014888.

258. Xian M, Wang Q, Xiao L, Zhong L, Xiong W, Ye L, Su P, Zhang C, Li Y, Orlowski RZ, Zhan F, Ganguly S, Zu Y, Qian J, Yi Q. Leukocyte immunoglobulin-like receptor B1 (LILRB1) protects human multiple myeloma cells from ferroptosis by maintaining cholesterol homeostasis. **Nat Commun** 15: 5767, 2024. doi:10.1038/s41467-024-50073-x.

259. Gill S, Stevenson J, Kristiana I, Brown AJ. Cholesterol-dependent degradation of squalene monooxygenase, a control point in cholesterol synthesis beyond HMG-CoA reductase. **Cell Metab** 13: 260–273, 2011. doi:10.1016/j.cmet.2011.01.015.

260. Foresti O, Ruggiano A, Hannibal-Bach HK, Ejsing CS, Carvalho P. Sterol homeostasis requires regulated degradation of squalene monooxygenase by the ubiquitin ligase Doa10/Teb4. **Elife** 2: e00953, 2013. doi:10.7554/eLife.00953.

261. Zelcer N, Sharpe LJ, Loregger A, Kristiana I, Cook EC, Phan L, Stevenson J, Brown AJ. The E3 ubiquitin ligase MARCH6 degrades squalene monooxygenase and affects 3-hydroxy-3-methyl-glutaryl coenzyme A reductase and the cholesterol synthesis pathway. **Mol Cell Biol** 34: 1262–1270, 2014. doi:10.1128/MCB.01140-13.

262. Magtanong L, Ko PJ, To M, Cao JY, Forcina GC, Tarangelo A, Ward CC, Cho K, Patti GJ, Nomura DK, Olzmann JA, Dixon SJ. Exogenous monounsaturated fatty acids promote a ferroptosis-resistant cell state. **Cell Chem Biol** 26: 420–432.e9, 2019. doi:10.1016/j.chembiol.2018.11.016.

263. Cui W, Liu D, Gu W, Chu B. Peroxisome-driven ether-linked phospholipids biosynthesis is essential for ferroptosis. **Cell Death Differ** 28: 2536–2551, 2021. doi:10.1038/s41418-021-00769-0.

264. Conrad M, Pratt DA. The chemical basis of ferroptosis. **Nat Chem Biol** 15: 1137–1147, 2019. doi:10.1038/s41589-019-0408-1.

265. Ma X, Xiao L, Liu L, Ye L, Su P, Bi E, Wang Q, Yang M, Qian J, Yi Q. CD36-mediated ferroptosis dampens intratumoral $CD8^+$ T cell effector function and impairs their antitumor ability. **Cell Metab** 33: 1001–1012.e5, 2021. doi:10.1016/j.cmet.2021.02.015.

266. Xu S, Chaudhary O, Rodríguez-Morales P, Sun X, Chen D, Zappasodi R, Xu Z, Pinto AF, Williams A, Schulze I, Farsakoglu Y, Varanasi SK, Low JS, Tang W, Wang H, McDonald B, Tripple V, Downes M, Evans RM, Abumrad NA, Merghoub T, Wolchok JD, Shokhirev MN, Ho PC, Witztum JL, Emu B, Cui G, Kaech SM. Uptake of oxidized lipids by the scavenger receptor CD36 promotes lipid peroxidation and dysfunction in $CD8^+$ T cells in tumors. **Immunity** 54: 1561–1577.e7, 2021. doi:10.1016/j.immuni.2021.05.003.

267. Veglia F, Tyurin VA, Blasi M, De Leo A, Kossenkov AV, Donthireddy L, To TK, Schug Z, Basu S, Wang F, Ricciotti E, DiRusso C, Murphy ME, Vonderheide RH, Lieberman PM, Mulligan C, Nam B, Hockstein N, Masters G, Guarino M, Lin C, Nefedova Y, Black P, Kagan VE, Gabrilovich DI. Fatty acid transport protein 2 reprograms neutrophils in cancer. **Nature** 569: 73–78, 2019. doi:10.1038/s41586-019-1118-2.

268. Kim R, Hashimoto A, Markosyan N, Tyurin VA, Tyurina YY, Kar G, Fu S, Sehgal M, Garcia-Gerique L, Kossenkov A, Gebregziabher BA, Tobias JW, Hicks K, Halpin RA, Cvetesic N, Deng H, Donthireddy L, Greenberg A, Nam B, Vonderheide RH, Nefedova Y, Kagan VE, Gabrilovich DI. Ferroptosis of tumour neutrophils causes immune suppression in cancer. **Nature** 612: 338–346, 2022. doi:10.1038/s41586-022-05443-0.

269. Ubellacker JM, Tasdogan A, Ramesh V, Shen B, Mitchell EC, Martin-Sandoval MS, Gu Z, McCormick ML, Durham AB, Spitz DR, Zhao Z, Mathews TP, Morrison SJ. Lymph protects metastasizing melanoma cells from ferroptosis. **Nature** 585: 113–118, 2020. doi:10.1038/s41586-020-2623-z.

270. Pascual G, Domínguez D, Elosúa-Bayes M, Beckedorff F, Laudanna C, Bigas C, Douillet D, Greco C, Symeonidi A, Hernández I, Gil SR, Prats N, Bescós C, Shiekhattar R, Amit M, Heyn H, Shilatifard A, Benitah SA. Dietary palmitic acid promotes a prometastatic memory via Schwann cells. **Nature** 599: 485–490, 2021. doi:10.1038/s41586-021-04075-0.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

271. Li Z, Liao X, Hu Y, Li M, Tang M, Zhang S, Mo S, Li X, Chen S, Qian W, Feng R, Yu R, Xu Y, Yuan S, Xie C, Li J. SLC27A4-mediated selective uptake of mono-unsaturated fatty acids promotes ferroptosis defense in hepatocellular carcinoma. **Free Radic Biol Med** 201: 41–54, 2023. doi:10.1016/j.freeradbiomed.2023.03.013.

272. Terry AR, Nogueira V, Rho H, Ramakrishnan G, Li J, Kang S, Pathmasiri KC, Bhat SA, Jiang L, Kuchay S, Cologna SM, Hay N. CD36 maintains lipid homeostasis via selective uptake of mono-unsaturated fatty acids during matrix detachment and tumor progression. **Cell Metab** 35: 2060–2076.e9, 2023. doi:10.1016/j.cmet.2023.09.012.

273. Sokol KH, Lee CJ, Rogers TJ, Waldhart A, Ellis AE, Madireddy S, Daniels SR, House RR, Ye X, Olesnavich M, Johnson A, Furness BR, Sheldon RD, Lien EC. Lipid availability influences ferroptosis sensitivity in cancer cells by regulating polyunsaturated fatty acid trafficking. **Cell Chem Biol** 2024. doi:10.1016/j.chembiol.2024.09.008.

274. Bartolacci C, Andreani C, Vale G, Berto S, Melegari M, Crouch AC, Baluya DL, Kemble G, Hodges K, Starrett J, Politi K, Starnes SL, Lorenzini D, Raso MG, Solis Soto LM, Behrens C, Kadara H, Gao B, Wistuba II, Minna JD, McDonald JG, Scaglioni PP. Targeting de novo lipogenesis and the Lands cycle induces ferroptosis in KRAS-mutant lung cancer. **Nat Commun** 13: 4327, 2022. doi:10.1038/s41467-022-31963-4.

275. Ates G, Goldberg J, Currais A, Maher P. CMS121, a fatty acid synthase inhibitor, protects against excess lipid peroxidation and inflammation and alleviates cognitive loss in a transgenic mouse model of Alzheimer's disease. **Redox Biol** 36: 101648, 2020. doi:10.1016/j.redox.2020.101648.

276. Wakil SJ. Fatty acid synthase, a proficient multifunctional enzyme. **Biochemistry** 28: 4523–4530, 1989. doi:10.1021/bi00437a001.

277. Lee JY, Nam M, Son HY, Hyun K, Jang SY, Kim JW, Kim MW, Jung Y, Jang E, Yoon SJ, Kim J, Kim J, Seo J, Min JK, Oh KJ, Han BS, Kim WK, Bae KH, Song J, Kim J, Huh YM, Hwang GS, Lee EW, Lee SC. Polyunsaturated fatty acid biosynthesis pathway determines ferroptosis sensitivity in gastric cancer. **Proc Natl Acad Sci USA** 117: 32433–32442, 2020. doi:10.1073/pnas.2006828117.

278. Yamane D, Hayashi Y, Matsumoto M, Nakanishi H, Imagawa H, Kohara M, Lemon SM, Ichi I. FADS2-dependent fatty acid desaturation dictates cellular sensitivity to ferroptosis and permissiveness for hepatitis C virus replication. **Cell Chem Biol** 29: 799–810.e4, 2022. doi:10.1016/j.chembiol.2021.07.022.

279. Lorito N, Subbiani A, Smiriglia A, Bacci M, Bonechi F, Tronci L, Romano E, Corrado A, Longo DL, Iozzo M, Ippolito L, Comito G, Giannoni E, Meattini I, Avgustinova A, Chiarugi P, Bachi A, Morandi A. FADS1/2 control lipid metabolism and ferroptosis susceptibility in triple-negative breast cancer. **EMBO Mol Med** 16: 1533–1559, 2024. doi:10.1038/s44321-024-00090-6.

280. Xin S, Mueller C, Pfeiffer S, Kraft VA, Merl-Pham J, Bao X, Feederle R, Jin X, Hauck SM, Schmitt-Kopplin P, Schick JA. MS4A15 drives ferroptosis resistance through calcium-restricted lipid remodeling. **Cell Death Differ** 29: 670–686, 2022. doi:10.1038/s41418-021-00883-z.

281. Tesfay L, Paul BT, Konstorum A, Deng Z, Cox AO, Lee J, Furdui CM, Hegde P, Torti FM, Torti SV. Stearoyl-CoA desaturase 1 protects ovarian cancer cells from ferroptotic cell death. **Cancer Res** 79: 5355–5366, 2019. doi:10.1158/0008-5472.CAN-19-0369.

282. Luis G, Godfroid A, Nishiumi S, Cimino J, Blacher S, Maquoi E, Wery C, Collignon A, Longuespée R, Montero-Ruiz L, Dassoul I, Maloujahmoum N, Pottier C, Mazzucchelli G, Depauw E, Bellahcène A, Yoshida M, Noel A, Sounni NE. Tumor resistance to ferroptosis driven by Stearoyl-CoA Desaturase-1 (SCD1) in cancer cells and Fatty Acid Biding Protein-4 (FABP4) in tumor microenvironment promote tumor recurrence. **Redox Biol** 43: 102006, 2021. doi:10.1016/j.redox.2021.102006.

283. Wohlhieter CA, Richards AL, Uddin F, Hulton CH, Quintanal-Villalonga A, Martin A, de Stanchina E, Bhanot U, Asher M, Shah NS, Hayatt O, Buonocore DJ, Rekhtman N, Shen R, Arbour KC, Donoghue M, Poirier JT, Sen T, Rudin CM. Concurrent mutations in STK11 and KEAP1 promote ferroptosis protection and SCD1 dependence in lung cancer. **Cell Rep** 33: 108444, 2020. doi:10.1016/j.celrep.2020.108444.

284. Vriens K, Christen S, Parik S, Broekaert D, Yoshinaga K, Talebi A, et al. Evidence for an alternative fatty acid desaturation pathway increasing cancer plasticity. **Nature** 566: 403–406, 2019. doi:10.1038/s41586-019-0904-1.

285. Yi J, Zhu J, Wu J, Thompson CB, Jiang X. Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis via SREBP-mediated lipogenesis. **Proc Natl Acad Sci USA** 117: 31189–31197, 2020. doi:10.1073/pnas.2017152117.

286. Chen H, Qi Q, Wu N, Wang Y, Feng Q, Jin R, Jiang L. Aspirin promotes RSL3-induced ferroptosis by suppressing mTOR/SREBP-1/SCD1-mediated lipogenesis in PIK3CA-mutant colorectal cancer. **Redox Biol** 55: 102426, 2022. doi:10.1016/j.redox.2022.102426.

287. Sabatier M, Birsen R, Lauture L, Mouche S, Angelino P, Dehairs J, et al. C/EBPalpha confers dependence to fatty acid anabolic pathways and vulnerability to lipid oxidative stress-induced ferroptosis in FLT3-mutant leukemia. **Cancer Discov** 13: 1720–1747, 2023. doi:10.1158/2159-8290.CD-22-0411.

288. Ding Z, Pan Y, Shang T, Jiang T, Lin Y, Yang C, Pang S, Cui X, Wang Y, Feng XF, Xu M, Pei M, Chen Y, Li X, Ding J, Tan Y, Wang H, Dong L, Wang L. URI alleviates tyrosine kinase inhibitors-induced ferroptosis by reprogramming lipid metabolism in p53 wild-type liver cancers. **Nat Commun** 14: 6269, 2023. doi:10.1038/s41467-023-41852-z.

289. Liu MY, Li HM, Wang XY, Xia R, Li X, Ma YJ, Wang M, Zhang HS. TIGAR drives colorectal cancer ferroptosis resistance through ROS/AMPK/SCD1 pathway. **Free Radic Biol Med** 182: 219–231, 2022. doi:10.1016/j.freeradbiomed.2022.03.002.

290. Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, Prokisch H, Trümbach D, Mao G, Qu F, Bayir H, Füllekrug J, Scheel CH, Wurst W, Schick JA, Kagan VE, Angeli JP, Conrad M. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. **Nat Chem Biol** 13: 91–98, 2017. doi:10.1038/nchembio.2239.

291. Kagan VE, Mao G, Qu F, Angeli JP, Doll S, Croix CS, Dar HH, Liu B, Tyurin VA, Ritov VB, Kapralov AA, Amoscato AA, Jiang J, Anthonymuthu T, Mohammadyani D, Yang Q, Proneth B, Klein-Seetharaman J, Watkins S, Bahar I, Greenberger J, Mallampalli RK, Stockwell BR, Tyurina YY, Conrad M, Bayır H. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. **Nat Chem Biol** 13: 81–90, 2017. doi:10.1038/nchembio.2238.

292. Beatty A, Singh T, Tyurina YY, Tyurin VA, Samovich S, Nicolas E, Maslar K, Zhou Y, Cai KQ, Tan Y, Doll S, Conrad M, Subramanian A, Bayır H, Kagan VE, Rennefahrt U, Peterson JR. Ferroptotic cell death triggered by conjugated linolenic acids is mediated by ACSL1. **Nat Commun** 12: 2244, 2021. doi:10.1038/s41467-021-22471-y.

293. Ma L, Chen C, Zhao C, Li T, Ma L, Jiang J, Duan Z, Si Q, Chuang TH, Xiang R, Luo Y. Targeting carnitine palmitoyl transferase 1A (CPT1A) induces ferroptosis and synergizes with immunotherapy in lung cancer. **Signal Transduct Target Ther** 9: 64, 2024. doi:10.1038/s41392-024-01772-w.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

294. Miess H, Dankworth B, Gouw AM, Rosenfeldt M, Schmitz W, Jiang M, Saunders B, Howell M, Downward J, Felsher DW, Peck B, Schulze A. The glutathione redox system is essential to prevent ferroptosis caused by impaired lipid metabolism in clear cell renal cell carcinoma. **Oncogene** 37: 5435–5450, 2018. doi:10.1038/s41388-018-0315-z.

295. Xiao L, Ma X, Ye L, Su P, Xiong W, Bi E, Wang Q, Xian M, Yang M, Qian J, Yi Q. IL-9/STAT3/fatty acid oxidation–mediated lipid peroxidation contributes to Tc9 cell longevity and enhanced antitumor activity. **J Clin Invest** 132: e153247, 2022. doi:10.1172/JCI153247.

296. Loos M, Klampe B, Schulze T, Yin X, Theofilatos K, Ulmer BM, Schulz C, Behrens CS, van Bergen TD, Adami E, Maatz H, Schweizer M, Brodesser S, Skryabin BV, Rozhdestvensky TS, Bodbin S, Stathopoulou K, Christ T, Denning C, Hübner N, Mayr M, Cuello F, Eschenhagen T, Hansen A. Human model of primary carnitine deficiency cardiomyopathy reveals ferroptosis as a novel mechanism. **Stem Cell Reports** 18: 2123–2137, 2023. doi:10.1016/j.stemcr.2023.09.002.

297. Blomme A, Ford CA, Mui E, Patel R, Ntala C, Jamieson LE, Planque M, McGregor GH, Peixoto P, Hervouet E, Nixon C, Salji M, Gaughan L, Markert E, Repiscak P, Sumpton D, Blanco GR, Lilla S, Kamphorst JJ, Graham D, Faulds K, MacKay GM, Fendt SM, Zanivan S, Leung HY. 2,4-Dienoyl-CoA reductase regulates lipid homeostasis in treatment-resistant prostate cancer. **Nat Commun** 11: 2508, 2020. doi:10.1038/s41467-020-16126-7.

298. Nassar ZD, Mah CY, Dehairs J, Burvenich IJ, Irani S, Centenera MM, Helm M, Shrestha RK, Moldovan M, Don AS, Holst J, Scott AM, Horvath LG, Lynn DJ, Selth LA, Hoy AJ, Swinnen JV, Butler LM. Human DECR1 is an androgen-repressed survival factor that regulates PUFA oxidation to protect prostate tumor cells from ferroptosis. **Elife** 9: e54166, 2020. doi:10.7554/eLife.54166.

299. Tan SK, Mahmud I, Fontanesi F, Puchowicz M, Neumann CKA, Griswold AJ, Patel R, Dispagna M, Ahmed HH, Gonzalgo ML, Brown JM, Garrett TJ, Welford SM. Obesity-dependent adipokine chemerin suppresses fatty acid oxidation to confer ferroptosis resistance. **Cancer Discov** 11: 2072–2093, 2021. doi:10.1158/2159-8290.CD-20-1453.

300. Bailey AP, Koster G, Guillermier C, Hirst EM, MacRae JI, Lechene CP, Postle AD, Gould AP. Antioxidant role for lipid droplets in a stem cell niche of *Drosophila*. **Cell** 163: 340–353, 2015. doi:10.1016/j.cell.2015.09.020.

301. Li N, Sancak Y, Frasor J, Atilla-Gokcumen GE. A protective role for triacylglycerols during apoptosis. **Biochemistry** 57: 72–80, 2018. doi:10.1021/acs.biochem.7b00975.

302. Minami JK, Morrow D, Bayley NA, Fernandez EG, Salinas JJ, Tse C, Zhu H, Su B, Plawat R, Jones A, Sammarco A, Liau LM, Graeber TG, Williams KJ, Cloughesy TF, Dixon SJ, Bensinger SJ, Nathanson DA. CDKN2A deletion remodels lipid metabolism to prime glioblastoma for ferroptosis. **Cancer Cell** 41: 1048–1060.e9, 2023. doi:10.1016/j.ccell.2023.05.001.

303. Dierge E, Debock E, Guilbaud C, Corbet C, Mignolet E, Mignard L, Bastien E, Dessy C, Larondelle Y, Feron O. Peroxidation of n-3 and n-6 polyunsaturated fatty acids in the acidic tumor environment leads to ferroptosis-mediated anticancer effects. **Cell Metab** 33: 1701–1715.e5, 2021. doi:10.1016/j.cmet.2021.05.016.

304. Lee H, Horbath A, Kondiparthi L, Meena JK, Lei G, Dasgupta S, Liu X, Zhuang L, Koppula P, Li M, Mahmud I, Wei B, Lorenzi PL, Keyomarsi K, Poyurovsky MV, Olszewski K, Gan B. Cell cycle arrest induces lipid droplet formation and confers ferroptosis resistance. **Nat Commun** 15: 79, 2024. doi:10.1038/s41467-023-44412-7.

305. McLelland GL, Lopez-Osias M, Verzijl CR, Ellenbroek BD, Oliveira RA, Boon NJ, Dekker M, van den Hengel LG, Ali R, Janssen H, Song JY, Krimpenfort P, van Zutphen T, Jonker JW, Brummelkamp TR. Identification of an alternative triglyceride biosynthesis pathway. **Nature** 621: 171–178, 2023. doi:10.1038/s41586-023-06497-4.

306. Gnanapradeepan K, Indeglia A, Stieg DC, Clarke N, Shao C, Dougherty JF, Murali N, Murphy ME. PLTP is a p53 target gene with roles in cancer growth suppression and ferroptosis. **J Biol Chem** 298: 102637, 2022. doi:10.1016/j.jbc.2022.102637.

307. Bai Y, Meng L, Han L, Jia Y, Zhao Y, Gao H, Kang R, Wang X, Tang D, Dai E. Lipid storage and lipophagy regulates ferroptosis. **Biochem Biophys Res Commun** 508: 997–1003, 2019. doi:10.1016/j.bbrc.2018.12.039.

308. Cui S, Simmons G Jr, Vale G, Deng Y, Kim J, Kim H, Zhang R, McDonald JG, Ye J. FAF1 blocks ferroptosis by inhibiting peroxidation of polyunsaturated fatty acids. **Proc Natl Acad Sci USA** 119: e2107189119, 2022. doi:10.1073/pnas.2107189119.

309. Wiernicki B, Dubois H, Tyurina YY, Hassannia B, Bayir H, Kagan VE, Vandenabeele P, Wullaert A, Vanden Berghe T. Excessive phospholipid peroxidation distinguishes ferroptosis from other cell death modes including pyroptosis. **Cell Death Dis** 11: 922, 2020. doi:10.1038/s41419-020-03118-0.

310. Yang X, Wang Z, Samovich SN, Kapralov AA, Amoscato AA, Tyurin VA, Dar HH, Li Z, Duan S, Kon N, Chen D, Tycko B, Zhang Z, Jiang X, Bayir H, Stockwell BR, Kagan VE, Gu W. PHLDA2-mediated phosphatidic acid peroxidation triggers a distinct ferroptotic response during tumor suppression. **Cell Metab** 36: 762–777.e9, 2024. doi:10.1016/j.cmet.2024.01.006.

311. Phadnis VV, Snider J, Varadharajan V, Ramachandiran I, Deik AA, Lai ZW, Kunchok T, Eaton EN, Sebastiany C, Lyakisheva A, Vaccaro KD, Allen J, Yao Z, Wong V, Geng B, Weiskopf K, Clish CB, Brown JM, Stagljar I, Weinberg RA, Henry WS. MMD collaborates with ACSL4 and MBOAT7 to promote polyunsaturated phosphatidylinositol remodeling and susceptibility to ferroptosis. **Cell Rep** 42: 113023, 2023. doi:10.1016/j.celrep.2023.113023.

312. Lin Z, Liu J, Long F, Kang R, Kroemer G, Tang D, Yang M. The lipid flippase SLC47A1 blocks metabolic vulnerability to ferroptosis. **Nat Commun** 13: 7965, 2022. doi:10.1038/s41467-022-35707-2.

313. Liu X, Chen Z, Yan Y, Zandkarimi F, Nie L, Li Q, Horbath A, Olszewski K, Kondiparthi L, Mao C, Lee H, Zhuang L, Poyurovsky M, Stockwell BR, Chen J, Gan B. Proteomic analysis of ferroptosis pathways reveals a role of CEPT1 in suppressing ferroptosis. **Protein Cell** 15: 686–703, 2024. doi:10.1093/procel/pwae004.

314. Horibata Y, Ando H, Sugimoto H. Locations and contributions of the phosphotransferases EPT1 and CEPT1 to the biosynthesis of ethanolamine phospholipids. **J Lipid Res** 61: 1221–1231, 2020. doi:10.1194/jlr.RA120000898.

315. Horibata Y, Sugimoto H. Differential contributions of choline phosphotransferases CPT1 and CEPT1 to the biosynthesis of choline phospholipids. **J Lipid Res** 62: 100100, 2021. doi:10.1016/j.jlr.2021.100100.

316. Zhu Y, Fujimaki M, Snape L, Lopez A, Fleming A, Rubinsztein DC. Loss of WIPI4 in neurodegeneration causes autophagy-independent ferroptosis. **Nat Cell Biol** 26: 542–551, 2024. doi:10.1038/s41556-024-01373-3.

317. Zou Y, Henry WS, Ricq EL, Graham ET, Phadnis VV, Maretich P, Paradkar S, Boehnke N, Deik AA, Reinhardt F, Eaton JK, Ferguson B, Wang W, Fairman J, Keys HR, Dančík V, Clish CB, Clemons PA, Hammond PT, Boyer LA, Weinberg RA, Schreiber SL. Plasticity of

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

ether lipids promotes ferroptosis susceptibility and evasion. **Nature** 585: 603–608, 2020. doi:10.1038/s41586-020-2732-8.

318. Magtanong L, Mueller GD, Williams KJ, Billmann M, Chan K, Armenta DA, Pope LE, Moffat J, Boone C, Myers CL, Olzmann JA, Bensinger SJ, Dixon SJ**.** Context-dependent regulation of ferroptosis sensitivity. **Cell Chem Biol** 29: 1409–1418.e6, 2022. doi:10.1016/j.chembiol.2022.06.004.

319. Perez MA, Magtanong L, Dixon SJ, Watts JL**.** Dietary lipids induce ferroptosis in *Caenorhabditis elegans* and human cancer cells. **Dev Cell** 54: 447–454.e4, 2020. doi:10.1016/j.devcel.2020.06.019.

320. Braverman NE, Moser AB**.** Functions of plasmalogen lipids in health and disease. **Biochim Biophys Acta** 1822: 1442–1452, 2012. doi:10.1016/j.bbadis.2012.05.008.

321. Perez MA, Clostio AJ, Houston IR, Ruiz J, Magtanong L, Dixon SJ, Watts JL**.** Ether lipid deficiency disrupts lipid homeostasis leading to ferroptosis sensitivity. **PLoS Genet** 18: e1010436, 2022. doi:10.1371/journal.pgen.1010436.

322. Morgan PK, Pernes G, Huynh K, Giles C, Paul S, Smith AAT, Mellett NA, Liang A, van Buuren-Milne T, Veiga CB, Collins TJ, Xu Y, Lee MK, De Silva TM, Meikle PJ, Lancaster GI, Murphy AJ**.** A lipid atlas of human and mouse immune cells provides insights into ferroptosis susceptibility. **Nat Cell Biol** 26: 645–659, 2024. doi:10.1038/s41556-024-01377-z.

323. Henry WS, Müller S, Yang JS, Innes-Gold S, Das S, Reinhardt F, Sigmund K, Phadnis VV, Wan Z, Eaton E, Sampaio JL, Bell GW, Viravalli A, Hammond PT, Kamm RD, Cohen AE, Boehnke N, Hsu VW, Levental KR, Rodriguez R, Weinberg RA**.** Ether lipids influence cancer cell fate by modulating iron uptake (Preprint). **bioRxiv** 2024.03.29.585922, 2024. doi:10.1101/2024.03.20.585922.

324. Honsho M, Asaoku S, Fujiki Y**.** Posttranslational regulation of fatty acyl-CoA reductase 1, Far1, controls ether glycerophospholipid synthesis. **J Biol Chem** 285: 8537–8542, 2010. doi:10.1074/jbc.M109.083311.

325. Liang D, Feng Y, Zandkarimi F, Wang H, Zhang Z, Kim J, Cai Y, Gu W, Stockwell BR, Jiang X**.** Ferroptosis surveillance independent of GPX4 and differentially regulated by sex hormones. **Cell** 186: 2748–2764.e22, 2023. doi:10.1016/j.cell.2023.05.003.

326. Soula M, Weber RA, Zilka O, Alwaseem H, La K, Yen F, Molina H, Garcia-Bermudez J, Pratt DA, Birsoy K**.** Metabolic determinants of cancer cell sensitivity to canonical ferroptosis inducers. **Nat Chem Biol** 16: 1351–1360, 2020. doi:10.1038/s41589-020-0613-y.

327. Beharier O, Tyurin VA, Goff JP, Guerrero-Santoro J, Kajiwara K, Chu T, Tyurina YY, St Croix CM, Wallace CT, Parry S, Parks WT, Kagan VE, Sadovsky Y**.** PLA2G6 guards placental trophoblasts against ferroptotic injury. **Proc Natl Acad Sci USA** 117: 27319–27328, 2020, doi:10.1073/pnas.2009201117.

328. Chen D, Chu B, Yang X, Liu Z, Jin Y, Kon N, Rabadan R, Jiang X, Stockwell BR, Gu W**.** iPLA2beta-mediated lipid detoxification controls p53-driven ferroptosis independent of GPX4. **Nat Commun** 12: 3644, 2021. doi:10.1038/s41467-021-23902-6.

329. Sun WY, Tyurin VA, Mikulska-Ruminska K, Shrivastava IH, Anthonymuthu TS, Zhai YJ, Pan MH, Gong HB, Lu DH, Sun J, Duan WJ, Korolev S, Abramov AY, Angelova PR, Miller I, Beharier O, Mao GW, Dar HH, Kapralov AA, Amoscato AA, Hastings TG, Greenamyre TJ, Chu CT, Sadovsky Y, Bahar I, Bayır H, Tyurina YY, He RR, Kagan VE**.** Phospholipase iPLA$_2$beta averts ferroptosis by eliminating a redox lipid death signal. **Nat Chem Biol** 17: 465–476, 2021. doi:10.1038/s41589-020-00734-x.

330. Oh M, Jang SY, Lee JY, Kim JW, Jung Y, Kim J, Seo J, Han TS, Jang E, Son HY, Kim D, Kim MW, Park JS, Song KH, Oh KJ, Kim WK, Bae KH, Huh YM, Kim SH, Kim D, Han BS, Lee SC, Hwang GS, Lee EW**.** The lipoprotein-associated phospholipase A2 inhibitor Darapladib sensitises cancer cells to ferroptosis by remodelling lipid metabolism. **Nat Commun** 14: 5728, 2023. doi:10.1038/s41467-023-41462-9.

331. Luo X, Gong HB, Gao HY, Wu YP, Sun WY, Li ZQ, Wang G, Liu B, Liang L, Kurihara H, Duan WJ, Li YF, He RR**.** Oxygenated phosphatidylethanolamine navigates phagocytosis of ferroptotic cells by interacting with TLR2. **Cell Death Differ** 28: 1971–1989, 2021. doi:10.1038/s41418-020-00719-2.

332. Lu B, Chen XB, Hong YC, Zhu H, He QJ, Yang B, Ying MD, Cao J**.** Identification of PRDX6 as a regulator of ferroptosis. **Acta Pharmacol Sin** 40: 1334–1342, 2019. doi:10.1038/s41401-019-0233-9.

333. Torres-Velarde JM, Allen KN, Salvador-Pascual A, Leija RG, Luong D, Moreno-Santillán DD, Ensminger DC, Vázquez-Medina JP**.** Peroxiredoxin 6 suppresses ferroptosis in lung endothelial cells. **Free Radic Biol Med** 218: 82–93, 2024. doi:10.1016/j.freeradbiomed.2024.04.208.

334. Kathman SG, Boshart J, Jing H, Cravatt BF**.** Blockade of the lysophosphatidylserine lipase ABHD12 potentiates ferroptosis in cancer cells. **ACS Chem Biol** 15: 871–877, 2020. doi:10.1021/acschembio.0c00086.

335. Zhang Q, Sun T, Yu F, Liu W, Gao J, Chen J, et al**.** PAFAH2 suppresses synchronized ferroptosis to ameliorate acute kidney injury. **Nat Chem Biol** 20: 934, 2024. doi:10.1038/s41589-023-01528-7.

336. Dixon SJ, Winter GE, Musavi LS, Lee ED, Snijder B, Rebsamen M, Superti-Furga G, Stockwell BR**.** Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. **ACS Chem Biol** 10: 1604–1609, 2015. doi:10.1021/acschembio.5b00245.

337. Reed A, Ware T, Li H, Fernando Bazan J, Cravatt BF**.** TMEM164 is an acyltransferase that forms ferroptotic C20:4 ether phospholipids. **Nat Chem Biol** 19: 378–388, 2023. doi:10.1038/s41589-022-01253-7.

338. Li Z, Hu Y, Zheng H, Li M, Liu Y, Feng R, Li X, Zhang S, Tang M, Yang M, Yu R, Xu Y, Liao X, Chen S, Qian W, Zhang Q, Tang D, Li B, Song L, Li J**.** LPCAT1-mediated membrane phospholipid remodelling promotes ferroptosis evasion and tumour growth. **Nat Cell Biol** 26: 811–824, 2024. doi:10.1038/s41556-024-01405-y.

339. Hashidate-Yoshida T, Harayama T, Hishikawa D, Morimoto R, Hamano F, Tokuoka SM, Eto M, Tamura-Nakano M, Yanobu-Takanashi R, Mukumoto Y, Kiyonari H, Okamura T, Kita Y, Shindou H, Shimizu T**.** Fatty acid remodeling by LPCAT3 enriches arachidonate in phospholipid membranes and regulates triglyceride transport. **ELife** 4: e06328, 2015. doi:10.7554/eLife.06328.

340. Reed A, Ichu TA, Milosevich N, Melillo B, Schafroth MA, Otsuka Y, Scampavia L, Spicer TP, Cravatt BF**.** LPCAT3 inhibitors remodel the polyunsaturated phospholipid content of human cells and protect from ferroptosis. **ACS Chem Biol** 17: 1607–1618, 2022. doi:10.1021/acschembio.2c00317.

341. Qiu B, Zandkarimi F, Bezjian CT, Reznik E, Soni RK, Gu W, Jiang X, Stockwell BR**.** Phospholipids with two polyunsaturated fatty acyl tails promote ferroptosis. **Cell** 187: 1177–1190.e18, 2024. doi:10.1016/j.cell.2024.01.030.

342. Kagan VE, Tyurina YY, Sun WY, Vlasova II, Dar H, Tyurin VA, Amoscato AA, Mallampalli R, van der Wel PC, He RR, Shvedova AA, Gabrilovich DI, Bayir H**.** Redox phospholipidomics of enzymatically generated oxygenated phospholipids as specific signals of programmed cell death. **Free Radic Biol Med** 147: 231–241, 2020. doi:10.1016/j.freeradbiomed.2019.12.028.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

343. Song S, Su Z, Kon N, Chu B, Li H, Jiang X, Luo J, Stockwell BR, Gu W. ALOX5-mediated ferroptosis acts as a distinct cell death pathway upon oxidative stress in Huntington's disease. **Genes Dev** 37: 204–217, 2023. doi:10.1101/gad.350211.122.

344. Kuang F, Liu J, Xie Y, Tang D, Kang R. MGST1 is a redox-sensitive repressor of ferroptosis in pancreatic cancer cells. **Cell Chem Biol** 28: 765–775.e5, 2021. doi:10.1016/j.chembiol.2021.01.006.

345. Chu B, Kon N, Chen D, Li T, Liu T, Jiang L, Song S, Tavana O, Gu W. ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway. **Nat Cell Biol** 21: 579–591, 2019. doi:10.1038/s41556-019-0305-6.

346. Wenzel SE, Tyurina YY, Zhao J, St Croix CM, Dar HH, Mao G, Tyurin VA, Anthonymuthu TS, Kapralov AA, Amoscato AA, Mikulska-Ruminska K, Shrivastava IH, Kenny EM, Yang Q, Rosenbaum JC, Sparvero LJ, Emlet DR, Wen X, Minami Y, Qu F, Watkins SC, Holman TR, VanDemark AP, Kellum JA, Bahar I, Bayır H, Kagan VE. PEBP1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. **Cell** 171: 628–641.e6, 2017. doi:10.1016/j.cell.2017.09.044.

347. Matsushita M, Freigang S, Schneider C, Conrad M, Bornkamm GW, Kopf M. T cell lipid peroxidation induces ferroptosis and prevents immunity to infection. **J Exp Med** 212: 555–568, 2015. doi:10.1084/jem.20140857.

348. Brütsch SH, Wang CC, Li L, Stender H, Neziroglu N, Richter C, Kuhn H, Borchert A. Expression of inactive glutathione peroxidase 4 leads to embryonic lethality, and inactivation of the Alox15 gene does not rescue such knock-in mice. **Antioxid Redox Signal** 22: 281–293, 2015. doi:10.1089/ars.2014.5967.

349. Bishop-Bailey D, Thomson S, Askari A, Faulkner A, Wheeler-Jones C. Lipid-metabolizing CYPs in the regulation and dysregulation of metabolism. **Annu Rev Nutr** 34: 261–279, 2014. doi:10.1146/annurev-nutr-071813-105747.

350. Zou Y, Li H, Graham ET, Deik AA, Eaton JK, Wang W, Sandoval-Gomez G, Clish CB, Doench JG, Schreiber SL. Cytochrome P450 oxidoreductase contributes to phospholipid peroxidation in ferroptosis. **Nat Chem Biol** 16: 302–309, 2020. doi:10.1038/s41589-020-0472-6.

351. Yan B, Ai Y, Sun Q, Ma Y, Cao Y, Wang J, Zhang Z, Wang X. Membrane damage during ferroptosis is caused by oxidation of phospholipids catalyzed by the oxidoreductases POR and CYB5R1. **Mol Cell** 81: 355–369.e10, 2021. doi:10.1016/j.molcel.2020.11.024.

352. Poursaitidis I, Wang X, Crighton T, Labuschagne C, Mason D, Cramer SL, Triplett K, Roy R, Pardo OE, Seckl MJ, Rowlinson SW, Stone E, Lamb RF. Oncogene-selective sensitivity to synchronous cell death following modulation of the amino acid nutrient cystine. **Cell Rep** 18: 2547–2556, 2017. doi:10.1016/j.celrep.2017.02.054.

353. Xie Y, Zhu S, Song X, Sun X, Fan Y, Liu J, Zhong M, Yuan H, Zhang L, Billiar TR, Lotze MT, Zeh HJ 3rd, Kang R, Kroemer G, Tang D. The tumor suppressor p53 limits ferroptosis by blocking DPP4 activity. **Cell Rep** 20: 1692–1704, 2017. doi:10.1016/j.celrep.2017.07.055.

354. Yang WH, Ding CC, Sun T, Rupprecht G, Lin CC, Hsu D, Chi JT. The Hippo pathway effector TAZ regulates ferroptosis in renal cell carcinoma. **Cell Rep** 28: 2501–2508.e4, 2019. doi:10.1016/j.celrep.2019.07.107.

355. Verrey F, Closs EI, Wagner CA, Palacin M, Endou H, Kanai Y. CATs and HATs: the SLC7 family of amino acid transporters. **Pflugers Arch** 447: 532–542, 2004. doi:10.1007/s00424-003-1086-z.

356. Zheng J, Sato M, Mishima E, Sato H, Proneth B, Conrad M. Sorafenib fails to trigger ferroptosis across a wide range of cancer cell lines. **Cell Death Dis** 12: 698, 2021. doi:10.1038/s41419-021-03998-w.

357. Sato M, Onuma K, Domon M, Hasegawa S, Suzuki A, Kusumi R, Hino R, Kakihara N, Kanda Y, Osaki M, Hamada J, Bannai S, Feederle R, Buday K, Angeli JP, Proneth B, Conrad M, Okada F, Sato H. Loss of the cystine/glutamate antiporter in melanoma abrogates tumor metastasis and dramatically increases survival rates of mice. **Int J Cancer** 147: 3224–3235, 2020. doi:10.1002/ijc.33262.

358. Verbruggen L, Ates G, Lara O, De Munck J, Villers A, De Pauw L, Ottestad-Hansen S, Kobayashi S, Beckers P, Janssen P, Sato H, Zhou Y, Hermans E, Njemini R, Arckens L, Danbolt NC, De Bundel D, Aerts JL, Barbé K, Guillaume B, Ris L, Bentea E, Massie A. Lifespan extension with preservation of hippocampal function in aged system x_c^--deficient male mice. **Mol Psychiatry** 27: 2355–2368, 2022. doi:10.1038/s41380-022-01470-5.

359. Kobayashi S, Kuwata K, Sugimoto T, Igarashi K, Osaki M, Okada F, Fujii J, Bannai S, Sato H. Enhanced expression of cystine/glutamate transporter in the lung caused by the oxidative-stress-inducing agent paraquat. **Free Radic Biol Med** 53: 2197–2203, 2012. doi:10.1016/j.freeradbiomed.2012.09.040.

360. Shibasaki T, Iuchi Y, Okada F, Kuwata K, Yamanobe T, Bannai S, Tomita Y, Sato H, Fujii J. Aggravation of ischemia-reperfusion-triggered acute renal failure in xCT-deficient mice. **Arch Biochem Biophys** 490: 63–69, 2009. doi:10.1016/j.abb.2009.08.008.

361. Taguchi K, Tamba M, Bannai S, Sato H. Induction of cystine/glutamate transporter in bacterial lipopolysaccharide induced endotoxemia in mice. **J Inflamm (Lond)** 4: 20, 2007. doi:10.1186/1476-9255-4-20.

362. Kobayashi S, Sato M, Kasakoshi T, Tsutsui T, Sugimoto M, Osaki M, Okada F, Igarashi K, Hiratake J, Homma T, Conrad M, Fujii J, Soga T, Bannai S, Sato H. Cystathionine is a novel substrate of cystine/glutamate transporter: implications for immune function. **J Biol Chem** 290: 8778–8788, 2015. doi:10.1074/jbc.M114.625053.

363. Sasaki H, Sato H, Kuriyama-Matsumura K, Sato K, Maebara K, Wang H, Tamba M, Itoh K, Yamamoto M, Bannai S. Electrophile response element-mediated induction of the cystine/glutamate exchange transporter gene expression. **J Biol Chem** 277: 44765–44771, 2002. doi:10.1074/jbc.M208704200.

364. Chen D, Tavana O, Chu B, Erber L, Chen Y, Baer R, Gu W. NRF2 is a major target of ARF in p53-independent tumor suppression. **Mol Cell** 68: 224–232.e4, 2017. doi:10.1016/j.molcel.2017.09.009.

365. Chen D, Fan Z, Rauh M, Buchfelder M, Eyupoglu IY, Savaskan N. ATF4 promotes angiogenesis and neuronal cell death and confers ferroptosis in a xCT-dependent manner. **Oncogene** 36: 5593–5608, 2017. doi:10.1038/onc.2017.146.

366. Fan Z, Wirth AK, Chen D, Wruck CJ, Rauh M, Buchfelder M, Savaskan N. Nrf2-Keap1 pathway promotes cell proliferation and diminishes ferroptosis. **Oncogenesis** 6: e371, 2017. doi:10.1038/oncsis.2017.65.

367. Wang Z, Yang X, Chen D, Liu Y, Li Z, Duan S, Zhang Z, Jiang X, Stockwell BR, Gu W. GAS41 modulates ferroptosis by anchoring NRF2 on chromatin. **Nat Commun** 15: 2531, 2024. doi:10.1038/s41467-024-46857-w.

368. Ogiwara H, Takahashi K, Sasaki M, Kuroda T, Yoshida H, Watanabe R, Maruyama A, Makinoshima H, Chiwaki F, Sasaki H, Kato T, Okamoto A, Kohno T. targeting the vulnerability of glutathione metabolism in ARID1A-deficient cancers. **Cancer Cell** 35: 177–190.e8, 2019. doi:10.1016/j.ccell.2018.12.009.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

369. Lim JKM, Delaidelli A, Minaker SW, Zhang HF, Colovic M, Yang H, Negri GL, von Karstedt S, Lockwood WW, Schaffer P, Leprivier G, Sorensen PH. Cystine/glutamate antiporter xCT (SLC7A11) facilitates oncogenic RAS transformation by preserving intracellular redox balance. **Proc Natl Acad Sci USA** 116: 9433–9442, 2019. doi:10.1073/pnas.1821323116.

370. Chu J, Jiang J, Fan X, Liu J, Gao K, Jiang Y, Li M, Xi W, Zhang L, Bian K, Yang A, Zhang R. A novel MYC-ZNF706-SLC7A11 regulatory circuit contributes to cancer progression and redox balance in human hepatocellular carcinoma. **Cell Death Differ** 31: 1333–1348, 2024. doi:10.1038/s41418-024-01324-3.

371. Zheng H, Liu J, Cheng Q, Zhang Q, Zhang Y, Jiang L, Huang Y, Li W, Zhao Y, Chen G, Yu F, Liu L, Li Y, Liao X, Xu L, Xiao Y, Zheng Z, Li M, Wang H, Hu G, Du L, Chen Q. Targeted activation of ferroptosis in colorectal cancer via LGR4 targeting overcomes acquired drug resistance. **Nat Cancer** 5: 572–589, 2024. doi:10.1038/s43018-023-00715-8.

372. Zhang W, Sun Y, Bai L, Zhi L, Yang Y, Zhao Q, Chen C, Qi Y, Gao W, He W, Wang L, Chen D, Fan S, Chen H, Piao HL, Qiao Q, Xu Z, Zhang J, Zhao J, Zhang S, Yin Y, Peng C, Li X, Liu Q, Liu H, Wang Y. RBMS1 regulates lung cancer ferroptosis through translational control of SLC7A11. **J Clin Invest** 131: e152067, 2021. doi:10.1172/JCI152067.

373. Jiang L, Kon N, Li T, Wang SJ, Su T, Hibshoosh H, Baer R, Gu W. Ferroptosis as a p53-mediated activity during tumour suppression. **Nature** 520: 57–62, 2015. doi:10.1038/nature14344.

374. He Y, Ling Y, Zhang Z, Mertens RT, Cao Q, Xu X, Guo K, Shi Q, Zhang X, Huo L, Wang K, Guo H, Shen W, Shen M, Feng W, Xiao P. Butyrate reverses ferroptosis resistance in colorectal cancer by inducing c-Fos-dependent xCT suppression. **Redox Biol** 65: 102822, 2023. doi:10.1016/j.redox.2023.102822.

375. Wang L, Liu Y, Du T, Yang H, Lei L, Guo M, Ding HF, Zhang J, Wang H, Chen X, Yan C. ATF3 promotes erastin-induced ferroptosis by suppressing system Xc. **Cell Death Differ** 27: 662–675, 2020. doi:10.1038/s41418-019-0380-z.

376. Nishizawa H, Matsumoto M, Shindo T, Saigusa D, Kato H, Suzuki K, Sato M, Ishii Y, Shimokawa H, Igarashi K. Ferroptosis is controlled by the coordinated transcriptional regulation of glutathione and labile iron metabolism by the transcription factor BACH1. **J Biol Chem** 295: 69–82, 2020. doi:10.1074/jbc.RA119.009548.

377. Zhang X, Duan Y, Li S, Zhang Z, Peng L, Ma X, Wang T, Xiang S, Chen G, Zhou D, Lu D, Qian M, Wang Z. CRISPR screening identifies PRMT1 as a key pro-ferroptotic gene via a two-layer regulatory mechanism. **Cell Rep** 43: 114662, 2024. doi:10.1016/j.celrep.2024.114662.

378. Kim DH, Kim WD, Kim SK, Moon DH, Lee SJ. TGF-beta1-mediated repression of SLC7A11 drives vulnerability to GPX4 inhibition in hepatocellular carcinoma cells. **Cell Death Dis** 11: 406, 2020. doi:10.1038/s41419-020-2618-6.

379. Zhang Y, Shi J, Liu X, Feng L, Gong Z, Koppula P, Sirohi K, Li X, Wei Y, Lee H, Zhuang L, Chen G, Xiao ZD, Hung MC, Chen J, Huang P, Li W, Gan B. BAP1 links metabolic regulation of ferroptosis to tumour suppression. **Nat Cell Biol** 20: 1181–1192, 2018. doi:10.1038/s41556-018-0178-0.

380. Jiang D, Guo Y, Wang T, Wang L, Yan Y, Xia L, Bam R, Yang Z, Lee H, Iwawaki T, Gan B, Koong AC. IRE1alpha determines ferroptosis sensitivity through regulation of glutathione synthesis. **Nat Commun** 15: 4114, 2024. doi:10.1038/s41467-024-48330-0.

381. Ishimoto T, Nagano O, Yae T, Tamada M, Motohara T, Oshima H, Oshima M, Ikeda T, Asaba R, Yagi H, Masuko T, Shimizu T, Ishikawa T, Kai K, Takahashi E, Imamura Y, Baba Y, Ohmura M, Suematsu M, Baba H, Saya H. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc^- and thereby promotes tumor growth. **Cancer Cell** 19: 387–400, 2011. doi:10.1016/j.ccr.2011.01.038.

382. Tsuchihashi K, Okazaki S, Ohmura M, Ishikawa M, Sampetrean O, Onishi N, Wakimoto H, Yoshikawa M, Seishima R, Iwasaki Y, Morikawa T, Abe S, Takao A, Shimizu M, Masuko T, Nagane M, Furnari FB, Akiyama T, Suematsu M, Baba E, Akashi K, Saya H, Nagano O. The EGF receptor promotes the malignant potential of glioma by regulating amino acid transport system xc^-. **Cancer Res** 76: 2954–2963, 2016. doi:10.1158/0008-5472.CAN-15-2121.

383. Liu T, Jiang L, Tavana O, Gu W. The deubiquitylase OTUB1 mediates ferroptosis via stabilization of SLC7A11. **Cancer Res** 79: 1913–1924, 2019. doi:10.1158/0008-5472.CAN-18-3037.

384. Zhou Q, Yu H, Chen Y, Ren J, Lu Y, Sun Y. The $CRL3^{KCTD10}$ ubiquitin ligase-USP18 axis coordinately regulates cystine uptake and ferroptosis by modulating SLC7A11. **Proc Natl Acad Sci USA** 121: e2320655121, 2024. doi:10.1073/pnas.2320655121.

385. Chen Q, Zheng W, Guan J, Liu H, Dan Y, Zhu L, Song Y, Zhou Y, Zhao X, Zhang Y, Bai Y, Pan Y, Zhang J, Shao C. SOCS2-enhanced ubiquitination of SLC7A11 promotes ferroptosis and radiosensitization in hepatocellular carcinoma. **Cell Death Differ** 30: 137–151, 2023. doi:10.1038/s41418-022-01051-7.

386. Song X, Zhu S, Chen P, Hou W, Wen Q, Liu J, Xie Y, Liu J, Klionsky DJ, Kroemer G, Lotze MT, Zeh HJ, Kang R, Tang D. AMPK-mediated BECN1 phosphorylation promotes ferroptosis by directly blocking system X_c^- activity. **Curr Biol** 28: 2388–2399.e5, 2018. doi:10.1016/j.cub.2018.05.094.

387. Gu Y, Albuquerque CP, Braas D, Zhang W, Villa GR, Bi J, Ikegami S, Masui K, Gini B, Yang H, Gahman TC, Shiau AK, Cloughesy TF, Christofk HR, Zhou H, Guan KL, Mischel PS. mTORC2 regulates amino acid metabolism in cancer by phosphorylation of the cystine-glutamate antiporter xCT. **Mol Cell** 67: 128–138.e7, 2017. doi:10.1016/j.molcel.2017.05.030.

388. Kang YP, Mockabee-Macias A, Jiang C, Falzone A, Prieto-Farigua N, Stone E, Harris IS, DeNicola GM. Non-canonical glutamate-cysteine ligase activity protects against ferroptosis. **Cell Metab** 33: 174–189.e7, 2021. doi:10.1016/j.cmet.2020.12.007.

389. Yang Z, Zou S, Zhang Y, Zhang J, Zhang P, Xiao L, Xie Y, Meng M, Feng J, Kang L, Lee MH, Fang L. ACTL6A protects gastric cancer cells against ferroptosis through induction of glutathione synthesis. **Nat Commun** 14: 4193, 2023. doi:10.1038/s41467-023-39901-8.

390. Lv D, Zhong C, Dixit D, Yang K, Wu Q, Godugu B, Prager BC, Zhao G, Wang X, Xie Q, Bao S, He C, Heiland DH, Rosenfeld MG, Rich JN. EGFR promotes ALKBH5 nuclear retention to attenuate N6-methyladenosine and protect against ferroptosis in glioblastoma. **Mol Cell** 83: 4334–4351.e7, 2023. doi:10.1016/j.molcel.2023.10.025.

391. Shi ZZ, Osei-Frimpong J, Kala G, Kala SV, Barrios RJ, Habib GM, Lukin DJ, Danney CM, Matzuk MM, Lieberman MW. Glutathione synthesis is essential for mouse development but not for cell growth in culture. **Proc Natl Acad Sci USA** 97: 5101–5106, 2000. doi:10.1073/pnas.97.10.5101.

392. Dalton TP, Dieter MZ, Yang Y, Shertzer HG, Nebert DW. Knockout of the mouse glutamate cysteine ligase catalytic subunit (Gclc) gene: embryonic lethal when homozygous, and proposed model for moderate glutathione deficiency when heterozygous. **Biochem**

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

Biophys Res Commun 279: 324–329, 2000. doi:10.1006/bbrc.2000.3930.

393. Winkler A, Njålsson R, Carlsson K, Elgadi A, Rozell B, Abraham L, Ercal N, Shi ZZ, Lieberman MW, Larsson A, Norgren S**.** Glutathione is essential for early embryogenesis–analysis of a glutathione synthetase knockout mouse. **Biochem Biophys Res Commun** 412: 121–126, 2011. doi:10.1016/j.bbrc.2011.07.056.

394. Yang Y, Dieter MZ, Chen Y, Shertzer HG, Nebert DW, Dalton TP**.** Initial characterization of the glutamate-cysteine ligase modifier subunit Gclm$^{-/-}$ knockout mouse. Novel model system for a severely compromised oxidative stress response. **J Biol Chem** 277: 49446–49452, 2002. doi:10.1074/jbc.M209372200.

395. Chen Y, Yang Y, Miller ML, Shen D, Shertzer HG, Stringer KF, Wang B, Schneider SN, Nebert DW, Dalton TP**.** Hepatocyte-specific Gclc deletion leads to rapid onset of steatosis with mitochondrial injury and liver failure. **Hepatology** 45: 1118–1128, 2007. doi:10.1002/hep.21635.

396. Davidson EA, Chen Y, Singh S, Orlicky DJ, Thompson B, Wang Y, Charkoftaki G, Furnary TA, Cardone RL, Kibbey RG, Shearn CT, Nebert DW, Thompson DC, Vasiliou V**.** Endocrine pancreas-specific Gclc gene deletion causes a severe diabetes phenotype (Preprint). **bioRxiv** 2023.06.13.544855, 2023. doi:10.1101/2023.06.13.544855.

397. Espinosa-Díez C, Miguel V, Vallejo S, Sánchez FJ, Sandoval E, Blanco E, Cannata P, Peiró C, Sánchez-Ferrer CF, Lamas S**.** Role of glutathione biosynthesis in endothelial dysfunction and fibrosis. **Redox Biol** 14: 88–99, 2018. doi:10.1016/j.redox.2017.08.019.

398. Telorack M, Meyer M, Ingold I, Conrad M, Bloch W, Werner S**.** A glutathione-Nrf2-thioredoxin cross-talk ensures keratinocyte survival and efficient wound repair. **PLoS Genet** 12: e1005800, 2016. doi:10.1371/journal.pgen.1005800.

399. Feng W, Rosca M, Fan Y, Hu Y, Feng P, Lee HG, Monnier VM, Fan X**.** Gclc deficiency in mouse CNS causes mitochondrial damage and neurodegeneration. **Hum Mol Genet** 26: 1376–1390, 2017. doi:10.1093/hmg/ddx040.

400. Almusafri F, Elamin HE, Khalaf TE, Ali A, Ben-Omran T, El-Hattab AW**.** Clinical and molecular characterization of 6 children with glutamate-cysteine ligase deficiency causing hemolytic anemia. **Blood Cells Mol Dis** 65: 73–77, 2017. doi:10.1016/j.bcmd.2017.05.011.

401. Ekuni S, Hirayama K, Nagasaka M, Osumi K, Kondo H, Nakahara E, Shimojima Yamamoto K, Kanno H, Katayama Y**.** Severe hemolytic anemia and metabolic acidosis at birth with glutathione synthetase deficiency and progressive neurological symptoms on follow-up. **Am J Case Rep** 24: e938396, 2023. doi:10.12659/AJCR.938396.

402. Spielberg SP, Boxer LA, Corash LM, Schulman JD**.** Improved erythrocyte survival with high-dose vitamin E in chronic hemolyzing G6PD and glutathione synthetase deficiencies. **Ann Intern Med** 90: 53–54, 1979. doi:10.7326/0003-4819-90-1-53.

403. Rogers LK, Tamura T, Rogers BJ, Welty SE, Hansen TN, Smith CV**.** Analyses of glutathione reductase hypomorphic mice indicate a genetic knockout. **Toxicol Sci** 82: 367–373, 2004. doi:10.1093/toxsci/kfh268.

404. Kamerbeek NM, van Zwieten R, de Boer M, Morren G, Vuil H, Bannink N, Lincke C, Dolman KM, Becker K, Schirmer RH, Gromer S, Roos D**.** Molecular basis of glutathione reductase deficiency in human blood cells. **Blood** 109: 3560–3566, 2007. doi:10.1182/blood-2006-08-042531.

405. Dixon SJ, Patel DN, Welsch M, Skouta R, Lee ED, Hayano M, Thomas AG, Gleason CE, Tatonetti NP, Slusher BS, Stockwell BR**.** Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. **Elife** 3: e02523, 2014. doi:10.7554/eLife.02523.

406. Ward NP, Yoon SJ, Flynn T, Sherwood AM, Olley MA, Madej J, DeNicola GM**.** Mitochondrial respiratory function is preserved under cysteine starvation via glutathione catabolism in NSCLC. **Nat Commun** 15: 4244, 2024. doi:10.1038/s41467-024-48695-2.

407. Chen MS, Wang SF, Hsu CY, Yin PH, Yeh TS, Lee HC, Tseng LM**.** CHAC1 degradation of glutathione enhances cystine-starvation-induced necroptosis and ferroptosis in human triple negative breast cancer cells via the GCN2-eIF2alpha-ATF4 pathway. **Oncotarget** 8: 114588–114602, 2017. doi:10.18632/oncotarget.23055.

408. Ichihara G, Katsumata Y, Sugiura Y, Matsuoka Y, Maeda R, Endo J, Anzai A, Shirakawa K, Moriyama H, Kitakata H, Hiraide T, Goto S, Ko S, Iwasawa Y, Sugai K, Daigo K, Goto S, Sato K, Yamada K-I, Suematsu M, Ieda M, Sano M**.** MRP1-dependent extracellular release of glutathione induces cardiomyocyte ferroptosis after ischemia-reperfusion. **Circ Res** 133: 861–876, 2023. doi:10.1161/CIRCRESAHA.123.323517.

409. Cao JY, Poddar A, Magtanong L, Lumb JH, Mileur TR, Reid MA, Dovey CM, Wang J, Locasale JW, Stone E, Cole SP, Carette JE, Dixon SJ**.** A genome-wide haploid genetic screen identifies regulators of glutathione abundance and ferroptosis sensitivity. **Cell Rep** 26: 1544–1556.e8, 2019. doi:10.1016/j.celrep.2019.01.043.

410. de Souza I, Monteiro LK, Guedes CB, Silva MM, Andrade-Tomaz M, Contieri B, Latancia MT, Mendes D, Porchia B, Lazarini M, Gomes LR, Rocha CR**.** High levels of NRF2 sensitize temozolomide-resistant glioblastoma cells to ferroptosis via ABCC1/MRP1 upregulation. **Cell Death Dis** 13: 591, 2022. doi:10.1038/s41419-022-05044-9.

411. Liang H, Yoo SE, Na R, Walter CA, Richardson A, Ran Q**.** Short form glutathione peroxidase 4 is the essential isoform required for survival and somatic mitochondrial functions. **J Biol Chem** 284: 30836–30844, 2009. doi:10.1074/jbc.M109.032839.

412. Maiorino M, Mauri P, Roveri A, Benazzi L, Toppo S, Bosello V, Ursini F**.** Primary structure of the nuclear forms of phospholipid hydroperoxide glutathione peroxidase (PHGPx) in rat spermatozoa. **FEBS Lett** 579: 667–670, 2005. doi:10.1016/j.febslet.2004.12.041.

413. Imai H, Hirao F, Sakamoto T, Sekine K, Mizukura Y, Saito M, Kitamoto T, Hayasaka M, Hanaoka K, Nakagawa Y**.** Early embryonic lethality caused by targeted disruption of the mouse PHGPx gene. **Biochem Biophys Res Commun** 305: 278–286, 2003. doi:10.1016/s0006-291x(03)00734-4.

414. Schneider M, Förster H, Boersma A, Seiler A, Wehnes H, Sinowatz F, Neumüller C, Deutsch MJ, Walch A, Hrabé de Angelis M, Wurst W, Ursini F, Roveri A, Maleszewski M, Maiorino M, Conrad M**.** Mitochondrial glutathione peroxidase 4 disruption causes male infertility. **FASEB J** 23: 3233–3242, 2009. doi:10.1096/fj.09-132795.

415. Conrad M, Moreno SG, Sinowatz F, Ursini F, Kölle S, Roveri A, Brielmeier M, Wurst W, Maiorino M, Bornkamm GW**.** The nuclear form of phospholipid hydroperoxide glutathione peroxidase is a protein thiol peroxidase contributing to sperm chromatin stability. **Mol Cell Biol** 25: 7637–7644, 2005. doi:10.1128/MCB.25.17.7637-7644.2005.

416. Azuma K, Koumura T, Iwamoto R, Matsuoka M, Terauchi R, Yasuda S, Shiraya T, Watanabe S, Aihara M, Imai H, Ueta T**.** Mitochondrial glutathione peroxidase 4 is indispensable for photoreceptor development and survival in mice. **J Biol Chem** 298: 101824, 2022. doi:10.1016/j.jbc.2022.101824.

417. Mishima E, Nakamura T, Zheng J, Zhang W, Mourão AS, Sennhenn P, Conrad M**.** DHODH inhibitors sensitize to ferroptosis by

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

FSP1 inhibition. **Nature** 619: E9–E18, 2023. doi:10.1038/s41586-023-06269-0.

418. Wu S, Mao C, Kondiparthi L, Poyurovsky MV, Olszewski K, Gan B. A ferroptosis defense mechanism mediated by glycerol-3-phosphate dehydrogenase 2 in mitochondria. **Proc Natl Acad Sci USA** 119: e2121987119, 2022. doi:10.1073/pnas.2121987119.

419. Mao C, Liu X, Zhang Y, Lei G, Yan Y, Lee H, Koppula P, Wu S, Zhuang L, Fang B, Poyurovsky MV, Olszewski K, Gan B. DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. **Nature** 593: 586–590, 2021. doi:10.1038/s41586-021-03539-7.

420. Imai H, Saito M, Kirai N, Hasegawa J, Konishi K, Hattori H, Nishimura M, Naito S, Nakagawa Y. Identification of the positive regulatory and distinct core regions of promoters, and transcriptional regulation in three types of mouse phospholipid hydroperoxide glutathione peroxidase. **J Biochem** 140: 573–590, 2006. doi:10.1093/jb/mvj186.

421. Burk RF, Hill KE. Regulation of selenium metabolism and transport. **Annu Rev Nutr** 35: 109–134, 2015. doi:10.1146/annurev-nutr-071714-034250.

422. Lei XG, Evenson JK, Thompson KM, Sunde RA. Glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase are differentially regulated in rats by dietary selenium. **J Nutr** 125: 1438–1446, 1995. doi:10.1093/jn/125.6.1438.

423. Weitzel F, Ursini F, Wendel A. Phospholipid hydroperoxide glutathione peroxidase in various mouse organs during selenium deficiency and repletion. **Biochim Biophys Acta** 1036: 88–94, 1990. doi:10.1016/0304-4165(90)90018-r.

424. Sneddon AA, Wu HC, Farquharson A, Grant I, Arthur JR, Rotondo D, Choe SN, Wahle KW. Regulation of selenoprotein GPx4 expression and activity in human endothelial cells by fatty acids, cytokines and antioxidants. **Atherosclerosis** 171: 57–65, 2003. doi:10.1016/j.atherosclerosis.2003.08.008.

425. Alim I, Caulfield JT, Chen Y, Swarup V, Geschwind DH, Ivanova E, Seravalli J, Ai Y, Sansing LH, Ste Marie EJ, Hondal RJ, Mukherjee S, Cave JW, Sagdullaev BT, Karuppagounder SS, Ratan RR. Selenium drives a transcriptional adaptive program to block ferroptosis and treat stroke. **Cell** 177: 1262–1279.e25, 2019. doi:10.1016/j.cell.2019.03.032.

426. Ursini F, Maiorino M. Lipid peroxidation and ferroptosis: The role of GSH and GPx4. **Free Radic Biol Med** 152: 175–185, 2020. doi:10.1016/j.freeradbiomed.2020.02.027.

427. Ufer C, Borchert A, Kuhn H. Functional characterization of cis- and trans-regulatory elements involved in expression of phospholipid hydroperoxide glutathione peroxidase. **Nucleic Acids Res** 31: 4293–4303, 2003. doi:10.1093/nar/gkg650.

428. Huang HS, Chen CJ, Chang WC. The CCAAT-box binding factor NF-Y is required for the expression of phospholipid hydroperoxide glutathione peroxidase in human epidermoid carcinoma A431 cells. **FEBS Lett** 455: 111–116, 1999. doi:10.1016/s0014-5793(99)00866-2.

429. Speckmann B, Bidmon HJ, Pinto A, Anlauf M, Sies H, Steinbrenner H. Induction of glutathione peroxidase 4 expression during enterocytic cell differentiation. **J Biol Chem** 286: 10764–10772, 2011. doi:10.1074/jbc.M110.216028.

430. Hattori H, Imai H, Kirai N, Furuhama K, Sato O, Konishi K, Nakagawa Y. Identification of a responsible promoter region and a key transcription factor, CCAAT/enhancer-binding protein epsilon, for up-regulation of PHGPx in HL60 cells stimulated with TNF alpha. **Biochem J** 408: 277–286, 2007. doi:10.1042/BJ20070245.

431. Wang Z, Zhang X, Tian X, Yang Y, Ma L, Wang J, Yu Y. CREB stimulates GPX4 transcription to inhibit ferroptosis in lung adenocarcinoma. **Oncol Rep** 45: 88, 2021. doi:10.3892/or.2021.8039.

432. Li P, Jiang M, Li K, Li H, Zhou Y, Xiao X, Xu Y, Krishfield S, Lipsky PE, Tsokos GC, Zhang X. Glutathione peroxidase 4-regulated neutrophil ferroptosis induces systemic autoimmunity. **Nat Immunol** 22: 1107–1117, 2021. doi:10.1038/s41590-021-00993-3.

433. Huang P, Duan W, Ruan C, Wang L, Hosea R, Wu Z, Zeng J, Wu S, Kasim V. NeuroD1-GPX4 signaling leads to ferroptosis resistance in hepatocellular carcinoma. **PLoS Genet** 19: e1011098, 2023. doi:10.1371/journal.pgen.1011098.

434. Wang Y, Zheng L, Shang W, Yang Z, Li T, Liu F, Shao W, Lv L, Chai L, Qu L, Xu Q, Du J, Liang X, Zeng J, Jia J. Wnt/beta-catenin signaling confers ferroptosis resistance by targeting GPX4 in gastric cancer. **Cell Death Differ** 29: 2190–2202, 2022. doi:10.1038/s41418-022-01008-w.

435. Yang F, Xiao Y, Ding JH, Jin X, Ma D, Li DQ, Shi JX, Huang W, Wang YP, Jiang YZ, Shao ZM. Ferroptosis heterogeneity in triple-negative breast cancer reveals an innovative immunotherapy combination strategy. **Cell Metab** 35: 84–100.e8, 2023. doi:10.1016/j.cmet.2022.09.021.

436. Han X, Duan X, Liu Z, Long Y, Liu C, Zhou J, Li N, Qin J, Wang Y. ZEB1 directly inhibits GPX4 transcription contributing to ROS accumulation in breast cancer cells. **Breast Cancer Res Treat** 188: 329–342, 2021. doi:10.1007/s10549-021-06301-9.

437. Zhang L, Chen F, Dong J, Wang R, Bi G, Xu D, Zhang Y, Deng Y, Lin W, Yang Z, Cao W. HDAC3 aberration-incurred GPX4 suppression drives renal ferroptosis and AKI-CKD progression. **Redox Biol** 68: 102939, 2023. doi:10.1016/j.redox.2023.102939.

438. Zhang Y, Zhang J, Feng D, Zhou H, Gui Z, Zheng M, Hang Z, Wang Z, Wang Z, Gu M, Tan R. IRF1/ZNF350/GPX4-mediated ferroptosis of renal tubular epithelial cells promote chronic renal allograft interstitial fibrosis. **Free Radic Biol Med** 193: 579–594, 2022. doi:10.1016/j.freeradbiomed.2022.11.002.

439. Ursini F, Bosello Travain V, Cozza G, Miotto G, Roveri A, Toppo S, Maiorino M. A white paper on phospholipid hydroperoxide glutathione peroxidase (GPx4) forty years later. **Free Radic Biol Med** 188: 117–133, 2022. doi:10.1016/j.freeradbiomed.2022.06.227.

440. Wei C, Xiao Z, Zhang Y, Luo Z, Liu D, Hu L, Shen D, Liu M, Shi L, Wang X, Lan T, Dai Q, Liu J, Chen W, Zhang Y, Sun Q, Wu W, Wang P, Zhang C, Hu J, Wang C, Yang F, Li Q. Itaconate protects ferroptotic neurons by alkylating GPx4 post stroke. **Cell Death Differ** 31: 983–998, 2024. doi:10.1038/s41418-024-01303-8.

441. Kerins MJ, Milligan J, Wohlschlegel JA, Ooi A. Fumarate hydratase inactivation in hereditary leiomyomatosis and renal cell cancer is synthetic lethal with ferroptosis induction. **Cancer Sci** 109: 2757–2766, 2018. doi:10.1111/cas.13701.

442. Li H, Sun Y, Yao Y, Ke S, Zhang N, Xiong W, Shi J, He C, Xiao X, Yu H, Dai P, Xiang B, Xing X, Xu G, Song W, Song J, Zhang J. USP8-governed GPX4 homeostasis orchestrates ferroptosis and cancer immunotherapy. **Proc Natl Acad Sci USA** 121: e2315541121, 2024. doi:10.1073/pnas.2315541121.

443. Li D, Wang Y, Dong C, Chen T, Dong A, Ren J, Li W, Shu G, Yang J, Shen W, Qin L, Hu L, Zhou J. CST1 inhibits ferroptosis and promotes gastric cancer metastasis by regulating GPX4 protein stability via OTUB1. **Oncogene** 42: 83–98, 2023. doi:10.1038/s41388-022-02537-x.

444. Chu LK, Cao X, Wan L, Diao Q, Zhu Y, Kan Y, Ye LL, Mao YM, Dong XQ, Xiong QW, Fu MC, Zhang T, Zhou HT, Cai SZ, Ma ZR, Hsu SW, Wu R, Chen CH, Yan XM, Liu J. Autophagy of OTUD5 destabilizes

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

GPX4 to confer ferroptosis-dependent kidney injury. **Nat Commun** 14: 8393, 2023. doi:10.1038/s41467-023-44228-5.

445. Sun J, Lin XM, Lu DH, Wang M, Li K, Li SR, Li ZQ, Zhu CJ, Zhang ZM, Yan CY, Pan MH, Gong HB, Feng JC, Cao YF, Huang F, Sun WY, Kurihara H, Li YF, Duan WJ, Jiao GL, Zhang L, He RR**.** Midbrain dopamine oxidation links ubiquitination of glutathione peroxidase 4 to ferroptosis of dopaminergic neurons. **J Clin Invest** 133: e173110, 2023. doi:10.1172/JCI173110.

446. Li J, Liu J, Zhou Z, Wu R, Chen X, Yu C, Stockwell B, Kroemer G, Kang R, Tang D**.** Tumor-specific GPX4 degradation enhances ferroptosis-initiated antitumor immune response in mouse models of pancreatic cancer. **Sci Transl Med** 15: eadg3049, 2023. doi:10.1126/scitranslmed.adg3049.

447. Tracz M, Bialek W**.** Beyond K48 and K63: non-canonical protein ubiquitination. **Cell Mol Biol Lett** 26: 1, 2021. doi:10.1186/s11658-020-00245-6.

448. Wang Z, Xia Y, Wang Y, Zhu R, Li H, Liu Y, Shen N**.** The E3 ligase TRIM26 suppresses ferroptosis through catalyzing K63-linked ubiquitination of GPX4 in glioma. **Cell Death Dis** 14: 695, 2023. doi:10.1038/s41419-023-06222-z.

449. Dong K, Wei R, Jin T, Zhang M, Shen J, Xiang H, Shan B, Yuan J, Li Y**.** HOIP modulates the stability of GPx4 by linear ubiquitination. **Proc Natl Acad Sci USA** 119: e2214227119, 2022. doi:10.1073/pnas.2214227119.

450. Wu Z, Geng Y, Lu X, Shi Y, Wu G, Zhang M, Shan B, Pan H, Yuan J**.** Chaperone-mediated autophagy is involved in the execution of ferroptosis. **Proc Natl Acad Sci USA** 116: 2996–3005, 2019. doi:10.1073/pnas.1819728116.

451. Woo MS, Mayer C, Binkle-Ladisch L, Sonner JK, Rosenkranz SC, Shaposhnykov A, Rothammer N, Tsvilovskyy V, Lorenz SM, Raich L, Bal LC, Vieira V, Wagner I, Bauer S, Glatzel M, Conrad M, Merkler D, Freichel M, Friese MA**.** STING orchestrates the neuronal inflammatory stress response in multiple sclerosis. **Cell** 187: 4043–4060. e30, 2024. doi:10.1016/j.cell.2024.05.031.

452. Thayyullathil F, Cheratta AR, Alakkal A, Subburayan K, Pallichankandy S, Hannun YA, Galadari S**.** Acid sphingomyelinase-dependent autophagic degradation of GPX4 is critical for the execution of ferroptosis. **Cell Death Dis** 12: 26, 2021. doi:10.1038/s41419-020-03297-w.

453. Wu K, Yan M, Liu T, Wang Z, Duan Y, Xia Y, Ji G, Shen Y, Wang L, Li L, Zheng P, Dong B, Wu Q, Xiao L, Yang X, Shen H, Wen T, Zhang J, Yi J, Deng Y, Qian X, Ma L, Fang J, Zhou Q, Lu Z, Xu D**.** Creatine kinase B suppresses ferroptosis by phosphorylating GPX4 through a moonlighting function. **Nat Cell Biol** 25: 714–725, 2023. doi:10.1038/s41556-023-01133-9.

454. Carlson BA, Tobe R, Yefremova E, Tsuji PA, Hoffmann VJ, Schweizer U, Gladyshev VN, Hatfield DL, Conrad M**.** Glutathione peroxidase 4 and vitamin E cooperatively prevent hepatocellular degeneration. **Redox Biol** 9: 22–31, 2016. doi:10.1016/j.redox.2016.05.003.

455. Chen L, Hambright WS, Na R, Ran Q**.** Ablation of the ferroptosis inhibitor glutathione peroxidase 4 in neurons results in rapid motor neuron degeneration and paralysis. **J Biol Chem** 290: 28097–28106, 2015. doi:10.1074/jbc.M115.680090.

456. Hu Q, Zhang Y, Lou H, Ou Z, Liu J, Duan W, Wang H, Ge Y, Min J, Wang F, Ju Z**.** GPX4 and vitamin E cooperatively protect hematopoietic stem and progenitor cells from lipid peroxidation and ferroptosis. **Cell Death Dis** 12: 706, 2021. doi:10.1038/s41419-021-04008-9.

457. Wortmann M, Schneider M, Pircher J, Hellfritsch J, Aichler M, Vegi N, Kölle P, Kuhlencordt P, Walch A, Pohl U, Bornkamm GW, Conrad M, Beck H**.** Combined deficiency in glutathione peroxidase 4 and vitamin E causes multiorgan thrombus formation and early death in mice. **Circ Res** 113: 408–417, 2013. doi:10.1161/CIRCRESAHA.113.279984.

458. Canli Ö, Alankuş YB, Grootjans S, Vegi N, Hültner L, Hoppe PS, Schroeder T, Vandenabeele P, Bornkamm GW, Greten FR**.** Glutathione peroxidase 4 prevents necroptosis in mouse erythroid precursors. **Blood** 127: 139–148, 2016. doi:10.1182/blood-2015-06-654194.

459. Canli Ö, Nicolas AM, Gupta J, Finkelmeier F, Goncharova O, Pesic M, Neumann T, Horst D, Löwer M, Sahin U, Greten FR**.** Myeloid cell-derived reactive oxygen species induce epithelial mutagenesis. **Cancer Cell** 32: 869–883.e865, 2017. doi:10.1016/j.ccell.2017.11.004.

460. Muri J, Thut H, Bornkamm GW, Kopf M**.** B1 and marginal Zone B cells but not follicular B2 cells require Gpx4 to prevent lipid peroxidation and ferroptosis. **Cell Rep** 29: 2731–2744.e4, 2019. doi:10.1016/j.celrep.2019.10.070.

461. Yao Y, Chen Z, Zhang H, Chen C, Zeng M, Yunis J, et al**.** Selenium-GPX4 axis protects follicular helper T cells from ferroptosis. **Nat Immunol** 22: 1127–1139, 2021. doi:10.1038/s41590-021-00996-0.

462. Sok SPM, Pipkin K, Popescu NI, Reidy M, Li B, Van Remmen H, Kinter M, Sun XH, Fan Z, Zhao M**.** Gpx4 regulates invariant NKT cell homeostasis and function by preventing lipid peroxidation and ferroptosis. **J Immunol** 213: 941–951, 2024. doi:10.4049/jimmunol.2400246.

463. Schriever SC, Zimprich A, Pfuhlmann K, Baumann P, Giesert F, Klaus V, Kabra DG, Hafen U, Romanov A, Tschöp MH, Wurst W, Conrad M, Hölter SM, Vogt Weisenhorn D, Pfluger PT**.** Alterations in neuronal control of body weight and anxiety behavior by glutathione peroxidase 4 deficiency. **Neuroscience** 357: 241–254, 2017. doi:10.1016/j.neuroscience.2017.05.050.

464. Hambright WS, Fonseca RS, Chen L, Na R, Ran Q**.** Ablation of ferroptosis regulator glutathione peroxidase 4 in forebrain neurons promotes cognitive impairment and neurodegeneration. **Redox Biol** 12: 8–17, 2017. doi:10.1016/j.redox.2017.01.021.

465. Sedaghatian MR**.** Congenital lethal metaphyseal chondrodysplasia: a newly recognized complex autosomal recessive disorder. **Am J Med Genet** 6: 269–274, 1980. doi:10.1002/ajmg.1320060403.

466. Smith AC, Mears AJ, Bunker R, Ahmed A, MacKenzie M, Schwartzentruber JA, Beaulieu CL, Ferretti E, Consortium FC, Majewski J, Bulman DE, Celik FC, Boycott KM, Graham GE; FORGE Canada Consortium. Mutations in the enzyme glutathione peroxidase 4 cause Sedaghatian-type spondylometaphyseal dysplasia. **J Med Genet** 51: 470–474, 2014. doi:10.1136/jmedgenet-2013-102218.

467. Fedida A, Ben Harouch S, Kalfon L, Abunassar Z, Omari H, Mandel H, Falik-Zaccai TC**.** Sedaghatian-type spondylometaphyseal dysplasia: whole exome sequencing in neonatal dry blood spots enabled identification of a novel variant in GPX4. **Eur J Med Genet** 63: 104020, 2020. doi:10.1016/j.ejmg.2020.104020.

468. Liu H, Forouhar F, Seibt T, Saneto R, Wigby K, Friedman J, Xia X, Shchepinov MS, Ramesh SK, Conrad M, Stockwell BR**.** Characterization of a patient-derived variant of GPX4 for precision therapy. **Nat Chem Biol** 18: 91–100, 2022. doi:10.1038/s41589-021-00915-2.

469. Roveri A, Di Giacinto F, Rossetto M, Cozza G, Cheng Q, Miotto G, Zennaro L, Di Paolo ML, Arnér ES, De Spirito M, Maiorino M, Ursini F**.** Cardiolipin drives the catalytic activity of GPX4 on membranes: insights from the R152H mutant. **Redox Biol** 64: 102806, 2023. doi:10.1016/j.redox.2023.102806.

470. Peshimam N, Farah H, Caswell R, Ellard S, Jan W, Calder AD, Cobben J, Kariholu U, Leitch HG**.** Sedaghatian spondylometaphyseal

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

dysplasia in two siblings. **Eur J Med Genet** 65: 104541, 2022. doi:10.1016/j.ejmg.2022.104541.

471. Lv Y, Liang C, Sun Q, Zhu J, Xu H, Li X, Li YY, Wang Q, Yuan H, Chu B, Zhu D. Structural insights into FSP1 catalysis and ferroptosis inhibition. **Nat Commun** 14: 5933, 2023. doi:10.1038/s41467-023-41626-7.

472. Nguyen HP, Yi D, Lin F, Viscarra JA, Tabuchi C, Ngo K, Shin G, Lee AY, Wang Y, Sul HS. Aifm2, a NADH oxidase, supports robust glycolysis and is required for cold- and diet-induced thermogenesis. **Mol Cell** 77: 600–617.e4, 2020. doi:10.1016/j.molcel.2019.12.002.

473. Horikoshi N, Cong J, Kley N, Shenk T. Isolation of differentially expressed cDNAs from p53-dependent apoptotic cells: activation of the human homologue of the *Drosophila* peroxidasin gene. **Biochem Biophys Res Commun** 261: 864–869, 1999. doi:10.1006/bbrc.1999.1123.

474. Ohiro Y, Garkavtsev I, Kobayashi S, Sreekumar KR, Nantz R, Higashikubo BT, Duffy SL, Higashikubo R, Usheva A, Gius D, Kley N, Horikoshi N. A novel p53-inducible apoptogenic gene, PRG3, encodes a homologue of the apoptosis-inducing factor (AIF). **FEBS Lett** 524: 163–171, 2002. doi:10.1016/s0014-5793(02)03049-1.

475. Wu M, Xu LG, Li X, Zhai Z, Shu HB. AMID, an apoptosis-inducing factor-homologous mitochondrion-associated protein, induces caspase-independent apoptosis. **J Biol Chem** 277: 25617–25623, 2002. doi:10.1074/jbc.M202285200.

476. Brielmeier M, Béchet JM, Suppmann S, Conrad M, Laux G, Bornkamm GW. Cloning of phospholipid hydroperoxide glutathione peroxidase (PHGPx) as an anti-apoptotic and growth promoting gene of Burkitt lymphoma cells. **Biofactors** 14: 179–190, 2001. doi:10.1002/biof.5520140123.

477. Nguyen HP, Villivalam SD, Jung BC, You D, Lin F, Yi D, Pi A, Ma K, Jung S, Park SH, Jang C, Sul HS, Kang S. AIFM2 is required for high-intensity aerobic exercise in promoting glucose utilization. **Diabetes** 71: 2084–2093, 2022. doi:10.2337/db21-1114.

478. Melo AM, Bandeiras TM, Teixeira M. New insights into type II NAD(P)H:quinone oxidoreductases. **Microbiol Mol Biol Rev** 68: 603–616, 2004. doi:10.1128/MMBR.68.4.603-616.2004.

479. Nakamura T, Hipp C, Santos Dias Mourão A, Borggräfe J, Aldrovandi M, Henkelmann B, Wanninger J, Mishima E, Lytton E, Emler D, Proneth B, Sattler M, Conrad M. Phase separation of FSP1 promotes ferroptosis. **Nature** 619: 371–377, 2023. doi:10.1038/s41586-023-06255-6.

480. Koppula P, Lei G, Zhang Y, Yan Y, Mao C, Kondiparthi L, Shi J, Liu X, Horbath A, Das M, Li W, Poyurovsky MV, Olszewski K, Gan B. A targetable CoQ-FSP1 axis drives ferroptosis- and radiation-resistance in KEAP1 inactive lung cancers. **Nat Commun** 13: 2206, 2022. doi:10.1038/s41467-022-29905-1.

481. Müller F, Lim JK, Bebber CM, Seidel E, Tishina S, Dahlhaus A, Stroh J, Beck J, Yapici FI, Nakayama K, Torres Fernández L, Brägelmann J, Leprivier G, von Karstedt S. Elevated FSP1 protects KRAS-mutated cells from ferroptosis during tumor initiation. **Cell Death Differ** 30: 442–456, 2023. doi:10.1038/s41418-022-01096-8.

482. Schmitt A, Grimm M, Kreienkamp N, Junge H, Labisch J, Schuhknecht L, Schönfeld C, Görsch E, Tibello A, Menck K, Bleckmann A, Lengerke C, Rosenbauer F, Grau M, Zampieri M, Schulze-Osthoff K, Klener P, Dolnikova A, Lenz G, Hailfinger S. BRD4 inhibition sensitizes diffuse large B-cell lymphoma cells to ferroptosis. **Blood** 142: 1143–1155, 2023. doi:10.1182/blood.2022019274.

483. Pontel LB, Bueno-Costa A, Morellato AE, Carvalho Santos J, Roué G, Esteller M. Acute lymphoblastic leukemia necessitates GSH-dependent ferroptosis defenses to overcome FSP1-epigenetic silencing. **Redox Biol** 55: 102408, 2022. doi:10.1016/j.redox.2022.102408.

484. Gong J, Liu Y, Wang W, He R, Xia Q, Chen L, Zhao C, Gao Y, Shi Y, Bai Y, Liao Y, Zhang Q, Zhu F, Wang M, Li X, Qin R. TRIM21-promoted FSP1 plasma membrane translocation confers ferroptosis resistance in human cancers. **Adv Sci (Weinh)** 10: e2302318, 2023. doi:10.1002/advs.202302318.

485. Xie W, Wang J, Tian S, Zhao H, Cao L, Liang Z, Yang J, Zhao Y, Wang B, Jiang F, Ma J. RNF126-mediated ubiquitination of FSP1 affects its subcellular localization and ferroptosis. **Oncogene** 43: 1463–1475, 2024. doi:10.1038/s41388-024-02949-x.

486. Yuan J, Lv T, Yang J, Wu Z, Yan L, Yang J, Shi Y. HDLBP-stabilized lncFAL inhibits ferroptosis vulnerability by diminishing Trim69-dependent FSP1 degradation in hepatocellular carcinoma. **Redox Biol** 58: 102546, 2022. doi:10.1016/j.redox.2022.102546.

487. Deshwal S, Onishi M, Tatsuta T, Bartsch T, Cors E, Ried K, Lemke K, Nolte H, Giavalisco P, Langer T. Mitochondria regulate intracellular coenzyme Q transport and ferroptotic resistance via STARD7. **Nat Cell Biol** 25: 246–257, 2023. doi:10.1038/s41556-022-01071-y.

488. Nakagawa K, Hirota Y, Sawada N, Yuge N, Watanabe M, Uchino Y, Okuda N, Shimomura Y, Suhara Y, Okano T. Identification of UBIAD1 as a novel human menaquinone-4 biosynthetic enzyme. **Nature** 468: 117–121, 2010. doi:10.1038/nature09464.

489. Mugoni V, Postel R, Catanzaro V, De Luca E, Turco E, Digilio G, Silengo L, Murphy MP, Medana C, Stainier DY, Bakkers J, Santoro MM. Ubiad1 is an antioxidant enzyme that regulates eNOS activity by CoQ10 synthesis. **Cell** 152: 504–518, 2013. doi:10.1016/j.cell.2013.01.013.

490. Arslanbaeva L, Tosi G, Ravazzolo M, Simonato M, Tucci FA, Pece S, Cogo P, Santoro MM. UBIAD1 and CoQ10 protect melanoma cells from lipid peroxidation-mediated cell death. **Redox Biol** 51: 102272, 2022. doi:10.1016/j.redox.2022.102272.

491. Mishima E, Wahida A, Seibt T, Conrad M. Diverse biological functions of vitamin K: from coagulation to ferroptosis. **Nat Metab** 5: 924–932, 2023. doi:10.1038/s42255-023-00821-y.

492. Kraft VA, Bezjian CT, Pfeiffer S, Ringelstetter L, Müller C, Zandkarimi F, Merl-Pham J, Bao X, Anastasov N, Kössl J, Brandner S, Daniels JD, Schmitt-Kopplin P, Hauck SM, Stockwell BR, Hadian K, Schick JA. GTP cyclohydrolase 1/tetrahydrobiopterin counteract ferroptosis through lipid remodeling. **ACS Cent Sci** 6: 41–53, 2020. doi:10.1021/acscentsci.9b01063.

493. Ma Y, Yi M, Wang W, Liu X, Wang Q, Liu C, Chen Y, Deng H. Oxidative degradation of dihydrofolate reductase increases CD38-mediated ferroptosis susceptibility. **Cell Death Dis** 13: 944, 2022. doi:10.1038/s41419-022-05383-7.

494. Wang D, Liang W, Huo D, Wang H, Wang Y, Cong C, Zhang C, Yan S, Gao M, Su X, Tan X, Zhang W, Han L, Zhang D, Feng H. SPY1 inhibits neuronal ferroptosis in amyotrophic lateral sclerosis by reducing lipid peroxidation through regulation of GCH1 and TFR1. **Cell Death Differ** 30: 369–382, 2023. doi:10.1038/s41418-022-01089-7.

495. Yang G, Yang Y, Song Z, Chen L, Liu F, Li Y, Jiang S, Xue S, Pei J, Wu Y, He Y, Chu B, Wu H. Spliceosomal GTPase Eftud2 deficiency-triggered ferroptosis leads to Purkinje cell degeneration. **Neuron** 112: 3452–3469.e9, 2024. doi:10.1016/j.neuron.2024.07.020.

496. Fisher AB, Dodia C, Manevich Y, Chen JW, Feinstein SI. Phospholipid hydroperoxides are substrates for non-selenium glutathione

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

peroxidase. **J Biol Chem** 274: 21326–21334, 1999. doi:10.1074/jbc.274.30.21326.

497. Manevich Y, Feinstein SI, Fisher AB**.** Activation of the antioxidant enzyme 1-CYS peroxiredoxin requires glutathionylation mediated by heterodimerization with pi GST. **Proc Natl Acad Sci USA** 101: 3780–3785, 2004. doi:10.1073/pnas.0400181101.

498. Zhou S, Lien YC, Shuvaeva T, DeBolt K, Feinstein SI, Fisher AB**.** Functional interaction of glutathione S-transferase pi and peroxiredoxin 6 in intact cells. **Int J Biochem Cell Biol** 45: 401–407, 2013. doi:10.1016/j.biocel.2012.11.005.

499. Zhang W, Dai J, Hou G, Liu H, Zheng S, Wang X, Lin Q, Zhang Y, Lu M, Gong Y, Xiang Z, Yu Y, Hu Y**.** SMURF2 predisposes cancer cell toward ferroptosis in GPX4-independent manners by promoting GSTP1 degradation. **Mol Cell** 83: 4352–4369.e8, 2023. doi:10.1016/j.molcel.2023.10.042.

500. Yang X, Wang Z, Zandkarimi F, Liu Y, Duan S, Li Z, Kon N, Zhang Z, Jiang X, Stockwell BR, Gu W**.** Regulation of VKORC1L1 is critical for p53-mediated tumor suppression through vitamin K metabolism. **Cell Metab** 35: 1474–1490.e8, 2023. doi:10.1016/j.cmet.2023.06.014.

501. Kanner J, Harel S, Granit R**.** Nitric oxide, an inhibitor of lipid oxidation by lipoxygenase, cyclooxygenase and hemoglobin. **Lipids** 27: 46–49, 1992. doi:10.1007/BF02537058.

502. Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA**.** Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. **J Biol Chem** 269: 26066–26075, 1994. doi:10.1016/S0021-9258(18)47160-8.

503. Hogg N, Kalyanaraman B, Joseph J, Struck A, Parthasarathy S**.** Inhibition of low-density lipoprotein oxidation by nitric oxide. Potential role in atherogenesis. **FEBS Lett** 334: 170–174, 1993. doi:10.1016/0014-5793(93)81706-6.

504. Homma T, Kobayashi S, Conrad M, Konno H, Yokoyama C, Fujii J**.** Nitric oxide protects against ferroptosis by aborting the lipid peroxidation chain reaction. **Nitric Oxide** 115: 34–43, 2021. doi:10.1016/j.niox.2021.07.003.

505. Kapralov AA, Yang Q, Dar HH, Tyurina YY, Anthonymuthu TS, Kim R, St Croix CM, Mikulska-Ruminska K, Liu B, Shrivastava IH, Tyurin VA, Ting HC, Wu YL, Gao Y, Shurin GV, Artyukhova MA, Ponomareva LA, Timashev PS, Domingues RM, Stoyanovsky DA, Greenberger JS, Mallampalli RK, Bahar I, Gabrilovich DI, Bayır H, Kagan VE**.** Redox lipid reprogramming commands susceptibility of macrophages and microglia to ferroptotic death. **Nat Chem Biol** 16: 278–290, 2020. doi:10.1038/s41589-019-0462-8.

506. Dar HH, Anthonymuthu TS, Ponomareva LA, Souryavong AB, Shurin GV, Kapralov AO, Tyurin VA, Lee JS, Mallampalli RK, Wenzel SE, Bayir H, Kagan VE**.** A new thiol-independent mechanism of epithelial host defense against *Pseudomonas aeruginosa*: iNOS/NO· sabotage of theft-ferroptosis. **Redox Biol** 45: 102045, 2021. doi:10.1016/j.redox.2021.102045.

507. Wang X, Chen Y, Yang X, Cheng L, He Z, Xin Y, Huang S, Meng F, Zhang P, Luo L**.** Activation of ALOX12 by a multi-organelle-orienting photosensitizer drives ACSL4-independent cell ferroptosis. **Cell Death Dis** 13: 1040, 2022. doi:10.1038/s41419-022-05462-9.

508. Shui S, Zhao Z, Wang H, Conrad M, Liu G**.** Non-enzymatic lipid peroxidation initiated by photodynamic therapy drives a distinct ferroptosis-like cell death pathway. **Redox Biol** 45: 102056, 2021. doi:10.1016/j.redox.2021.102056.

509. Li Y, Feng D, Wang Z, Zhao Y, Sun R, Tian D, Liu D, Zhang F, Ning S, Yao J, Tian X**.** Ischemia-induced ACSL4 activation contributes to ferroptosis-mediated tissue injury in intestinal ischemia/reperfusion. **Cell Death Differ** 26: 2284–2299, 2019. doi:10.1038/s41418-019-0299-4.

510. Wang Y, Zhang M, Bi R, Su Y, Quan F, Lin Y, Yue C, Cui X, Zhao Q, Liu S, Yang Y, Zhang D, Cao Q, Gao X**.** ACSL4 deficiency confers protection against ferroptosis-mediated acute kidney injury. **Redox Biol** 51: 102262, 2022. doi:10.1016/j.redox.2022.102262.

511. Falk MH, Meier T, Issels RD, Brielmeier M, Scheffer B, Bornkamm GW**.** Apoptosis in Burkitt lymphoma cells is prevented by promotion of cysteine uptake. **Int J Cancer** 75: 620–625, 1998. doi:10.1002/(SICI)1097-0215(19980209)75:4<620::AID-IJC21>3.0.CO;2-B.

512. Wu J, Minikes AM, Gao M, Bian H, Li Y, Stockwell BR, Chen ZN, Jiang X**.** Intercellular interaction dictates cancer cell ferroptosis via NF2-YAP signalling. **Nature** 572: 402–406, 2019. doi:10.1038/s41586-019-1426-6.

513. Wang ME, Chen J, Lu Y, Bawcom AR, Wu J, Ou J, Asara JM, Armstrong AJ, Wang Q, Li L, Wang Y, Huang J, Chen M**.** RB1-deficient prostate tumor growth and metastasis are vulnerable to ferroptosis induction via the E2F/ACSL4 axis. **J Clin Invest** 133: e166647, 2023. doi:10.1172/JCI166647.

514. Schwab A, Rao Z, Zhang J, Gollowitzer A, Siebenkäs K, Bindel N, D'Avanzo E, van Roey R, Hajjaj Y, Özel E, Armstark I, Bereuter L, Su F, Grander J, Bonyadi Rad E, Groenewoud A, Engel FB, Bell GW, Henry WS, Angeli JP, Stemmler MP, Brabletz S, Koeberle A, Brabletz T**.** Zeb1 mediates EMT/plasticity-associated ferroptosis sensitivity in cancer cells by regulating lipogenic enzyme expression and phospholipid composition. **Nat Cell Biol** 26: 1470–1481, 2024. doi:10.1038/s41556-024-01464-1.

515. Liao P, Wang W, Wang W, Kryczek I, Li X, Bian Y, Sell A, Wei S, Grove S, Johnson JK, Kennedy PD, Gijón M, Shah YM, Zou W**.** $CD8^+$ T cells and fatty acids orchestrate tumor ferroptosis and immunity via ACSL4. **Cancer Cell** 40: 365–378.e6, 2022. doi:10.1016/j.ccell.2022.02.003.

516. Zhang HL, Hu BX, Li ZL, Du T, Shan JL, Ye ZP, Peng XD, Li X, Huang Y, Zhu XY, Chen YH, Feng GK, Yang D, Deng R, Zhu XF**.** PKCbetaII phosphorylates ACSL4 to amplify lipid peroxidation to induce ferroptosis. **Nat Cell Biol** 24: 88–98, 2022. doi:10.1038/s41556-021-00818-3.

517. Li Z, Xu ZM, Chen WP, Du XJ, Ou CX, Luo ZK, Wang R, Zhang CQ, Ge CD, Han M, Wang F, He RR, Sun WY, Ma J, Liang XY, Liu ZW**.** Tumor-repopulating cells evade ferroptosis via PCK2-dependent phospholipid remodeling. **Nat Chem Biol** 20: 1341–1352, 2024. doi:10.1038/s41589-024-01612-6.

518. Feng S, Rao Z, Zhang J, She X, Chen Y, Wan K, Li H, Zhao C, Feng Y, Wang G, Hu J, Luo X**.** Inhibition of CARM1-mediated methylation of ACSL4 promotes ferroptosis in colorectal cancer. **Adv Sci (Weinh)** 10: e2303484, 2023. doi:10.1002/advs.202303484.

519. Bao Z, Liu Y, Chen B, Miao Z, Tu Y, Li C, Chao H, Ye Y, Xu X, Sun G, Zhao P, Liu N, Liu Y, Wang X, Lam SM, Kagan VE, Bayır H, Ji J**.** Prokineticin-2 prevents neuronal cell deaths in a model of traumatic brain injury. **Nat Commun** 12: 4220, 2021. doi:10.1038/s41467-021-24469-y.

520. Cho YY, Kang MJ, Sone H, Suzuki T, Abe M, Igarashi M, Tokunaga T, Ogawa S, Takei YA, Miyazawa T, Sasano H, Fujino T, Yamamoto TT**.** Abnormal uterus with polycysts, accumulation of uterine prostaglandins, and reduced fertility in mice heterozygous for acyl-CoA synthetase 4 deficiency. **Biochem Biophys Res Commun** 284: 993–997, 2001. doi:10.1006/bbrc.2001.5065.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

521. Kuwata H, Nakatani E, Shimbara-Matsubayashi S, Ishikawa F, Shibanuma M, Sasaki Y, Yoda E, Nakatani Y, Hara S**.** Long-chain acyl-CoA synthetase 4 participates in the formation of highly unsaturated fatty acid-containing phospholipids in murine macrophages. **Biochim Biophys Acta Mol Cell Biol Lipids** 1864: 1606–1618, 2019. doi:10.1016/j.bbalip.2019.07.013.

522. Tomitsuka Y, Imaeda H, Ito H, Asou I, Ohbayashi M, Ishikawa F, Kuwata H, Hara S**.** Gene deletion of long-chain acyl-CoA synthetase 4 attenuates xenobiotic chemical-induced lung injury via the suppression of lipid peroxidation. **Redox Biol** 66: 102850, 2023. doi:10.1016/j.redox.2023.102850.

523. Yamada N, Karasawa T, Kimura H, Watanabe S, Komada T, Kamata R, Sampilvanjil A, Ito J, Nakagawa K, Kuwata H, Hara S, Mizuta K, Sakuma Y, Sata N, Takahashi M**.** Ferroptosis driven by radical oxidation of n-6 polyunsaturated fatty acids mediates acetaminophen-induced acute liver failure. **Cell Death Dis** 11: 144, 2020. doi:10.1038/s41419-020-2334-2.

524. Reeves AR, Sansbury BE, Pan M, Han X, Spite M, Greenberg AS**.** Myeloid-specific deficiency of long-chain acyl CoA synthetase 4 reduces inflammation by remodeling phospholipids and reducing production of arachidonic acid-derived proinflammatory lipid mediators. **J Immunol** 207: 2744–2753, 2021. doi:10.4049/jimmunol.2100393.

525. Killion EA, Reeves AR, El Azzouny MA, Yan QW, Surujon D, Griffin JD, Bowman TA, Wang C, Matthan NR, Klett EL, Kong D, Newman JW, Han X, Lee MJ, Coleman RA, Greenberg AS**.** A role for long-chain acyl-CoA synthetase-4 (ACSL4) in diet-induced phospholipid remodeling and obesity-associated adipocyte dysfunction. **Mol Metab** 9: 43–56, 2018. doi:10.1016/j.molmet.2018.01.012.

526. Kuwata H, Nakatani E, Tomitsuka Y, Ochiai T, Sasaki Y, Yoda E, Hara S**.** Deficiency of long-chain acyl-CoA synthetase 4 leads to lipopolysaccharide-induced mortality in a mouse model of septic shock. **FASEB J** 37: e23330, 2023. doi:10.1096/fj.202301314R.

527. Jenkins NL, James SA, Salim A, Sumardy F, Speed TP, Conrad M, Richardson DR, Bush AI, McColl G**.** Changes in ferrous iron and glutathione promote ferroptosis and frailty in aging *Caenorhabditis elegans*. **Elife** 9: e56580, 2020. doi:10.7554/eLife.56580.

528. Papsdorf K, Miklas JW, Hosseini A, Cabruja M, Morrow CS, Savini M, Yu Y, Silva-García CG, Haseley NR, Murphy LM, Yao P, de Launoit E, Dixon SJ, Snyder MP, Wang MC, Mair WB, Brunet A**.** Lipid droplets and peroxisomes are co-regulated to drive lifespan extension in response to mono-unsaturated fatty acids. **Nat Cell Biol** 25: 672–684, 2023. doi:10.1038/s41556-023-01136-6.

529. Sun DY, Wu WB, Wu JJ, Shi Y, Xu JJ, Ouyang SX, Chi C, Shi Y, Ji QX, Miao JH, Fu JT, Tong J, Zhang PP, Zhang JB, Li ZY, Qu LF, Shen FM, Li DJ, Wang P**.** Pro-ferroptotic signaling promotes arterial aging via vascular smooth muscle cell senescence. **Nat Commun** 15: 1429, 2024. doi:10.1038/s41467-024-54088-2.

530. Miao Y, Zhang S, Liang Z, Wang Y, Tian D, Jin S, Guo Q, Xue H, Teng X, Xiao L, Wu Y**.** Hydrogen sulfide ameliorates endothelial dysfunction in aging arteries by regulating ferroptosis. **Nitric Oxide** 140-141: 77–90, 2023. doi:10.1016/j.niox.2023.10.002.

531. Zheng H, Jiang L, Tsuduki T, Conrad M, Toyokuni S**.** Embryonal erythropoiesis and aging exploit ferroptosis. **Redox Biol** 48: 102175, 2021. doi:10.1016/j.redox.2021.102175.

532. Li T, Kon N, Jiang L, Tan M, Ludwig T, Zhao Y, Baer R, Gu W**.** Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. **Cell** 149: 1269–1283, 2012. doi:10.1016/j.cell.2012.04.026.

533. Moon SH, Huang CH, Houlihan SL, Regunath K, Freed-Pastor WA, Morris JP, Tschaharganeh DF, Kastenhuber ER, Barsotti AM, Culp-Hill R, Xue W, Ho YJ, Baslan T, Li X, Mayle A, de Stanchina E, Zender L, Tong DR, D'Alessandro A, Lowe SW, Prives C**.** p53 represses the mevalonate pathway to mediate tumor suppression. **Cell** 176: 564–580.e19, 2019. doi:10.1016/j.cell.2018.11.011.

534. Tarangelo A, Magtanong L, Bieging-Rolett KT, Li Y, Ye J, Attardi LD, Dixon SJ**.** p53 suppresses metabolic stress-induced ferroptosis in cancer cells. **Cell Rep** 22: 569–575, 2018. doi:10.1016/j.celrep.2017.12.077.

535. Kuganesan N, Dlamini S, Tillekeratne LM, Taylor WR**.** Tumor suppressor p53 promotes ferroptosis in oxidative stress conditions independent of modulation of ferroptosis by p21, CDKs, RB, and E2F. **J Biol Chem** 297: 101365, 2021. doi:10.1016/j.jbc.2021.101365.

536. Cahuzac KM, Lubin A, Bosch K, Stokes N, Shoenfeld SM, Zhou R, Lemon H, Asara J, Parsons RE**.** AKT activation because of PTEN loss upregulates xCT via GSK3β/NRF2, leading to inhibition of ferroptosis in PTEN-mutant tumor cells. **Cell Rep** 42: 112536, 2023. doi:10.1016/j.celrep.2023.112536.

537. Wang W, Green M, Choi JE, Gijón M, Kennedy PD, Johnson JK, Liao P, Lang X, Kryczek I, Sell A, Xia H, Zhou J, Li G, Li J, Li W, Wei S, Vatan L, Zhang H, Szeliga W, Gu W, Liu R, Lawrence TS, Lamb C, Tanno Y, Cieslik M, Stone E, Georgiou G, Chan TA, Chinnaiyan A, Zou W**.** CD8+ T cells regulate tumour ferroptosis during cancer immunotherapy. **Nature** 569: 270–274, 2019. doi:10.1038/s41586-019-1170-y.

538. Jiang X, Stockwell BR, Conrad M**.** Ferroptosis: mechanisms, biology and role in disease. **Nat Rev Mol Cell Biol** 22: 266–282, 2021. doi:10.1038/s41580-020-00324-8.

539. Beavers WN, Monteith AJ, Amarnath V, Mernaugh RL, Roberts LJ 2nd, Chazin WJ, Davies SS, Skaar EP**.** Arachidonic acid kills *Staphylococcus aureus* through a lipid peroxidation mechanism. **mBio** 10: 2019. doi:10.1128/mBio.01333-19.

540. Svahn SL, Grahnemo L, Pálsdóttir V, Nookaew I, Wendt K, Gabrielsson B, Schéle E, Benrick A, Andersson N, Nilsson S, Johansson ME, Jansson JO**.** Dietary polyunsaturated fatty acids increase survival and decrease bacterial load during septic *Staphylococcus aureus* infection and improve neutrophil function in mice. **Infect Immun** 83: 514–521, 2015. doi:10.1128/IAI.02349-14.

541. Blot C, Lavernhe M, Lugo-Villarino G, Coulson K, Salon M, Tertrais M, Planès R, Santoni K, Authier H, Jacquemin G, Rahabi M, Parny M, Letron IR, Meunier E, Lefèvre L, Coste A**.** Leishmania infantum exploits the anti-ferroptosis effects of Nrf2 to escape cell death in macrophages. **Cell Rep** 43: 114720, 2024. doi:10.1016/j.celrep.2024.114720.

542. Yamane D, McGivern DR, Wauthier E, Yi M, Madden VJ, Welsch C, Antes I, Wen Y, Chugh PE, McGee CE, Widman DG, Misumi I, Bandyopadhyay S, Kim S, Shimakami T, Oikawa T, Whitmire JK, Heise MT, Dittmer DP, Kao CC, Pitson SM, Merrill AH, Reid LM, Lemon SM**.** Regulation of the hepatitis C virus RNA replicase by endogenous lipid peroxidation. **Nat Med** 20: 927–935, 2014. doi:10.1038/nm.3610.

543. Zhao J, Wang Q, Liu Z, Zhang M, Li J, Fu ZF, Zhao L, Zhou M**.** Neuroinvasive virus facilitates viral replication by employing lipid droplets to reduce arachidonic acid-induced ferroptosis. **J Biol Chem** 300: 107168, 2024. doi:10.1016/j.jbc.2024.107168.

544. Kain HS, Glennon EK, Vijayan K, Arang N, Douglass AN, Fortin CL, Zuck M, Lewis AJ, Whiteside SL, Dudgeon DR, Johnson JS, Aderem A, Stevens KR, Kaushansky A**.** Liver stage malaria infection is

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

controlled by host regulators of lipid peroxidation. **Cell Death Differ** 27: 44–54, 2020. doi:10.1038/s41418-019-0338-1.

545. Guan J, Tang L, Wang Y, Fu M, Xia T, Zheng K, Sabi MM, Cong H, Wang J, Zhou C, Zhou H, Weiss LM, Qu H, Han B**.** Microsporidian EnP1 alters host cell H2B monoubiquitination and prevents ferroptosis facilitating microsporidia survival. **Proc Natl Acad Sci USA** 121: e2400657121, 2024. doi:10.1073/pnas.2400657121.

546. Zhang G, Hu H, Yin Y, Tian M, Bu Z, Ding C, Yu S**.** *Brucella* manipulates host cell ferroptosis to facilitate its intracellular replication and egress in RAW264.7 macrophages. **Antioxidants (Basel)** 13: 577, 2024. doi:10.3390/antiox13050577.

547. Jennis M, Kung CP, Basu S, Budina-Kolomets A, Leu JI, Khaku S, Scott JP, Cai KQ, Campbell MR, Porter DK, Wang X, Bell DA, Li X, Garlick DS, Liu Q, Hollstein M, George DL, Murphy ME**.** An African-specific polymorphism in the TP53 gene impairs p53 tumor suppressor function in a mouse model. **Genes Dev** 30: 918–930, 2016. doi:10.1101/gad.275891.115.

548. Singh KS, Leu JI, Barnoud T, Vonteddu P, Gnanapradeepan K, Lin C, Liu Q, Barton JC, Kossenkov AV, George DL, Murphy ME, Dotiwala F**.** African-centric TP53 variant increases iron accumulation and bacterial pathogenesis but improves response to malaria toxin. **Nat Commun** 11: 473, 2020. doi:10.1038/s41467-019-14151-9.

549. Bell HN, Stockwell BR, Zou W**.** Ironing out the role of ferroptosis in immunity. **Immunity** 57: 941–956, 2024. doi:10.1016/j.immuni.2024.03.019.

550. Berndt C, Alborzinia H, Amen VS, Ayton S, Barayeu U, Bartelt A, et al**.** Ferroptosis in health and disease. **Redox Biol** 75: 103211, 2024. doi:10.1016/j.redox.2024.103211.

551. Amaral EP, Costa DL, Namasivayam S, Riteau N, Kamenyeva O, Mittereder L, Mayer-Barber KD, Andrade BB, Sher A**.** A major role for ferroptosis in *Mycobacterium tuberculosis*-induced cell death and tissue necrosis. **J Exp Med** 216: 556–570, 2019. doi:10.1084/jem.20181776.

552. Dar HH, Tyurina YY, Mikulska-Ruminska K, Shrivastava I, Ting HC, Tyurin VA, Krieger J, St Croix CM, Watkins S, Bayir E, Mao G, Armbruster CR, Kapralov A, Wang H, Parsek MR, Anthonymuthu TS, Ogunsola AF, Flitter BA, Freedman CJ, Gaston JR, Holman TR, Pilewski JM, Greenberger JS, Mallampalli RK, Doi Y, Lee JS, Bahar I, Bomberger JM, Bayır H, Kagan VE**.** *Pseudomonas aeruginosa* utilizes host polyunsaturated phosphatidylethanolamines to trigger theft-ferroptosis in bronchial epithelium. **J Clin Invest** 128: 4639–4653, 2018. doi:10.1172/JCI99490.

553. Qiu B, Zandkarimi F, Saqi A, Castagna C, Tan H, Sekulic M, Miorin L, Hibshoosh H, Toyokuni S, Uchida K, Stockwell BR**.** Fatal COVID-19 pulmonary disease involves ferroptosis. **Nat Commun** 15: 3816, 2024. doi:10.1038/s41467-024-48055-0.

554. Han Y, Zhu J, Yang L, Nilsson-Payant BE, Hurtado R, Lacko LA, Sun X, Gade AR, Higgins CA, Sisso WJ, Dong X, Wang M, Chen Z, Ho DD, Pitt GS, Schwartz RE, tenOever BR, Evans T, Chen S**.** SARS-CoV-2 infection induces ferroptosis of sinoatrial node pacemaker cells. **Circ Res** 130: 963–977, 2022. doi:10.1161/CIRCRESAHA.121.320518.

555. Martin-Sanchez D, Ruiz-Andres O, Poveda J, Carrasco S, Cannata-Ortiz P, Sanchez-Niño MD, Ruiz Ortega M, Egido J, Linkermann A, Ortiz A, Sanz AB**.** Ferroptosis, but not necroptosis, is important in nephrotoxic folic acid-induced AKI. **J Am Soc Nephrol** 28: 218–229, 2017. doi:10.1681/ASN.2015121376.

556. Jaeschke H, Adelusi OB, Ramachandran A**.** Ferroptosis and acetaminophen hepatotoxicity: are we going down another rabbit hole? **Gene Expr** 20: 169–178, 2021. doi:10.3727/105221621X16104581979144.

557. Knight TR, Fariss MW, Farhood A, Jaeschke H**.** Role of lipid peroxidation as a mechanism of liver injury after acetaminophen overdose in mice. **Toxicol Sci** 76: 229–236, 2003. doi:10.1093/toxsci/kfg220.

558. Linkermann A, Skouta R, Himmerkus N, Mulay SR, Dewitz C, De Zen F, Prokai A, Zuchtriegel G, Krombach F, Welz PS, Weinlich R, Vanden Berghe T, Vandenabeele P, Pasparakis M, Bleich M, Weinberg JM, Reichel CA, Bräsen JH, Kunzendorf U, Anders HJ, Stockwell BR, Green DR, Krautwald S**.** Synchronized renal tubular cell death involves ferroptosis. **Proc Natl Acad Sci USA** 111: 16836–16841, 2014. doi:10.1073/pnas.1415518111.

559. Xu Y, Li X, Cheng Y, Yang M, Wang R**.** Inhibition of ACSL4 attenuates ferroptotic damage after pulmonary ischemia-reperfusion. **FASEB J** 34: 16262–16275, 2020. doi:10.1096/fj.202001758R.

560. Tuo QZ, Lei P, Jackman KA, Li XL, Xiong H, Li XL, Liuyang ZY, Roisman L, Zhang ST, Ayton S, Wang Q, Crouch PJ, Ganio K, Wang XC, Pei L, Adlard PA, Lu YM, Cappai R, Wang JZ, Liu R, Bush AI**.** Tau-mediated iron export prevents ferroptotic damage after ischemic stroke. **Mol Psychiatry** 22: 1520–1530, 2017. doi:10.1038/mp.2017.171.

561. Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord EN, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa AS, Brookes PS, Davidson SM, Duchen MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T, Murphy MP**.** Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. **Nature** 515: 431–435, 2014. doi:10.1038/nature13909.

562. Ma XH, Liu JH, Liu CY, Sun WY, Duan WJ, Wang G, Kurihara H, He RR, Li YF, Chen Y, Shang H**.** ALOX15-launched PUFA-phospholipids peroxidation increases the susceptibility of ferroptosis in ischemia-induced myocardial damage. **Signal Transduct Target Ther** 7: 288, 2022. doi:10.1038/s41392-022-01090-z.

563. Cai W, Liu L, Shi X, Liu Y, Wang J, Fang X, Chen Z, Ai D, Zhu Y, Zhang X**.** Alox15/15-HpETE aggravates myocardial ischemia-reperfusion injury by promoting cardiomyocyte ferroptosis. **Circulation** 147: 1444–1460, 2023. doi:10.1161/CIRCULATIONAHA.122.060257.

564. Zhao J, Li J, Wei D, Gao F, Yang X, Yue B, Xiong D, Liu M, Xu H, Hu C, Chen J**.** Liproxstatin-1 alleviates lung transplantation-induced cold ischemia-reperfusion injury by inhibiting ferroptosis. **Transplantation** 107: 2190–2202, 2023. doi:10.1097/TP.0000000000004638.

565. Kojima H, Hirao H, Kadono K, Ito T, Yao S, Torgerson T, Dery KJ, Kitajima H, Ogawa T, Kaldas FM, Farmer DG, Kupiec-Weglinski JW**.** Cold stress-induced ferroptosis in liver sinusoidal endothelial cells determines liver transplant injury and outcomes. **JCI Insight** 9: e174354, 2024. doi:10.1172/jci.insight.174354.

566. Li W, Feng G, Gauthier JM, Lokshina I, Higashikubo R, Evans S, Liu X, Hassan A, Tanaka S, Cicka M, Hsiao HM, Ruiz-Perez D, Bredemeyer A, Gross RW, Mann DL, Tyurina YY, Gelman AE, Kagan VE, Linkermann A, Lavine KJ, Kreisel D**.** Ferroptotic cell death and TLR4/Trif signaling initiate neutrophil recruitment after heart transplantation. **J Clin Invest** 129: 2293–2304, 2019. doi:10.1172/JCI126428.

567. Liao CM, Wulfmeyer VC, Chen R, Erlangga Z, Sinning J, von Mässenhausen A, Sörensen-Zender I, Beer K, von Vietinghoff S, Haller H, Linkermann A, Melk A, Schmitt R**.** Induction of ferroptosis selectively eliminates senescent tubular cells. **Am J Transplant** 22: 2158–2168, 2022. doi:10.1111/ajt.17102.

568. Ward RJ, Zucca FA, Duyn JH, Crichton RR, Zecca L**.** The role of iron in brain ageing and neurodegenerative disorders. **Lancet Neurol** 13: 1045–1060, 2014. doi:10.1016/S1474-4422(14)70117-6.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

569. Chen L, Dar NJ, Na R, McLane KD, Yoo K, Han X, Ran Q. Enhanced defense against ferroptosis ameliorates cognitive impairment and reduces neurodegeneration in 5xFAD mice. **Free Radic Biol Med** 180: 1–12, 2022. doi:10.1016/j.freeradbiomed.2022.01.002.

570. Belaidi AA, Masaldan S, Southon A, Kalinowski P, Acevedo K, Appukuttan AT, Portbury S, Lei P, Agarwal P, Leurgans SE, Schneider J, Conrad M, Bush AI, Ayton S. Apolipoprotein E potently inhibits ferroptosis by blocking ferritinophagy. **Mol Psychiatry** 29: 211–220, 2024. doi:10.1038/s41380-022-01568-w.

571. Xian X, Pohlkamp T, Durakoglugil MS, Wong CH, Beck JK, Lane-Donovan C, Plattner F, Herz J. Reversal of ApoE4-induced recycling block as a novel prevention approach for Alzheimer's disease. **Elife** 7: e40048, 2018. doi:10.7554/eLife.40048.

572. Angelova PR, Choi ML, Berezhnov AV, Horrocks MH, Hughes CD, De S, Rodrigues M, Yapom R, Little D, Dolt KS, Kunath T, Devine MJ, Gissen P, Shchepinov MS, Sylantyev S, Pavlov EV, Klenerman D, Abramov AY, Gandhi S. Alpha synuclein aggregation drives ferroptosis: an interplay of iron, calcium and lipid peroxidation. **Cell Death Differ** 27: 2747, 2020. doi:10.1038/s41418-020-0552-x.

573. Mahoney-Sanchez L, Bouchaoui H, Boussaad I, Jonneaux A, Timmerman K, Berdeaux O, Ayton S, Krüger R, Duce JA, Devos D, Devedjian JC. Alpha synuclein determines ferroptosis sensitivity in dopaminergic neurons via modulation of ether-phospholipid membrane composition. **Cell Rep** 40: 111231, 2022. doi:10.1016/j.celrep.2022.111231.

574. Zhang B, Chen K, Dai Y, Luo X, Xiong Z, Zhang W, Huang X, So KF, Zhang L. Human alpha-synuclein aggregation activates ferroptosis leading to parvalbumin interneuron degeneration and motor learning impairment. **Commun Biol** 7: 1227, 2024. doi:10.1038/s42003-024-06896-x.

575. Bellinger FP, Bellinger MT, Seale LA, Takemoto AS, Raman AV, Miki T, Manning-Boğ AB, Berry MJ, White LR, Ross GW. Glutathione peroxidase 4 is associated with neuromelanin in substantia nigra and dystrophic axons in putamen of Parkinson's brain. **Mol Neurodegener** 6: 8, 2011. doi:10.1186/1750-1326-6-8.

576. Devos D, Moreau C, Kyheng M, Garçon G, Rolland AS, Blasco H, Gelé P, Timothée Lenglet T, Veyrat-Durebex C, Corcia P, Dutheil M, Bede P, Jeromin A, Oeckl P, Otto M, Meininger V, Danel-Brunaud V, Devedjian JC, Duce JA, Pradat PF. A ferroptosis-based panel of prognostic biomarkers for amyotrophic lateral sclerosis. **Sci Rep** 9: 2918, 2019. doi:10.1038/s41598-019-39739-5.

577. Lee H, Lee JJ, Park NY, Dubey SK, Kim T, Ruan K, Lim SB, Park SH, Ha S, Kovlyagina I, Kim KT, Kim S, Oh Y, Kim H, Kang SU, Song MR, Lloyd TE, Maragakis NJ, Hong YB, Eoh H, Lee G. Multi-omic analysis of selectively vulnerable motor neuron subtypes implicates altered lipid metabolism in ALS. **Nat Neurosci** 24: 1673–1685, 2021. doi:10.1038/s41593-021-00944-z.

578. Wang T, Tomas D, Perera ND, Cuic B, Luikinga S, Viden A, Barton SK, McLean CA, Samson AL, Southon A, Bush AI, Murphy JM, Turner BJ. Ferroptosis mediates selective motor neuron death in amyotrophic lateral sclerosis. **Cell Death Differ** 29: 1187–1198, 2022. doi:10.1038/s41418-021-00910-z.

579. Chen L, Na R, Danae McLane K, Thompson CS, Gao J, Wang X, Ran Q. Overexpression of ferroptosis defense enzyme Gpx4 retards motor neuron disease of SOD1G93A mice. **Sci Rep** 11: 12890, 2021. doi:10.1038/s41598-021-92369-8.

580. Floros KV, Cai J, Jacob S, Kurupi R, Fairchild CK, Shende M, Coon CM, Powell KM, Belvin BR, Hu B, Puchalapalli M, Ramamoorthy S, Swift K, Lewis JP, Dozmorov MG, Glod J, Koblinski JE, Boikos SA, Faber AC. MYCN-amplified neuroblastoma is addicted to iron and vulnerable to inhibition of the system Xc-/glutathione axis. **Cancer Res** 81: 1896–1908, 2021. doi:10.1158/0008-5472.CAN-20-1641.

581. Kamphorst JJ, Cross JR, Fan J, de Stanchina E, Mathew R, White EP, Thompson CB, Rabinowitz JD. Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids. **Proc Natl Acad Sci USA** 110: 8882–8887, 2013. doi:10.1073/pnas.1307237110.

582. Padanad MS, Konstantinidou G, Venkateswaran N, Melegari M, Rindhe S, Mitsche M, Yang C, Batten K, Huffman KE, Liu J, Tang X, Rodriguez-Canales J, Kalhor N, Shay JW, Minna JD, McDonald J, Wistuba II, DeBerardinis RJ, Scaglioni PP. Fatty acid oxidation mediated by acyl-CoA synthetase long chain 3 is required for mutant KRAS lung tumorigenesis. **Cell Rep** 16: 1614–1628, 2016. doi:10.1016/j.celrep.2016.07.009.

583. Dai E, Han L, Liu J, Xie Y, Zeh HJ, Kang R, Bai L, Tang D. Ferroptotic damage promotes pancreatic tumorigenesis through a TMEM173/STING-dependent DNA sensor pathway. **Nat Commun** 11: 6339, 2020. doi:10.1038/s41467-020-20154-8.

584. Hangauer MJ, Viswanathan VS, Ryan MJ, Bole D, Eaton JK, Matov A, Galeas J, Dhruv HD, Berens ME, Schreiber SL, McCormick F, McManus MT. Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. **Nature** 551: 247–250, 2017. doi:10.1038/nature24297.

585. Tsoi J, Robert L, Paraiso K, Galvan C, Sheu KM, Lay J, Wong DJ, Atefi M, Shirazi R, Wang X, Braas D, Grasso CS, Palaskas N, Ribas A, Graeber TG. Multi-stage differentiation defines melanoma subtypes with differential vulnerability to drug-induced iron-dependent oxidative stress. **Cancer Cell** 33: 890–904.e5, 2018. doi:10.1016/j.ccell.2018.03.017.

586. Oliveira T, Hermann E, Lin D, Chowanadisai W, Hull E, Montgomery M. HDAC inhibition induces EMT and alterations in cellular iron homeostasis to augment ferroptosis sensitivity in SW13 cells. **Redox Biol** 47: 102149, 2021. doi:10.1016/j.redox.2021.102149.

587. Brown CW, Amante JJ, Goel HL, Mercurio AM. The alpha6beta4 integrin promotes resistance to ferroptosis. **J Cell Biol** 216: 4287–4297, 2017. doi:10.1083/jcb.201701136.

588. Goel HL, Karner ER, Kumar A, Mukhopadhyay D, Goel S, Mercurio AM. YAP/TAZ-mediated regulation of laminin 332 is enabled by beta4 integrin repression of ZEB1 to promote ferroptosis resistance. **J Biol Chem** 300: 107202, 2024. doi:10.1016/j.jbc.2024.107202.

589. Arensman MD, Yang XS, Leahy DM, Toral-Barza L, Mileski M, Rosfjord EC, Wang F, Deng S, Myers JS, Abraham RT, Eng CH. Cystine-glutamate antiporter xCT deficiency suppresses tumor growth while preserving antitumor immunity. **Proc Natl Acad Sci USA** 116: 9533–9542, 2019. doi:10.1073/pnas.1814932116.

590. Daher B, Parks SK, Durivault J, Cormerais Y, Baidarjad H, Tambutte E, Pouysségur J, Vučetić M. Genetic ablation of the cystine transporter xCT in PDAC cells inhibits mTORC1, growth, survival, and tumor formation via nutrient and oxidative stresses. **Cancer Res** 79: 3877–3890, 2019. doi:10.1158/0008-5472.CAN-18-3855.

591. Lei G, Zhang Y, Koppula P, Liu X, Zhang J, Lin SH, Ajani JA, Xiao Q, Liao Z, Wang H, Gan B. The role of ferroptosis in ionizing radiation-induced cell death and tumor suppression. **Cell Res** 30: 146–162, 2020. doi:10.1038/s41422-019-0263-3.

592. Ye LF, Chaudhary KR, Zandkarimi F, Harken AD, Kinslow CJ, Upadhyayula PS, Dovas A, Higgins DM, Tan H, Zhang Y, Buonanno M, Wang TJ, Hei TK, Bruce JN, Canoll PD, Cheng SK, Stockwell BR. Radiation-induced lipid peroxidation triggers ferroptosis and synergizes with ferroptosis inducers. **ACS Chem Biol** 15: 469–484, 2020. doi:10.1021/acschembio.9b00939.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.

593. Yang P, Li J, Zhang T, Ren Y, Zhang Q, Liu R, Li H, Hua J, Wang WA, Wang J, Zhou H. Ionizing radiation-induced mitophagy promotes ferroptosis by increasing intracellular free fatty acids. **Cell Death Differ** 30: 2432–2445, 2023. doi:10.1038/s41418-023-01230-0.

594. Zhang Y, Tan H, Daniels JD, Zandkarimi F, Liu H, Brown LM, Uchida K, O'Connor OA, Stockwell BR. Imidazole ketone erastin induces ferroptosis and slows tumor growth in a mouse lymphoma model. **Cell Chem Biol** 26: 623–633.e9, 2019. doi:10.1016/j.chembiol.2019.01.008.

595. Woo JH, Shimoni Y, Yang WS, Subramaniam P, Iyer A, Nicoletti P, Rodríguez Martínez M, López G, Mattioli M, Realubit R, Karan C, Stockwell BR, Bansal M, Califano A. Elucidating compound mechanism of action by network perturbation analysis. **Cell** 162: 441–451, 2015. doi:10.1016/j.cell.2015.05.056.

596. Fujihara KM, Zhang BZ, Jackson TD, Ogunkola MO, Nijagal B, Milne JV, Sallman DA, Ang CS, Nikolic I, Kearney CJ, Hogg SJ, Cabalag CS, Sutton VR, Watt S, Fujihara AT, Trapani JA, Simpson KJ, Stojanovski D, Leimkühler S, Haupt S, Phillips WA, Clemons NJ. Eprenetapopt triggers ferroptosis, inhibits NFS1 cysteine desulfurase, and synergizes with serine and glycine dietary restriction. **Sci Adv** 8: eabm9427, 2022. doi:10.1126/sciadv.abm9427.

597. Bruedigam C, Porter AH, Song A, Vroeg In de Wei G, Stoll T, Straube J, Cooper L, Cheng G, Kahl VFS, Sobinoff AP, Ling VY, Jebaraj BMC, Janardhanan Y, Haldar R, Bray LJ, Bullinger L, Heidel FH, Kennedy GA, Hill MM, Pickett HA, Abdel-Wahab O, Hartel G, Lane SW. Imetelstat-mediated alterations in fatty acid metabolism to induce ferroptosis as a therapeutic strategy for acute myeloid leukemia. **Nat Cancer** 5: 47–65, 2024. doi:10.1038/s43018-023-00653-5.

598. Cui S, Ghai A, Deng Y, Li S, Zhang R, Egbulefu C, Liang G, Achilefu S, Ye J. Identification of hyperoxidized PRDX3 as a ferroptosis marker reveals ferroptotic damage in chronic liver diseases. **Mol Cell** 83: 3931–3939.e5, 2023. doi:10.1016/j.molcel.2023.09.025.

599. Evans RC, Chen L, Na R, Yoo K, Ran Q. The Gpx4NIKO mouse is a versatile model for testing interventions targeting ferroptotic cell death of spinal motor neurons. **Neurotox Res** 40: 373–383, 2022. doi:10.1007/s12640-021-00469-0.

600. Cramer SL, Saha A, Liu J, Tadi S, Tiziani S, Yan W, Triplett K, Lamb C, Alters SE, Rowlinson S, Zhang YJ, Keating MJ, Huang P, DiGiovanni J, Georgiou G, Stone E. Systemic depletion of L-cyst(e)ine with cyst(e)inase increases reactive oxygen species and suppresses tumor growth. **Nat Med** 23: 120–127, 2017. doi:10.1038/nm.4232.

601. Griffith OW, Meister A. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (S-n-butyl homocysteine sulfoximine). **J Biol Chem** 254: 7558–7560, 1979. doi:10.1016/S0021-9258(18)35980-5.

602. Chen T, Leng J, Tan J, Zhao Y, Xie S, Zhao S, Yan X, Zhu L, Luo J, Kong L, Yin Y. Discovery of novel potent covalent glutathione peroxidase 4 inhibitors as highly selective ferroptosis inducers for the treatment of triple-negative breast cancer. **J Med Chem** 66: 10036–10059, 2023. doi:10.1021/acs.jmedchem.3c00967.

603. Randolph JT, O'Connor MJ, Han F, Hutchins CW, Siu YA, Cho M, Zheng Y, Hickson JA, Markley JL, Manaves V, Algire M, Baker KA, Chapman AM, Gopalakrishnan SM, Panchal SC, Foster-Duke K, Stolarik DF, Kempf-Grote A, Dammeier D, Fossey S, Sun Q, Sun C, Shen Y, Dart MJ, Kati WM, Lai A, Firestone AJ, Kort ME. Discovery of a potent chloroacetamide GPX4 inhibitor with bioavailability to enable target engagement in mice, a potential tool compound for inducing ferroptosis in vivo. **J Med Chem** 66: 3852–3865, 2023. doi:10.1021/acs.jmedchem.2c01415.

604. Rodencal J, Kim N, He A, Li VL, Lange M, He J, Tarangelo A, Schafer ZT, Olzmann JA, Long JZ, Sage J, Dixon SJ. Sensitization of cancer cells to ferroptosis coincident with cell cycle arrest. **Cell Chem Biol** 31: 234–248.e13, 2024. doi:10.1016/j.chembiol.2023.10.011.

605. Devos D, Labreuche J, Rascol O, Corvol JC, Duhamel A, Guyon Delannoy P, et al. Trial of deferiprone in Parkinson's disease. **N Engl J Med** 387: 2045–2055, 2022. doi:10.1056/NEJMoa2209254.

606. Ayton S, Barton D, Brew B, Brodtmann A, Clarnette R, Desmond P, Devos D, Ellis KA, Fazlollahi A, Fradette C, Goh AM, Kalinowski P, Kyndt C, Lai R, Lim YY, Maruff P, O'Brien TJ, Rowe C, Salvado O, Schofield PW, Spino M, Tricta F, Wagen A, Williams R, Woodward M, Bush AI. Deferiprone in Alzheimer disease: a randomized clinical trial. **JAMA Neurol** 2024: e243733, 2024. doi:10.1001/jamaneurol.2024.3733.

607. Violi F, Nocella C, Loffredo L, Carnevale R, Pignatelli P. Interventional study with vitamin E in cardiovascular disease and meta-analysis. **Free Radic Biol Med** 178: 26–41, 2022. doi:10.1016/j.freeradbiomed.2021.11.027.

608. Klein EA, Thompson IM Jr, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, Minasian LM, Ford LG, Parnes HL, Gaziano JM, Karp DD, Lieber MM, Walther PJ, Klotz L, Parsons JK, Chin JL, Darke AK, Lippman SM, Goodman GE, Meyskens FL Jr, Baker LH. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). **JAMA** 306: 1549–1556, 2011. doi:10.1001/jama.2011.1437.

609. Jakaria M, Belaidi AA, Bush AI, Ayton S. Vitamin A metabolites inhibit ferroptosis. **Biomed Pharmacother** 164: 114930, 2023. doi:10.1016/j.biopha.2023.114930.

610. Bi G, Liang J, Shan G, Bian Y, Chen Z, Huang Y, Lu T, Li M, Besskaya V, Zhao M, Fan H, Wang Q, Gan B, Zhan C. Retinol saturase mediates retinoid metabolism to impair a ferroptosis defense system in cancer cells. **Cancer Res** 83: 2387–2404, 2023. doi:10.1158/0008-5472.CAN-22-3977.

611. Tschuck J, Padmanabhan Nair V, Galhoz A, Zaratiegui C, Tai HM, Ciceri G, Rothenaigner I, Tchieu J, Stockwell BR, Studer L, Cabianca DS, Menden MP, Vincendeau M, Hadian K. Suppression of ferroptosis by vitamin A or radical-trapping antioxidants is essential for neuronal development. **Nat Commun** 15: 7611, 2024. doi:10.1038/s41467-024-51996-1.

612. Zilka O, Shah R, Li B, Friedmann Angeli JP, Griesser M, Conrad M, Pratt DA. On the mechanism of cytoprotection by Ferrostatin-1 and Liproxstatin-1 and the role of lipid peroxidation in ferroptotic cell death. **ACS Cent Sci** 3: 232–243, 2017. doi:10.1021/acscentsci.7b00028.

613. Devisscher L, Van Coillie S, Hofmans S, Van Rompaey D, Goossens K, Meul E, Maes L, De Winter H, Van Der Veken P, Vandenabeele P, Berghe TV, Augustyns K. Discovery of novel, drug-like ferroptosis inhibitors with in vivo efficacy. **J Med Chem** 61: 10126–10140, 2018. doi:10.1021/acs.jmedchem.8b01299.

Downloaded from journals.physiology.org/journal/physrev at Helmholtz Zentrum Muenchen (146.107.213.240) on March 3, 2025.