HHS Public Access

Author manuscript

Curr Opin Cell Biol. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

Curr Opin Cell Biol. 2018 April ; 51: 58–64. doi:10.1016/j.ceb.2017.11.001.

To Eat or Not to Eat – The Metabolic Flavor of Ferroptosis

Minghui Gao[1,2,*] and **Xuejun Jiang**[2,*]

[1]HIT Center for Life Sciences, School of Life Science and Technology, Harbin Institute of Technology, Harbin 150080, China

[2]Cell Biology Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

Abstract

Ferroptosis is a newly defined iron-dependent, non-apoptotic mode of cell death with necrotic morphology. Distinctive from other death mechanisms, ferroptosis requires cellular iron and lipid peroxides, and is dictated by specific cellular metabolic processes. Importantly, ferroptosis has been implicated in a plethora of human diseases. This paper reviews the recent advances and outstanding questions of the field by focusing on the role of cellular metabolism in ferroptosis. The relevance of ferroptosis to disease and therapy is also discussed.

Introduction

Death is the common fate of all living matters, including every cell in our bodies. While often detrimental and a sign of deterioration, cell death can also be an integral part of life and be harnessed, or programmed, to benefit the multicellular organism, thus the concept of “programmed cell death” (PCD). For example, spatially and temporally orchestrated PCD is critical in shaping the structure and function of developing tissues and organs, and precise PCD of immune cells enables proper immune response without yielding autoimmune disorders. Conversely, malfunction of PCD contributes to the development of various diseases, such as cancer, immune diseases, and neurodegeneration[1–5].

Two criteria need to be satisfied for the strict qualification of PCD: (1) the death process is genetically programmed, *i.e.*, the function of certain gene product(s) is required for the execution (NOT only inhibition) of cell death; and (2) the death process benefits the organism under specific biological contexts. However, in this review and numerous other publications in the PCD field, the term “PCD” is used in a more loose way to refer to genetically regulated cell death processes (the first criteria). A reasonable, although quite naïve, assumption is: if a delicate and highly regulated molecular pathway is required for the execution of a particular cell death process, this process most likely is relevant to, and

*Correspondence: gaominghui@hotmail.com, jiangx@mskcc.org.

contributes to, normal biology of the organism; and final validation of such life-benefiting functions of the death process might simply be a matter of time.

Historically, cell death had been divided into three categories based on their distinctive morphological features: type I (apoptosis), type II (autophagy), and type III (necrosis)[6]. Apoptosis and autophagy were considered to be “programmed”, while necrosis was believed to be passive, unregulated, and pathological [6]. In early days, programmed cell death had been synonymous with apoptosis, owing to the elegant discoveries made by Horvitz and colleagues using C. elegans model system, as well as subsequent studies in other organisms[7,8]. These studies unveiled the precise molecular program of apoptosis and its crucial function in a plethora of normal biological processes. More recently, it has been demonstrated that there are multiple forms of PCD in addition to apoptosis. Importantly, growing evidence shows that even necrosis, previously believed to be “non-programmed”, can also be molecularly programmed and might even play physiologically beneficial roles under specific biological conditions[9–12]. One such example is RIPK3-dependent necrosis, also known as necroptosis [13–15]. In this review, we will focus on another type of cell death known as ferroptosis, which is a blend of necrosis (morphologically) and autophagy (autophagy promotes ferroptotic death, as described later).

The emergence of the concept of ferroptosis

In 2012, the Stockwell lab reported that a small molecule, erastin, which they identified by a high throughput screening, can induce potent cell death in oncogenic KRas-expressing fibroblasts[16–18]. Distinctive from apoptotic cell death, erastin-induced cell death does not require caspase activation, and during cell death there is no typical apoptotic morphological features such as chromatin condensation or nuclear fragmentation. Their counter screening led to the discovery of iron chelators, among other chemical compounds, as inhibitors of such erastin-induced cell death, suggesting iron is essential for this non-apoptotic cell death. The term of ferroptosis is thus coined to describe this iron-dependent, non-apoptotic cell death process. Further, the molecular target of erastin has been identified as glutamate-cystine antiport system Xc^-. System Xc^- is a plasma membrane-localized amino acid transporter, which mediates the import of cystine in exchange of the export of glutamate. Once inside the cell, cystine is reduced to cysteine, providing substrate for glutathione syntheses and maintaining cellular redox homeostasis. Upon erastin treatment, the system Xc^- is inactive and import of cystine is blocked, leading to cysteine starvation and glutathione depletion; and this is the cause of ferroptotic cell death[18,19].

Ferroptosis is reminiscent of a form of metabolically controlled cell death described decades earlier. In 1950s, it was reported that extracellular cysteine and cystine are essential for growth of various types of cultured cells, cystine starvation leads to glutathione depletion and triggers cell death[20]. As expected, this death can be prevented by the antioxidant atocopherol [21]. Intriguingly, iron chelators, such as deferoxamine, were found to be able to prevent cystine deprivation-induced cell death[22]. Taken together, what these early investigators had observed is probably the cell death process we now know as ferroptosis.

Another effort to define the role of metabolism in cell death and cell survival also led to the discovery of ferroptosis[23,24]. The discovery started with a surprising and counter-intuitive observation: in multiple types of cultured cells, total amino acid starvation can induce rapid and potent cell death, which is non-apoptotic and non-necroptotic, but only in the presence of serum. Subsequently, it was revealed that deprivation of cystine was sufficient (as total amino acid starvation) to induce such cell death in the presence of serum, and serum iron carrier protein transferrin is essential for the killing[23,24]. These findings naturally led to the idea, and subsequent experimental validation, that the death mode is ferroptosis. Importantly, another serum factor, the amino acid glutamine (which is present in regular medium but not in total amino acid-free medium) as well as its cellular metabolism (glutaminolysis), was also found to be essential for ferroptosis induced either by amino acid starvation plus serum or by erastin.

Remarkably, both transferrin and glutamine are crucial for cell viability under normal conditions, but they are nonetheless essential for the execution of ferroptotic cell death under specific conditions. Taken together, this work further demonstrated an intimate communication between ferroptosis and cellular metabolism[23,24].

Below we will discuss the role of various cellular metabolic processes in ferroptosis by focusing on the metabolism of reactive oxygen species (particularly lipid peroxides), iron, and amino acids (Figure 1).

ROS and lipid peroxidation in ferroptosis

Reactive oxygen species (ROS) are partially reduced oxygen-containing molecules, including superoxide (O_{2+}^{-}), peroxides (H_2O_2, ROOH) and free radicals (HO_+ and RO_+) [25]. Although a low and controlled level of ROS is crucial for normal cellular and organismal function, the aberrant accumulation of ROS is linked to a number of acute organ injuries and chronic degenerative conditions. Mitochondria are one of the most important organelles where significant amounts of ROS are generated from normal metabolism and energy production through the electron transport chain (ETC).

The observation that cystine deprivation (or equivalently, system Xc^- inhibition) can trigger ferroptosis immediately suggested a role of (ROS) in ferroptosis [18,23]. Indeed, ferroptosis can be inhibited by various antioxidants and ROS scavengers. But what is the exact source of lethal ROS production upon ferroptosis induction? This is still a highly debated question. For example, the role of mitochondria in ferroptosis-associated ROS generation is controversial. On one hand, oligomycin, a mitochondrial oxidative phosphorylation inhibitor was reported to partially inhibit ferroptosis, suggesting ETC is one of ROS sources[23]. On the other hand, cells with mitochondrial DNA depleted can still undergo potent ferroptosis, suggesting that mitochondrion is dispensable for ferroptosis[18].

Not all ROS molecules function equally in ferroptosis. It is generally accepted that eventual executioners of ferroptosis are lipid peroxides, and it takes excess lipid peroxide generation to cause plasma membrane damage and ultimately, ferroptotic cell death[26]. Several commonly used ferroptosis inhibitors, including the original ferrostatin-1, are believed to

function by trapping lipid peroxides. Consistently, inhibition of phospholipid glutathione peroxidase 4 (GPX4, an antioxidant enzyme that uses glutathione as a cofactor to catalyze the reduction of lipid peroxides) genetically or pharmacologically can lead to ferroptosis even when cells have ample supply of cystine/cysteine[19,27]. Inhibition of GPX4 results in uncontrolled polyunsaturated fatty acid (PUFA) oxidation and fatty acid radical generation, thereby leading to ferroptotic cell death[28] (Figure 1). More recently, a study using a genome-wide CRISPR-based genetic screen and microarray analysis of ferroptosis-resistant cell lines unveiled that acyl-CoA synthetase long-chain family member 4 (ACSL4) drives ferroptosis via accumulating oxidized cellular membrane phospholipids[29,30]. Further, a series of experiments suggest the following specific lipid metabolic pathway plays a central role in ferroptosis: ACSL4 promotes ferroptosis by producing oxidized phosphatidylethanolamines (PE) in endoplasmic reticulum-associated oxygenation center; ACSL4 catalyzes the ligation of an arachidonoyl (AA) or adrenoyl (AdA) to produce AA or AdA *acyl* Co-A derivatives; these derivatives are then esterified into phosphatidylethanolamines (AA-PE and AdA-PE) by lysophosphatidylcholine acyltransferase 3 (LPCAT3), and AA-PE and AdA-PE are subsequently oxidized by 15-lipoxygenase (15-LOX) to generate lipid hydroperoxides, which execute ferroptosis[29,30] (Figure 1).

While the essential and necessary role of lipid peroxides in ferroptosis has been established beyond doubt, there is no definitive evidence proving that this class of ROS is the most downstream molecules that drive ferroptosis. Therefore, the jury is still out on what the ultimate molecular executioner of ferroptotic cell death is (as caspases for apoptosis).

It should be noted that recent evidence indicates a role of the antioxidant defensive system, coordinated by the transcriptional factor NRF2, in the regulation of ferroptosis[31,32]. This is not unexpected, considering the central role of ROS in ferroptosis.

Iron metabolism and ferroptosis

As the name “ferroptosis” infers, the requirement of intracellular iron is a fundamental property of ferroptosis. Be the death triggered from the most upstream of the pathway (such as by erastin or direct cystine deprivation) or at a downstream step (such as by inhibiting GPX4), ferroptosis and associated lipid peroxide accumulation can always be completely abrogated by iron chelators[22].

Intracellular iron metabolism and homeostasis is under delicate regulation. A sophisticated network, involving iron-binding and mRNA-regulatory proteins IRP1 and IRP2 (iron-regulatory protein 1 and 2), can directly sense the concentration of free iron (Fe^{2+}) in cells, and respond by altering the synthesis of a series of proteins governing iron export, import, storage and release[33]. During ferroptosis, this fine-tuned iron homeostasis is probably disrupted, and an undesired increase of free cellular iron contents (also know as “labile iron portion” or “LIP”) occurs. Such abnormal increase of LIP requires transferrin and transferrin receptor (to import iron from extracellular environment), as well as autophagic-lysosomal degradation of ferritin (to release the stored intracellular iron)[34,35] (Figure 1).

The finding that autophagy promotes ferroptosis is also important for the field of autophagy. It has been a long-standing and highly debated question in the autophagy field whether autophagy, commonly a stress-responsive mechanism for cell survival, can also be pro-death as the term “autophagic cell death” implies. The finding that autophagic degradation of ferritin (also known as ferritinophagy, mediated by the specific autophagy cargo receptor NCOA4[36,37]) mediates ferroptosis provides a defined mechanism that indeed autophagy can promote cell death under specific biological conditions[34,35] (Figure 1).

Although intracellular iron is essential for ferroptosis, the key question – what is the exact function of iron in ferroptosis – remains to be answered. One proposed model is that iron is involved in the generation of lipid ROS, either through Fenton chemistry, or via the action of iron-dependent oxidases. Since iron is a cofactor of various metabolic enzymes (for example, multiple enzymes in the TCA cycle), the requirement of iron for ferroptosis may simply reflect the role of these enzymes in ROS generation. It should be noted though, that a redox-independent role of iron has not been completely ruled out, and that iron might have multiple functions in ferroptosis.

The essential role of iron in life can be dated back to billions years ago when oxygen just became available to drive central metabolic process in living cells. The oxygen/redox-centered metabolism, almost always with the involvement of iron (due to the unique physical chemical property of this transition metal), created a paradox: while iron-dependent biochemical reactions are essential for life, these highly efficient processes are associated with generation of ROS, which can be harmful and even lethal. Conversely and speculatively, organisms may take advantage of such ROS/iron-driven cell death and “program” it for their own benefit, *i.e.*, can ferroptosis be the most ancient form of PCD? And if so, under what contexts life can be benefitted from ferroptotic cell death?

Amino acid metabolism and ferroptosis

Amino acids play a central role in ferroptosis. Ferroptosis is induced by cystine/cysteine depletion when cells are treated with erastin or other system Xc^- inhibitors[18,23]. Some cells can use the transsulfuration pathway to biosynthesize cysteine from methionine when the system Xc^- is inactivated; as a result, these cells are resistance to ferroptosis induced by system Xc^- inhibitors [38]. Knockdown of cysteinyl- tRNA synthetase (*CARS*) results in upregulation of the transsulfuration pathway and resistance to erastin-induced ferroptosis. As expected, *CARS* knockdown cells are still sensitive to ferroptosis induced by GPX4 inhibitors[38] (Figure 1).

Both glutamate and glutamine play important roles in ferroptosis. High concentration of extracellular glutamate can block cystine uptake by inhibiting the activity of system Xc^-, thus inducing ferroptosis[18,39]. The role of glutamine in ferroptosis is more complexed[23]. Although glutamine can be converted to glutamate by glutaminases (GLS1 and GLS2) in cells, high dose of extracellular glutamine alone cannot induce ferroptosis. Instead, glutamine drives ferroptosis in combination of cystine deprivation. Glutamine fuels ferroptosis through its specific metabolism, glutaminolysis[23] (Figure 1). In the absence of glutamine, or when glutaminolysis is inhibited, cystine starvation and blockage of cystine

import cannot induce ferroptosis or the associated rapid accumulation of ROS and lipid peroxidation. The role of glutaminolysis in ferroptosis can be explained by the observation that α-ketoglutarate (aKG), a product of glutaminolysis, can replace the requirement of glutamine for ferroptosis. Interestingly, although both GLS1 and GLS2 can convert glutamine to glutamate, only GLS2 is required for ferroptosis[23]. Since GLS1 is a cytosolic protein whereas GLS2 localizes in the mitochondria[40], it is likely that mitochondria plays a role in ferroptosis-associated ROS accumulation.

Ferroptosis and human diseases

Although a life-beneficial or physiological function of ferroptosis has yet to be uncovered (genetic deletion of GPX4 in mouse model only gives rise to developmental defect and lethality[27,41–44]), the role of ferroptosis in various human diseases have been established.

Studies using ferroptosis inhibitors such as ferrostatin-1 and its improved analogs, as well as glutaminolysis inhibitors, have demonstrated the important role of ferroptosis in ischemia/reperfusion (I/R)-induced damage in organs such as kidney, liver, brain, and heart[18,21,23,27]. These studies suggest that ferroptosis is the major mode of cell death associated with I/R-induced organ damage, and thus ferroptosis is a promising therapeutic target for the treatment of these diseases.

Ferroptosis is also implicated in neuron degenerative diseases, as ferrostatins and iron chelators have been shown to be effective in models of Huntington’s disease and Parkinson’s disease [45]. Additionally, ferroptosis has been suggested to contribute to neuronal cell death triggered by high dose of glutamate, namely glutamate excitotoxicity, because cystine import can be inhibited by high dose of glutamate[18].

Can ferroptosis pathway be explored for cancer treatment? Indeed, the original study that led to the identification of erastin aimed for selective killing of cancer cells[16–18]. Since then, multiple lines of research suggest the important role of ferroptosis in cancer development and treatment. For example, it has been reported that a p53 mutant which loss the ability to induce apoptosis and senescence can still inhibit tumor growth by suppressing the expression of SLC7A11, a component of system xc^-, thus activating ferroptosis[46]. Additionally, since multiple cancer types are addicted to glutaminolysis, can these cancer cells be more sensitive to ferroptosis induction as a potential treatment? In glutaminolysis pathway, GLS2 but not GLS1 mediates ferroptosis[23]. Consistently, GLS1 is a putative oncoprotein whereas GLS2 has been suggested to be a tumor suppressor[43,47,48]. Further, the GLS2 but not GLS1 gene is a transcriptional target of p53, and upregulation of GLS2 contributes to p53-dependent ferroptosis[48,49]. Most recently, it was reported that a therapy-resistant, high-mesenchymal cell state depends on a druggable GPX4/lipid peroxidase pathway to evade ferroptosis, further supporting the notion that induction of ferroptosis represents a novel strategy for anti-tumor drug discovery[50].

Conclusions and perspectives

Distinctive from other forms of cell death, ferroptosis features lipid ROS and iron dependency. Recent advance has provided insights into the precise molecular mechanisms of

ferroptosis, particularly its relationship with cellular metabolism. The relevance of ferroptosis to various human diseases has also been well established. However, many critical questions remain. For example, what is the molecular executioner of ferroptosis (is it lipid peroxides, if so, any specific subclasses?)? What happens after lipid oxidation during ferroptosis, and what is the point of no-return of ferroptosis? What is the exact function of iron in ferroptosis? Further, how do the central metabolic processes in ferroptosis, namely, that of iron, redox, amino acids, and lipids, communicate with each other to dictate the output of survival versus ferroptotic cell death? Last but not the least, the normal physiological function of ferroptosis, if any, is still elusive. Answers to these questions will draw a clear picture about the ferroptotic pathway and should be instrumental in translating our knowledge of this basic cell biology to clinical settings.

Acknowledgments

The authors thank members of the Jiang lab for critical reading and suggestions. The ferroptosis research of the Jiang lab is supported by NIH R01CA204232 and a Geoffrey Beene Cancer Research fund. This work is also supported by NCI cancer center core grant P30 CA008748.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. Thompson CB. Apoptosis in the Pathogenesis and Treatment of Disease. Science. 1995; 267:1456–1462. [PubMed: 7878464]
2. Budihardjo I, Oliver H, Lutter M, Luo X, Wang X. Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol. 1999; 15:269–290. [PubMed: 10611963]
3. Danial NN, Korsmeyer SJ. Cell death: Critical control points. Cell. 2004; 116:205–219. [PubMed: 14744432]
4. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. Science. 2004; 305:626–629. [PubMed: 15286356]
5. Fuchs Y, Steller H. Programmed Cell Death in Animal Development and Disease. Cell. 2011; 147:742–758. [PubMed: 22078876]
6. Schweichel JU, Merker HJ. Morphology of Various Types of Cell Death in Prenatal Tissues. Teratology. 1973; 7:253–266. [PubMed: 4807128]
7. Vaux DL, Korsmeyer SJ. Cell death in development. Cell. 1999; 96:245–254. [PubMed: 9988219]
8. Meier P, Finch A, Evan G. Apoptosis in development. Nature. 2000; 407:796–801. [PubMed: 11048731]
9. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. Nat Rev Microbiol. 2009; 7:99–109. [PubMed: 19148178]
10. Yuan J, Kroemer G. Alternative cell death mechanisms in development and beyond. Genes Dev. 2010; 24:2592–2602. [PubMed: 21123646]
11. Blum ES, Abraham MC, Yoshimura S, Lu Y, Shaham S. Control of Nonapoptotic Developmental Cell Death in Caenorhabditis elegans by a Polyglutamine-Repeat Protein. Science. 2012; 335:970–973. [PubMed: 22363008]
12. Vanden Berghe T, Linkermann A, Jouan-Lanhouet S, Walczak H, Vandenabeele P. Regulated necrosis: the expanding network of non-apoptotic cell death pathways. Nat Rev Mol Cell Biol. 2014; 15:135–147. [PubMed: 24452471]

13. Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, Chan FK. Phosphorylation-driven assembly of the RIP1–RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell. 2009; 137:1112–1123. [PubMed: 19524513]

14. He S, Wang L, Miao L, Wang T, Du F, Zhao L, Wang X. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell. 2009; 137:1100–1111. [PubMed: 19524512]

15. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, Han J. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science. 2009; 325:332–336. [PubMed: 19498109]

16•. 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. 2003; 3:285–296. The authors identified erastin in a high throughput screening as a potent cell death inducer in oncogenic KRas-expressed fibroblast cells. [PubMed: 12676586]

17•. Yagoda N, von Rechenberg M, Zaganjor E, Bauer AJ, Yang WS, Fridman DJ, Wolpaw AJ, Smukste I, Peltier JM, Boniface JJ, et al. RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. Nature. 2007; 447:864–868. This paper demenstrates erastin can induce a ROS-dependent non-apoptotic cell death. [PubMed: 17568748]

18••. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012; 149:1060–1072. The peper coins the term ferroptosis and demenstrates that ferroptosis is a iron dependent, non-apoptotic cell death. The author demenstrates that erastin inhibits cystine import, leading to deregulated cellular redox homeostasis and ultimately cell death. They also develop small molecule ferrostatin-1 as a potent inhibitor of ferroptosis in cancer cells and unveil glutamate-induced cell death in organotypic rat brain slices, suggesting the potential function of ferroptosis in cancer and neurodegeneration diseases. [PubMed: 22632970]

19••. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, et al. Regulation of ferroptotic cancer cell death by GPX4. Cell. 2014; 156:317–331. The authors demenstate that erastin triggers ferroptosis by depleting cellular glutathione. They further demenenstrate that glutathione-dependent peroxidases 4 (GPX4), the target of RSL3, play a central role in the regulation of ferroptosis. They also develop a new erastin derivative that can induce ferroptosis in mouse tumor xenografts. [PubMed: 24439385]

20. Lorincz T, Jemnitz K, Kardon T, Mandl J, Szarka A. Ferroptosis is Involved in Acetaminophen Induced Cell Death. Pathol Oncol Res. 2015; 21:1115–1121. [PubMed: 25962350]

21•. Linkermann A, Skouta R, Himmerkus N, Mulay SR, Dewitz C, De Zen F, Prokai A, Zuchtriegel G, Krombach F, Welz PS, et al. Synchronized renal tubular cell death involves ferroptosis. Proc Natl Acad Sci USA. 2014; 111:16836–16841. This paper demenstrates that iron-dependent ferroptosis causes synchronized necrosis of renal tubules in models of IRI and oxalate crystal-induced acute kidney injury, suggesting that ferroptosis may be therapeutically targeted in postischemic and toxic renal necrosis. [PubMed: 25385600]

22. Dixon SJ, Stockwell BR. The role of iron and reactive oxygen species in cell death. Nat Chem Biol. 2014; 10:9–17. [PubMed: 24346035]

23••. Gao M, Monian P, Quadri N, Ramasamy R, Jiang X. Glutaminolysis and Transferrin Regulate Ferroptosis. Mol Cell. 2015; 59:298–308. The authors identify transferrin and glutamine as essential factors for the induction of ferroptosis. They further demenstrate that transferrin receptor mediated iron transpotation and glutaminolysis play crucial roles in the death process. The authors provide evidences that inhibition of ferroptosis by blockage of glutaminolysis can reduce heart damage triggered by ischemia/reperfusion. [PubMed: 26166707]

24. Gao MH, Monian P, Jiang XJ. Metabolism and iron signaling in ferroptotic cell death. Oncotarget. 2015; 6:35145–35146. [PubMed: 26387139]

25. Fridovich I. The biology of oxygen radicals. Science. 1978; 201:875–880. [PubMed: 210504]

26. Yang WS, Stockwell BR. Ferroptosis: Death by Lipid Peroxidation. Trends Cell Biol. 2016; 26:165–176. [PubMed: 26653790]

27••. Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, Herbach N, Aichler M, Walch A, Eggenhofer E, et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. Nat Cell Biol. 2014; 16:1180–1191. The paper provides direct genetic evidence that the knockout of Gpx4 triggers ferroptosis. The authors elucidate the essential role for the glutathione/Gpx4 axis in preventing lipid-oxidation- induced acute renal failure and associated death using inducible knockout mice model. The authors also develop a novel small molecular compound, liproxstatin-1, which can inhibit ferroptosis in cells, in Gpx4 knockout mice, and in a model of ischaemia/reperfusion-induced hepatic damage. [PubMed: 25402683]

28•. 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. 2016; 113:E4966–4975. The authors demenstrate that peroxidation of polyunsaturated fatty acids (PUFAs) by lipoxygenases mediated by a phosphorylase kinase G2 (PHKG2)-dependent iron pool play an important role in ferroptosis. [PubMed: 27506793]

29••. Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. Nat Chem Biol. 2017; 13:91–98. This paper as well as reference 30 demenstrates that acyl-CoA synthetase long-chain family member 4 (ACSL4) is an essential component for ferroptosis execution by regulating cell membrane long polyunsaturated ω6 fatty acids. The authors also provide evidence that Inhibition of ACSL4 is a viable therapeutic approach to preventing ferroptosis-related diseases. [PubMed: 27842070]

30••. Kagan VE, Mao G, Qu F, Angeli JP, Doll S, Croix CS, Dar HH, Liu B, Tyurin VA, Ritov VB, et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. Nat Chem Biol. 2017; 13:81–90. This paper as well as reference 29 demenstrates the important role of ACSL4 in ferroptosis execution. The authors discovered that the Lipoxygenase can directly oxidize acyls—arachidonoyl (AA)- and adrenoyl (AdA)- phosphatidylethanolamines (PE) into ferroptotic signals, which is facilitated by esterification of AA and AdA into PE driven by ACSL4. [PubMed: 27842066]

31. Fan Z, Wirth AK, Chen D, Wruck CJ, Rauh M, Buchfelder M, Savaskan N. Nrf2-Keap1 pathway promotes cell proliferation and diminishes ferroptosis. Oncogenesis. 2017; 6:e371. [PubMed: 28805788]

32•. Sun X, Ou Z, Chen R, Niu X, Chen D, Kang R, Tang D. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. Hepatology. 2016; 63:173–184. This paper demenstrates the NRF2, the master regulator of redox negatively regulates ferroptosis. [PubMed: 26403645]

33. Andrews NC, Schmidt PJ. Iron homeostasis. Annu Rev Physiol. 2007; 69:69–85. [PubMed: 17014365]

34••. Gao MH, Monian P, Pan QH, Zhang W, Xiang J, Jiang XJ. Ferroptosis is an autophagic cell death process. Cell Res. 2016; 26:1021–1032. This paper and reference 35 demenstrate the important role of autopahgy in promoting ferroptosis. The authors indentify multiple autophagy related genes as postive regulators of ferroptosis in a RNAi screening. They further elucidate that NCOA4-mediated autophagic ferroptinophagy promotes the accumulation of cellular ROS and consequent ferroptotic cell death by regulating cellular labile iron contents. [PubMed: 27514700]

35•. 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. 2016:0. This paper as well as reference 34 demenstrates the important role of autopahgy in promoting ferroptosis.

36. Dowdle WE, Nyfeler B, Nagel J, Elling RA, Liu S, Triantafellow E, Menon S, Wang Z, Honda A, Pardee G, et al. Selective VPS34 inhibitor blocks autophagy and uncovers a role for NCOA4 in ferritin degradation and iron homeostasis in vivo. Nat Cell Biol. 2014; 16:1069–1079. [PubMed: 25327288]

37. Mancias JD, Wang X, Gygi SP, Harper JW, Kimmelman AC. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. Nature. 2014; 509:105–109. [PubMed: 24695223]

38. Hayano M, Yang WS, Corn CK, Pagano NC, Stockwell BR. Loss of cysteinyl-tRNA synthetase (CARS) induces the transsulfuration pathway and inhibits ferroptosis induced by cystine deprivation. Cell Death Differ. 2016; 23:270–278. [PubMed: 26184909]

39. Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT. Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuron. 1989; 2:1547–1558. [PubMed: 2576375]

40. Cassago A, Ferreira AP, Ferreira IM, Fornezari C, Gomes ER, Greene KS, Pereira HM, Garratt RC, Dias SM, Ambrosio AL. Mitochondrial localization and structure-based phosphate activation mechanism of Glutaminase C with implications for cancer metabolism. Proc Natl Acad Sci USA. 2012; 109:1092–1097. [PubMed: 22228304]

41. Yant LJ, Ran QT, 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. 2003; 34:496–502. [PubMed: 12566075]

42. 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. 2003; 305:278–286. [PubMed: 12745070]

43. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958; 181:1199–1200.

44. Seiler A, Schneider M, Forster H, Roth S, Wirth EK, Culmsee C, Plesnila N, Kremmer E, Radmark O, Wurst W, et al. Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-Mediated cell death. Cell Metab. 2008; 8:237–248. [PubMed: 18762024]

45. Devos D, Moreau C, Devedjian JC, Kluza J, Petrault M, Laloux C, Jonneaux A, Ryckewaert G, Garcon G, Rouaix N, et al. Targeting Chelatable Iron as a Therapeutic Modality in Parkinson's Disease. Antioxid Redox Signal. 2014; 21:195–210. [PubMed: 24251381]

46••. Jiang L, Kon N, Li TY, Wang SJ, Su T, Hibshoosh H, Baer R, Gu W. Ferroptosis as a p53-mediated activity during tumour suppression. Nature. 2015; 520:57–62. This paper demenstrates the important role of p53 in regulation of ferroptosis. The authors elucidate that p53 represses expression of SLC7A11, a key component of the cystine/glutamate antiporter, resulting inhition of cystine uptake and sensitizing cells to ferroptosis. This study suggests an important funtion of ferroptosis in tumor suppression. [PubMed: 25799988]

47. Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature. 2009; 458:762–765. [PubMed: 19219026]

48. Hu W, Zhang C, Wu R, Sun Y, Levine A, Feng Z. Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. Proc Natl Acad Sci USA. 2010; 107:7455–7460. [PubMed: 20378837]

49. Suzuki S, Tanaka T, Poyurovsky MV, Nagano H, Mayama T, Ohkubo S, Lokshin M, Hosokawa H, Nakayama T, Suzuki Y, et al. Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. Proc Natl Acad Sci USA. 2010; 107:7461–7466. [PubMed: 20351271]

50•. Viswanathan VS, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, Seashore-Ludlow B, Kaffenberger SD, Eaton JK, Shimada K, Aguirre AJ, et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature. 2017; 547:453–457. This paper demenstrates the import role of GPX4-mediated lipid peroxidase pathway in cancer therapy resistance and provides evidences to support that induction of ferroptosis is a potential approach to overcome cancer therapy resistance. [PubMed: 28678785]

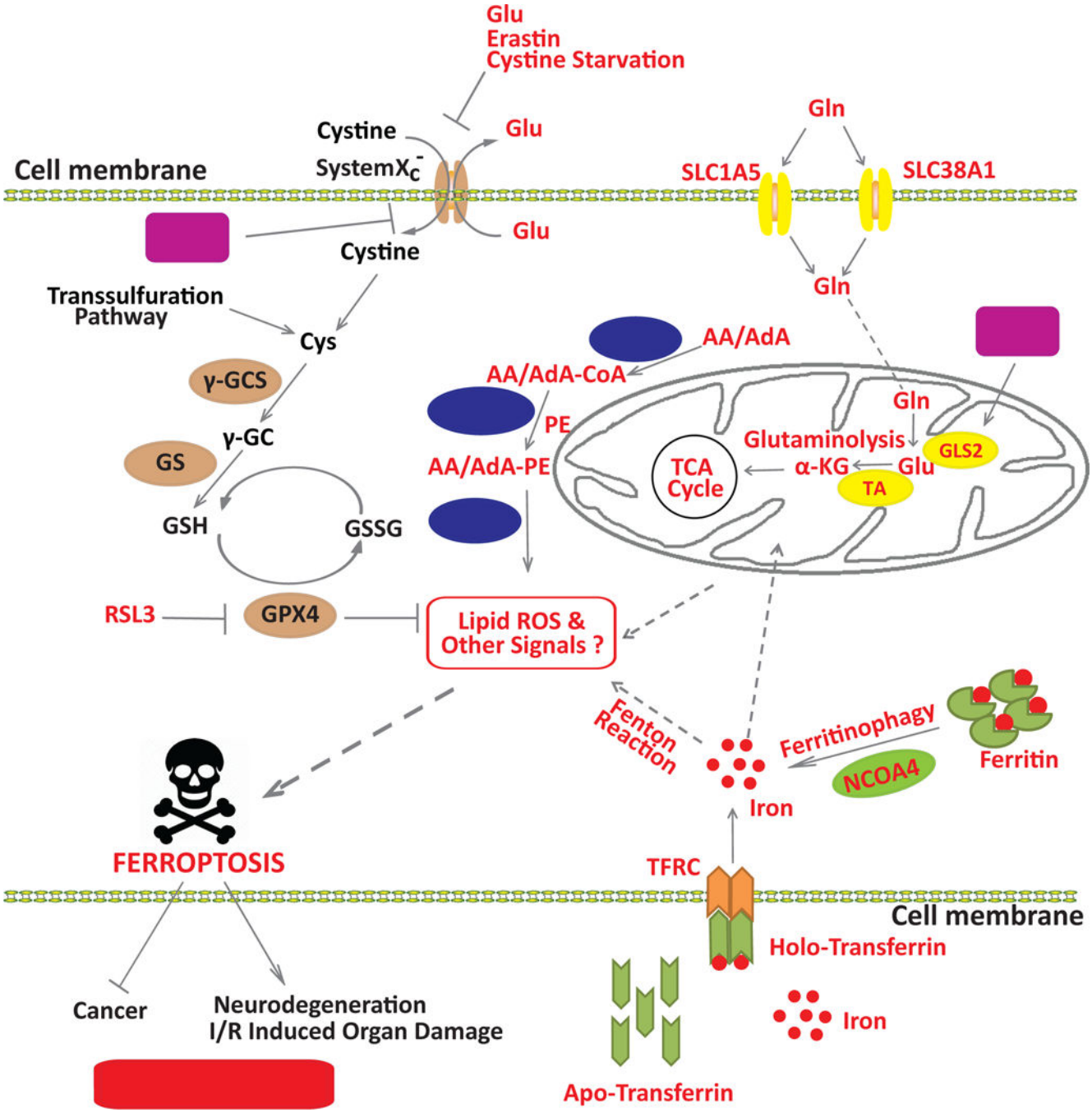

Figure 1. Regulation of ferroptosis by cellular metabolism

Ferroptosis is characterized by iron-dependent lipid peroxidation. Under normal conditions, glutathione synthesis axis, consisted of system Xc^-/γ-GCS-GS/GPX4, counteracts lipid peroxidation and thus prevents ferroptosis. Ferroptosis inducers inhibit the system Xc^-/γ-GCS-GS/GPX4 axis, leading to accumulation of lipid ROS (which is generated by the ACSL4/LPCTA3/LOX lipid peroxidation pathway). Transferrin receptor-mediated iron transportation and NCOA4-mediated ferritinophagy promote ferroptosis by sustaining cellular iron availability. Glutamine metabolic pathway, glutaminolysis, plays crucial roles in

the death process. Glutaminolysis may contribute to lipid peroxidation by providing precursors towards fatty acid or lipid synthesis.

Abbreviations: AA/AdA, arachidonic acid or adrenic acid; AA/AdA-CoAA, arachidonic acid or adrenic acid Coenzyme A; AA/AdA-PE, arachidonic acid or adrenic acid-phosphatidyl-ethanolamine; ACSL4, acyl-CoA synthetase long-chain family member 4; Cys, L-cysteine; γ-GC, gamma-glutamylcysteine; γ-GCS, γ- glutamylcysteine synthetase; GPX4, glutathione peroxidase 4; Gln, L-glutamine; GLS2, glutaminase 2; GS, Glutathione synthetase; GSH, reduced glutathione; GSSG, di-glutathione; Glu, L-glutamate; I/R Induced Organ Damage, ischemia/reperfusion induced organ damage; α-KG, α-ketoglutarate; LPCAT3, lysophosphatidylcholine acyltransferase 3; LOX, lipoxygenase; NCOA4, nuclear receptor coactivator 4; PE, phosphatidyl-ethanolamine; lipid ROS, lipid reactive oxygen species; TA, transaminases; TCA, tricarboxylic acid cycle; TFRC, transferrin receptor.