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Ferroptosis: role of lipid peroxidation, iron and ferritinophagy

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Abstract

Ferroptosis is a form of regulated cell death that is dependent on iron and reactive oxygen species (ROS) and is characterized by lipid peroxidation. It is morphologically and biochemically distinct and disparate from other processes of cell death. As ferroptosis is induced by inhibition of cysteine uptake or inactivation of the lipid repair enzyme glutathione peroxidase 4 (GPX4), the process is favoured by chemical or mutational inhibition of the cystine/glutamate antiporter and culminates in the accumulation of reactive oxygen species (ROS) in the form of lipid hydroperoxides. Excessive lipid peroxidation leads to death by ferroptosis and the phenotype is accentuated respectively by the repletion and depletion of iron and glutathione in cells. Furthermore, oxidized phosphatidylethanolamines (PE) harbouring arachidonoyl (AA) and adrenoyl moieties (AdA) have been shown as proximate executioners of ferroptosis. Induction of ferroptosis due to cysteine depletion leads to the degradation of ferritin, (ferritinophagy) which releases iron via NCOA4-mediated autophagy pathway. Evidence of the manifestation of ferroptosis *in vivo* in iron overload mice mutants is emerging. Thus, a concerted synchronization of iron-availability, ROS generation, glutamate excess and cysteine deficit leads to ferroptosis. A number of questions on the molecular mechanisms of some features and ferroptosis are highlighted as subjects for future investigations.

Key words: Apoptosis, Autophagy, Necrosis, Ferritinophagy, Ferroptosis, Peroxidation

Running Title: Ferroptosis, peroxidation and ferritinophagy

Regulated or programmed cell death essentially maintains homeostasis during physiological processes such as proliferation, growth, development and immunity. Dysregulation, disequilibrium and imbalance in cell death are associated with various disorders including different types of cancer. Consequently, apart from classical apoptosis, other forms of regulated death have been characterized as necroptosis, pyroptosis, autophagy, parthanatos, and lately ferroptosis, as discrete and disparate phenomena [1;2]. Table 1 shows the features of some forms of cell death processes. Ferroptosis, as the name implies, suggests the involvement of iron as a distinguishing feature of this form of cell death [3]. It has subsequently been characterized as a non-apoptotic peroxidation-induced cell death contingent upon the availability of iron and reactive oxygen species (ROS) [3;4]. Cell death manifestation of this process occurs without the involvement of apoptotic effectors such as caspases, BAX, and BAK. Moreover, it is independent of the inducer of necrosis, receptor-interacting protein kinase 3 (RIPK1/RIPK3) [3].. Consequently, morphological features of ferroptosis are intact cell membrane devoid of blebbing, normal-sized nucleus free of chromatin condensation and dense miniature mitochondria with vestigial cristae [5]. Biochemically, ferroptosis is characterized by the accumulation of ROS from iron metabolism, NADPH oxidase activity and lipid peroxidation products. It is induced by perturbations in signaling and membrane transport processes such as mitogen-activated protein kinases (MAPK), activation of mitochondrial voltage-dependent anion channels (VDAC) and inhibition of a specific light-chain subunit of the cystine/glutamate antiporter (SLC7A11, or system Xc-) particularly in cell culture. However, SLC7A11 knockout is not lethal due to the presence of residual cysteine in the plasma and tissue of mice [6].

Metabolically, glutaminolysis that is enhanced by the abundance of L-glutamine and transferrin, particularly when cysteine is deficient, promotes ferroptosis [7]. Furthermore, transcriptional inhibition of cystine-glutamate antiporter, SLC7A11 by mutant p53 enhances ferroptosis [8]. However, some mutant forms of p53 may not suppress SLC7A11 expression. For example, a p53 that harboured mutations in the N-terminal domain of the gene was not functional in the down-regulation of SLC7A11 [41]. Erastin and RAS-selective lethal small molecule 3 (RSL) were identified as compounds that were selectively lethal in a non-apoptotic manner to oncogenic Ras mutant cells before the concept of ferroptosis emerged [2]. Sorafenib and sulfasalazine also inhibit SLC7A11. Other inducers of ferroptosis, include buthionine sulfoximine, as well as pharmacological drugs such as, acetaminophen, lanperisone, and artesunate [2].

Ferroptosis has thus emerged recently as a paradigm characterized by a multiplicity of inducers and inhibitors that initiate increased accumulation of ROS and lipid peroxides to cause a discrete but distinct non-apoptotic cell death phenotype [9]. Conditional knockout of the key regulator GPX4 has revealed the association of ferroptosis to various disorders including cancer, neurodegenerative diseases, acute renal failure amongst others [4;10-14]. Although there are several reviews on ferroptosis to date, the current overview is an account that explores the experimentation strategies that led to the identification of the salient features of the mechanism of the cell death process.

Features of ferroptosis

Ferroptosis is initiated by events that are yet to be defined in a canonical signally pathway chronologically. The following are features of ferroptosis: (a) generation of reactive oxygen

species (b) depletion of GPX4 in cells (c) accumulation of lipid hydroperoxides and the (d) availability of iron.

(a) Reactive oxygen species (ROS) and ferroptosis

ROS are partially reduced oxygen-containing molecules, including superoxide ($O_2^{\bullet -}$), peroxides (H_2O_2 and ROOH) and free radicals ($HO^{\bullet}$ and $RO^{\bullet}$). Mitochondria generate significant amounts of ROS from normal metabolism and energy production in the electron transport chain (Figure 1). ROS are also endogenously generated from xanthine oxidase in the hydroxylation of hypoxanthine via xanthine to uric acid, cytochrome P450 catalytic reactions and by microsomes, NADPH oxidase, cyclooxygenases, uncoupled nitric oxide synthase (NOS), lipoxygenase and by peroxisomes in the oxidation of fatty acids particularly in the liver. Moreover, ROS are generated by activated neutrophils, eosinophils and macrophages during defence against infection. ROS are important as secondary messengers in intracellular signaling, defense against infection and in apoptosis of cancer cells [15]. Excess ROS are detoxified by antioxidants (enzymatic and non-enzymatic) and enzymes including superoxide dismutase (Cu, Zn-SOD, Mn-SOD), glutathione peroxidase (GPX) and catalase. Imbalance in the rate of ROS generation and detoxification leads to oxidative stress and consequent free radicals that can damage DNA, proteins and lipids. Redox-active metals, in particular Fe, could contribute to the ROS pool in the cell through Fenton reaction in which Fe catalyzes the breakdown of H_2O_2 to yield hydroxyl radicals (Figure 1). Superoxide radical eventually reduces Fe(III) to produce Fe(II) and O_2 (Haber-Weiss reaction). Under normal physiological conditions, redox-active Fe(II) in the form of the labile iron pool (LIP) is maintained at low concentrations of about 0.2–0.5 μM to sustain metabolic needs [16] while excess is sequestered in proteins, including ferritin, to avert

toxic repercussions. However, under conditions of oxidative stress, high levels of superoxide could induce Fe(II) release from iron compounds including [4Fe–4S] cluster, heme or ferritin. In ferroptosis, inactivation of cystine-glutamate antiporter, (SLC7A11) and glutathione depletion cause iron-dependent accumulation of ROS [17]. Moreover, generation of ROS, independent of iron could arise from glucose and glutamine metabolism that diminishes glutathione and GPX4 levels [3;18]. Although a vital hallmark of ferroptosis is the involvement of ROS in peroxidation, the identification of other effectors and molecular signaling events that lead to cell death by ferroptosis are yet to be characterized.

(b) **Depletion of GPX4 and ferroptosis**

GPX4, an antioxidant enzyme that neutralizes lipid peroxides and protects membrane fluidity. GPX4 uses glutathione as a cofactor to catalyze the reduction of lipid peroxides and protects cells and membranes against peroxidation. Oxidized glutathione disulfide (GSSG) is subsequently reduced by glutathione reductase and $NADPH/H^+$ to recirculate reduced glutathione (GSH). The catalytic site of this reaction is at the selenocysteine residue of GPX4 and the ligand binding of the electrophilic Ras-selective lethal small molecule (RSL3) to the nucleophile moiety of the selenocysteine on the active site of GPX4 has been demonstrated [19]. RSL3 therefore directly inactivates or inhibits GPX4 activity and induce ferroptosis. Inhibition of GPX4 initiates uncontrolled polyunsaturated fatty acid (PUFA) oxidation and fatty acid radical generation thereby causing ferroptotic cell death. Intracellular levels of glutathione (GSH), a cofactor of GPX4, are also influenced by the function of the system Xc^- SLC7A11 cystine-glutamate antiporter [11]. Erastin, sulfasalazine, sorafenib or glutamate can inhibit the transporter and induce ferroptosis.

It seems apparent that ferroptosis is triggered or induced mainly by reduced detoxification of lipid peroxides by the enzymatic activity of glutathione peroxidase 4 (GPX4) [11] or loss of this capacity. Moreover, peroxidation of PUFAs was shown to be the primary driver of ferroptosis in these studies. Oxidation of PUFAs by lipoxygenases leads to the accumulation of peroxides that may contribute to the generation of lipid peroxide breakdown products. While PUFAs including linoleic and arachidonic acids stimulated cells to RSL3–induced ferroptosis, oleic acid, a monounsaturated fatty acid (MUFA) had the contrasting effect of inhibition [18][19]. It presumably exerts a neutralizing or protective effect against ferroptosis.

Quite recently, Doll *et al* [9] reported that acyl-CoA synthetase long-chain family member 4 (ACSL4) drives ferroptosis via the accumulation of oxidized cellular membrane phospholipids. Subsequently, Kagan *et al* [20;21] identified oxidized phosphatidylethanolamines (PE) as the lethal lipid species produced by ACSL4 as inducers of ferroptosis. Doll *et al* [9] using CRISPR technology demonstrated that by knocking down the ACSL4 gene, lipid peroxides production and ferroptosis were inhibited in cells. A reversal of this observation was effected by transgenic overexpression of ACSL4 in cells in which GPX4 functionality was selectively inhibited to prevent confounding effect. Moreover, *in vivo* corroboration of this evidence was achieved by inhibiting ACSL4 with thiazolidinedione in a conditional knockout model of Gpx4 model. Furthermore, the revelation of the identity of PE as oxidized species driving ferroptosis was compelling, even though complex. Oxidized phospholipids that were produced during ferroptosis were identified by genetic, bioinformatics and LC-MS/MS analysis of extracts of RSL-3 sensitive cultured cells [22]. The lipid species specific to the induction of ferroptosis were stringently identified *in vitro* and *in vivo* in GPX4 models in only one class of phosphatidylethanolamines (PE) phospholipids.

PEs that harbour two fatty acyls, arachidonoyl (AA) and adrenoyl (AdA) activated by ACSL4 were shown to be death signals of ferroptosis particularly in the mitochondria. Gene knockout and pharmacological inhibition of ACSL4 attenuated the esterification of arachidonoyl (AA) or adrenoyl (AdA) into phosphatidylethanolamines (PE). Finally, the lethal species of double and triple-oxygenated (15-hydroperoxy)-diacylated PE peroxides are generated by 15-lipoxygenase (LOX). In essence, Acyl-CoA synthetase long-chain family member 4 (ACSL4) catalyzes the ligation of an arachidonoyl (AA) or adrenoyl (AdA) to produce AA or AdA *acyl* Co-A derivatives. These are then esterifed into phosphatidylethanolamines (AA-PE and AdA-PE) by lysophosphatidylcholine acyltransferase 3 (LPCTA3). Subsequently, AA-PE and AdA-PE, are oxidised by 15-lipoxygenase (15-LOX) to generate lipid hydroperoxides, the proximate executors of ferroptosis (Figure 2). Lipid hydroperoxides are reduced by GPX4 to inhibit ferroptosis. While the GPX4, an antioxidant enzyme neutralizes lipid peroxides, the antioxidant vitamin E (α-tocopherol) and α-tocotrienol were shown to regulate ferroptosis via LOX suppression [20]. Vitamin E inhibits LOX by also competing at the substrate binding site in addition to its ability to scavenge hydroxyl group radicals. Electron spin resonance (ESR) spectroscopy and LC-MS were employed to delineate the mechanism by which vitamin E inhibits LOX activity via competition with the substrate at the binding site of the enzyme. Esterified analogs of vitamin E, α-tocopherol succinate (TS) or α-tocopherol phosphate (TP) though unable to generate tocopheroxyl radicals, suppressed LOX activity nevertheless by competing for PUFA substrate binding site (the corking mechanism). Vitamin E has been shown to protect cells against ferroptotic death *in vitro* [14;23;24] and in vivo in *Gpx4*$^{-/-}$ knockout mice [41]. Tissue specific expression of 15-LOX and possibly vitamin E status underline disparate

organs and disorders that are associated with ferroptosis. Other inhibitors of ferroptosis include Liproxstatin-1, Ferostatin -1, ebselen, and Coenzyme Q. Furthermore, susceptibility and sensitivity to ferroptosis inducers vary with different cell types [25]. Some lymphoma cells have deficiencies in sulphur transfer pathways and rely on extracellular sources of cysteine and cystine. For example, sulfasalazine-induced ferroptosis was inhibited in Nb2 lymphoma cells when co-cultured with cysteine secreting fibroblasts or when supplemented with 2-mercaptoethanol. Moreover, hepatoma carcinoma cells, in response to sorafenib-induced ferroptosis exhibited elevation of NAD(P)H Quinone Dehydrogenase 1 (NQO), HO-1 and FTH1 mRNA levels [26] possibly to attenuate the effects of ROS. In essence, GPX4 inhibition, GSH depletion, or increased lipoxygenase activity enhances PUFA accumulation and fatty acid radicals including malondialdehyde and 4-hydroxynonenal which induce ferroptosis to cause cell death in pathological disorders such as Huntington's disease, acute kidney disease, B cell lymphoma, periventricular leukomalacia, and cancers [6].

Iron and ferroptosis

The involvement of iron from the onset in ferroptosis was revealed when the iron chelator, deferoxamine (DFO), abrogated erastin-induced lethality [27]. Evidence from Dixon et al., [3] revealed that iron regulatory protein 2 (IRP2) controls cellular iron levels to up-regulate erastin-induced ferroptosis. IRP2 binds to iron-responsive elements (IREs) which are stem-loop structures located in the 5'-UTR of ferritin and ferroportin to repress mRNA translation and in the 3'-UTR of transferrin receptor 1 and DMT1 leading to the inhibition of the ubiquitination of their mRNA. ALAS2, ACO2 and EPAS1 mRNAs amongst others are regulated by IREs. Consequently, shRNA knockdown of transferrin receptor reduced erastin-induced cytotoxicity.

In contrast, a knockdown of FBXL5, the E3 ubiquitin ligase that mediates IRP2 ubiquitination, promoted erastin-induced cell death [3]. Iron chelation could, however, target any of the diverse iron-containing or dependent proteins in cells. Incidentally, some of the proteins are iron-containing lipoxygenase and also NADPH oxidase, xanthine oxidase, cytochrome P450 amongst others. Interestingly, NADPH oxidase (NOX1) function, possibly, in the metabolism of Fe/heme binding proteins, is up-regulated in oncogenic RAS mutant cells [28]. The essentiality of iron in ferroptosis was confirmed further when transferrin, as holotransferrin, was reported to be vital for the induction of ferroptosis [29]. The study employed gene silencing, size exclusion fractionation and mass spectrometry to demonstrate that transferrin and glutamine are essential for ferroptosis when induced by amino acids or in particular during cystine deficiency . This scenario seems optimal for the induction of ferroptosis because reduced cystine, lower glutathione or antioxidant level and iron-loaded transferrin are ideal substrates and conditions for the generation of ROS in cell culture. The Tf-TfR1 axis and IRE-IRP2 regulation might be the link to IRP2 involvement in the initiation of ferroptosis in HT-1080 cells [27]. Moreover, TfR1 recycling was shown to be inhibited by heat shock protein B1 (HSPB1) with the consequent effect of decreased intracellular iron concentration [30;31]. On the contrary, however, inactivation of HSPB1 favored iron accumulation and erastin-induced ferroptosis [32]. The compelling evidence is that while iron elevation promotes ferroptosis, the process is inhibited by iron chelation [3]. Poignantly, L-glutamate has earlier been shown to inhibit cystine transport [33] and cause cell death in neuronal cells. Moreover, oxidative glutamate-induced toxicity in neurons is also abrogated by iron chelation [34] through inhibiting HIF proly hydroxylase 1, a 2-oxoglutarate and oxygen-dependent dioxygenase [28]. The authors [35]

proposed that iron chelators possibly abrogated ferroptosis by further inhibiting iron-dependent HIF prolyl hydroxylase. Of note, however, is that IRP2 lacks aconitase activity and does not sense iron directly [36]; rather the iron/oxygen sensor, akin to HIF prolyl hydroxylase, is FBXL5 ubiquitin ligase that modulates IRP2 degradation. Interestingly, mice with targeted ablation of the IRP-2 gene have been reported to have increased levels of iron and ferritin in the white matter of the brain before the onset of neurodegeneration [37]. Unlike IRP1, neurodegeneration disorders typify the phenotype of IRP2 knockout mice [38]. The role of iron, in modulating diverse ferroptosis-inducing compounds and signaling pathways of ferroptosis, remain to be elucidated. How does iron modulate the upstream or downstream regulators of ferroptosis in the signaling pathway?

Other features of ferroptosis

PHKG2, and p53

What is both intriguing and fascinating is the role of phosphorylase kinase G2 (PHKG2), an enzyme that induces the release of glucose-1-phosphate from glycogen, in ferroptosis. Phosphorylase kinase gamma2 is the liver and testicular isoform of PHK while PHKG1 is found mainly in the muscle. Mutations in the PHKG2 are associated with glycogen storage disease, liver cirrhosis and complications in type 1 diabetes in humans [39;40].

Silencing PHKG2 in cells was shown to decrease iron availability which inhibited lipid oxidation upon erastin treatment [19]. Thus far, PHKG2 has been suggested, tentatively, to modulate erastin sensitivity by its iron regulating function [19]. In essence, modulation of ferroptosis is regulated through PHKG2-induced iron availability. However, the manner by which PHKG2 regulates cytosolic iron levels to influence ferroptosis is still subject to further investigation.

One hypothesis is that this might be due to the link between PHKG2 and the recently reported non-canonical function of p53. Oncogenetic tumour-suppressive functions of p53 include cell cycle arrest, apoptosis, senescence and the newly identified activation of ferroptosis [41;42]. The latter is attributed to its inhibitory transcriptional regulation of cystine, cationic amino-acid transporter, member 11 (*SLC7A11*), causing reduced cellular cysteine levels, reduced GSH and induction of ferroptosis. In essence, inactivation of acetylation-defective p53 mutant inhibits transcription of *SLC7A11,* hence promoting ROS that facilitates ferroptosis and cell death in cancers cells [41]. Furthermore, glutaminase 2 (GLS2), a p53-regulated glutaminase was implicated in a glutaminolysis-ferroptosis nexus [7]. Quite remarkably, Jennis et al [43] identified a p53 variant that exhibited impaired ability to suppress SLC7A11 or transactivate GLS2 as incapable of ferroptosis induction. Consequently, two disparate phenotypic traits validated unequivocally the prime function of p53 in ferroptosis. The molecular mechanism by which p53 regulates ferroptosis was recently revealed [44] in a report that demonstrated that spermidine/spermine N1-acetyltransferase 1 (SAT1), a direct p53 target gene, enhances the levels of lipid oxygenase, arachidonate 15-lipoxygenase (ALOX15), an iron-binding enzyme that oxidizes polyunsaturated fatty acids (discussed above). Moreover, levels of SAT1 were found to be low in tumour samples and its knockdown significantly inhibited p53-induced ferroptosis. Thus, SAT1, in synchrony or (possibly in synergy) with ROS promotes lipid peroxidation that leads to ferroptosis. Yet, repression of p53 expression by iron, however, suppresses its canonical oncogenic cell death functions [45]. Consequently, a gain of function due to mutation and a loss of expression of a protein (p53) lead to contrasting phenotypic effects in different variants of the gene.

Ferritinophagy

Ferritinophagy is the autophagic degradation of the iron-storage ferritin protein that maintains homeostasis during iron depletion. The pathway is mediated, during low iron levels by nuclear receptor co-activator 4 (NCOA4), an autophagy cargo receptor that binds ferritin heavy chain 1 (FTH1) in autophagosome and it is delivered into the lysosome for degradation to release iron for systemic physiological requirements. However, when iron levels in cells are high, NCOA4 selectively binds to HERC2 (HECT and RLD domain containing E3 ubiquitin protein ligase 2) to initiate its proteosomal degradation and ubiquitination [46;47]. Initiation of ferroptosis was shown to activate ferritinophagy [48] in a process whereby ferritin catabolism increases the labile iron pool (LIP) which promotes ROS accumulation that drives ferroptosis. Gao *et al* [48] employed inhibitors of autophagy and NCOA4 gene knockdown to show that ferroptosis is an autophagic cell death process. Inhibition of NCOA4 repressed ferritin degradation and suppressed ferroptosis, while its overexpression had the opposite effects [48] . Induction of ferroptosis by erastin, independent of transferrin internalization, was shown [19] to cause a time-dependent increase of LIP, a process blocked by a potent inhibitor of autophagy, bafilomycin A1 (BafA1). It seems evident though that the acute phase response of ferritin synthesis from excess cellular labile iron overrides channeling of iron as a substrate for ROS generation, which paradoxically is a sine qua non for ferroptosis induction. The study showed an increase in endogenous ferritin heavy chain 1 (FTH1) expression during ferroptosis [19]. Subsequently, overexpression of FTH1 enhanced its degradation after induction of ferroptosis by erastin in the same study. In general, it was noted that autophagy-driven ferroptosis occurs during the early initiation stage [19]. The function of autophagy in cell death or survival remains

unclear. Autophagy is in general terms a physiological process that conserves metabolic mechanisms to preserve life by degrading nutrients in the lysosome during periods of stress, starvation and hypoxia [49]. Ferritinophagy, akin to this, is, an autophagic degradation of ferritin to release iron during situations of depletion [47] and in particular during an essential survival process of erythropoiesis [47]. The regulation of ferroptosis and the switch to ferritinophagy during pathological conditions lead to cell death [50] and this could also be beneficial therapeutically in the pathophysiology of cancer progression.

There has also been much debate and controversies about the physiological relevance of the Fenton reaction *in vivo* particularly during normal iron homeostasis. The rate constant for the Fenton reaction is reported to be considerable lower than that of the activity of catalase upon H_2O_2 interaction [51]. Moreover, a robust amalgam of glutathione, superoxide dismutase (SOD), catalase and peroxiredoxin protects cells from oxyradical damage. However, under conditions of iron loading disorders such as hemochromatosis and iron-loading anemias, such as thalassemia, sickle cell disease, hemolysis and myelodysplasia, excess non-transferrin bound iron (NTBI) may be sequestered as ferritin in the lysosomes. Ferritin is subsequently degraded or its iron reduced in the acidic pH of the lysosomes where the level of chelatable iron could be about 15 µM. Coupled with localised low antioxidant enzymes, this situation stimulates oxidative stress and the release of redox-active iron [52;53] into the cytosol from ruptured lysosomes. Although serum ferritin iron content is low it is released into blood circulation during inflammation and in autoimmune diseases may increase iron level locally and cause apoptosis [54;55] or possibly ferroptosis, the new member in an array of evolving cell death processes. The extracellular NTBI levels may increase intracellular LIP content, execute

oxyradical damage [56] and generate ROS that are features of iron overload disorders. Prolonged iron overload leads to the sequestration of Fe as ferritin in lysosomes and this forms hemosiderin which is possibly an adaptation of survival mechanism for tissue iron sequestration that is associated with low iron content of serum. An evidence of the role of ferroptosis, *in vivo* in iron-loading disorders was reported recently in severe iron overload Hjv-/- and $Smad4^{Alb/Alb}$ mice [57] where ferrostatin-1, a ferroptosis inhibitor attenuated liver damage in the mice. The study also reported that while $Slc7a11^{-/-}$ mice did not exhibit the phenotype of ferroptosis, primary hepatocytes and bone marrow-derived macrophages (BMDMs) from the mice when exposed to iron demonstrated increased Slc7a11 expression and the induction of ferroptosis [57]. Moreover, iron overload ($Hfe^{-/-}$, $Hjv^{-/-}$, and $Smad4^{Alb/Alb}$) had increased hepatic *Slc7a11* mRNA levels compared to wild-type mice. The implications of this and the antioxidant compensatory mechanisms in $Slc7a11^{-/-}$ mice require forther investigations.

Concluding remarks: Excessive accumulation of ROS principally due to the availability of iron causes lipid peroxidation that culminates in ferroptosis, a form of non-apoptotic cell death. Ferritinophagy contributes to Fe substrate for Fenton chemistry which in part causes peroxidation that culminates in ferroptosis (Figure.1). While the physiological function of ferroptosis is not clear, the etiology is associated with disequilibrium in redox metabolism and subsequent pathological disorders. The answers to a number of puzzling questions are relevant to our clear understanding of ferroptosis. Could non-iron sources of ROS or the depletion of antioxidant levels independently induce ferroptosis? Is the role of iron in ferroptosis limited

only to induction or are there other iron-dependent genes involved downstream during lipid peroxidation? Does transferrin saturation cause ferroptosis and the development of hepatocellular carcinoma? What are the consequences of effectors of ferroptosis that have pleiotropic functions in other forms of cell death processes? (See outstanding questions).

Concluding remarks: Ferroptosis has emerged as a new form of iron and ROS-dependent cell death. It is induced by inhibition of cysteine uptake that reduces intracellular levels of glutathione (GSH) and antioxidant status of the cell. Moreover, NADPH oxidase and mutant p53 regulate ferroptosis positively by enhancing ROS production and suppressing the expression of the light-chain subunit of the cystine/glutamate antiporter, SLC7A11. Transferrin as holotransferrin and amino acid glutamine, are also inducers of ferroptosis. Redox signaling in ferroptosis involves the generation of phosphatidylethanolamines (PE) by 15-lipoxygenase.

Ferritinophagy, a process of autophagic degradation of ferritin has been associated with ferroptosis as supplying Fe substrate in its initiation in systemic metabolism.

Derangement, imbalance and dysregulation that occur by genetic, pharmaceutical and pathological conditions [58] during ferroptosis could be confounding, complicated and complex (Figure 3). It is promising, however, that pharmaceutical compounds may be used to activate ferroptosis against cancer and other diseases, while its inhibition may be employed to attenuate degenerative disorders.

Outstanding Questions include the following

- Is ferroptosis a normal physiological process or is it a consequence of perturbed oxidative metabolism?

- What is the critical iron status that channels NCOA4 to either autophagy or ubiquitin degradation?
- Is it a pathological process that is associated with tissue specific expression of the different modulating components?

- What is the role ROS-induced lipid peroxidation in the signal cascades that lead to cell death by ferroptosis?
- What are the effector molecules downstream of the induction of ferroptosis?
- Are there other p53 target genes that could contribute to ferroptosis?
- How can ferroptosis be employed in the therapy of pathological conditions such as cancer, neurodegenerative diseases, heart ischemia/reperfusion injury, acute renal failure?

There is no conflict of interest.

Reference List

1 Hanson B: Necroptosis: A new way of dying? Cancer Biol Ther 2016;1-12.

2 Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, Kang R, Tang D: Ferroptosis: process and function. Cell Death Differ 2016;23:369-379.

3 Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison B, III, Stockwell BR: Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 2012;149:1060-1072.

4 Friedman BI, Schaefer JW, Schiff L, Olaniyan MFAA: Increased iron-59 absorption in patients with hepatic cirrhosis. J Nucl Med 1966;7:594-602.

5 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.

6 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 2005;280:37423-37429.

7 Gao M, Monian P, Quadri N, Ramasamy R, Jiang X: Glutaminolysis and Transferrin Regulate Ferroptosis. Mol Cell 2015;59:298-308.

8 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 2015;520:57-62.

9 Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, Prokisch H, Trumbach D, Mao G, Qu F, Bayir H, Fullekrug 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 2017;13:91-98.

10 Yang WS, Stockwell BR: Ferroptosis: Death by Lipid Peroxidation. Trends Cell Biol 2016;26:165-176.

11 Seiler A, Schneider M, Forster H, Roth S, Wirth EK, Culmsee C, Plesnila N, Kremmer E, Radmark 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 2008;8:237-248.

12 Wirth EK, Conrad M, Winterer J, Wozny C, Carlson BA, Roth S, Schmitz D, Bornkamm GW, Coppola V, Tessarollo L, Schomburg L, Kohrle J, Hatfield DL, Schweizer U: Neuronal selenoprotein expression is required for interneuron development and prevents seizures and neurodegeneration. FASEB J 2010;24:844-852.

13 Doll S, Conrad M: Iron and ferroptosis: A still ill-defined liaison. IUBMB Life 2017.

14 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 2015;290:28097-28106.

15 Frey PA, Reed GH: The ubiquity of iron. ACS Chem Biol 2012;7:1477-1481.

16 Petrat F, Rauen U, de GH: Determination of the chelatable iron pool of isolated rat hepatocytes by digital fluorescence microscopy using the fluorescent probe, phen green SK. Hepatology 1999;29:1171-1179.

17 Dixon SJ, Stockwell BR: The role of iron and reactive oxygen species in cell death. Nat Chem Biol 2014;10:9-17.

18 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 2014;156:317-331.

19 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 U S A 2016;113:E4966-E4975.

20 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, Bayir H: Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. Nat Chem Biol 2017;13:81-90.

21 D'Herde K, Krysko DV: Ferroptosis: Oxidized PEs trigger death. Nat Chem Biol 2017;13:4-5.

22 Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, Herbach N, Aichler M, Walch A, Eggenhofer E, Basavarajappa D,

Radmark O, Kobayashi S, Seibt T, Beck H, Neff F, Esposito I, Wanke R, Forster H, Yefremova O, Heinrichmeyer M, Bornkamm GW, Geissler EK, Thomas SB, Stockwell BR, O'Donnell VB, Kagan VE, Schick JA, Conrad M: Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. Nat Cell Biol 2014;16:1180-1191.

23 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 2016;9:22-31.

24 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 2015;212:555-568.

25 Gout PW, Simms CR, Robertson MC: In vitro studies on the lymphoma growth-inhibitory activity of sulfasalazine. Anticancer Drugs 2003;14:21-29.

26 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.

27 Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison B, III, Stockwell BR: Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 2012;149:1060-1072.

28 Siddiq A, Aminova LR, Troy CM, Suh K, Messer Z, Semenza GL, Ratan RR: Selective inhibition of hypoxia-inducible factor (HIF) prolyl-hydroxylase 1 mediates neuroprotection against normoxic oxidative death via HIF- and CREB-independent pathways. J Neurosci 2009;29:8828-8838.

29 Gao M, Monian P, Quadri N, Ramasamy R, Jiang X: Glutaminolysis and Transferrin Regulate Ferroptosis. Mol Cell 2015;59:298-308.

30 Arrigo AP, Virot S, Chaufour S, Firdaus W, Kretz-Remy C, Diaz-Latoud C: Hsp27 consolidates intracellular redox homeostasis by upholding glutathione in its reduced form and by decreasing iron intracellular levels. Antioxid Redox Signal 2005;7:414-422.

31 Chen H, Zheng C, Zhang Y, Chang YZ, Qian ZM, Shen X: Heat shock protein 27 downregulates the transferrin receptor 1-mediated iron uptake. Int J Biochem Cell Biol 2006;38:1402-1416.

32 Sun X, Ou Z, Xie M, Kang R, Fan Y, Niu X, Wang H, Cao L, Tang D: HSPB1 as a novel regulator of ferroptotic cancer cell death. Oncogene 2015;34:5617-5625.

33 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.

34 Zaman K, Ryu H, Hall D, O'Donovan K, Lin KI, Miller MP, Marquis JC, Baraban JM, Semenza GL, Ratan RR: Protection from oxidative stress-induced apoptosis in cortical neuronal cultures by iron chelators is associated with enhanced DNA binding of

hypoxia-inducible factor-1 and ATF-1/CREB and increased expression of glycolytic enzymes, p21(waf1/cip1), and erythropoietin. J Neurosci 1999;19:9821-9830.

35 Speer RE, Karuppagounder SS, Basso M, Sleiman SF, Kumar A, Brand D, Smirnova N, Gazaryan I, Khim SJ, Ratan RR: Hypoxia-inducible factor prolyl hydroxylases as targets for neuroprotection by "antioxidant" metal chelators: From ferroptosis to stroke. Free Radic Biol Med 2013;62:26-36.

36 Takahashi-Makise N, Ward DM, Kaplan J: On the mechanism of iron sensing by IRP2: new players, new paradigms. Nat Chem Biol 2009;5:874-875.

37 Lavaute T, Smith S, Cooperman S, Iwai K, Land W, Meyron-Holtz E, Drake SK, Miller G, Abu-Asab M, Tsokos M, Switzer R, III, Grinberg A, Love P, Tresser N, Rouault TA: Targeted deletion of the gene encoding iron regulatory protein-2 causes misregulation of iron metabolism and neurodegenerative disease in mice. Nat Genet 2001;27:209-214.

38 Salvatore MF, Fisher B, Surgener SP, Gerhardt GA, Rouault T: Neurochemical investigations of dopamine neuronal systems in iron-regulatory protein 2 (IRP-2) knockout mice. Brain Res Mol Brain Res 2005;139:341-347.

39 MacDonald MJ, Hasan NM, Ansari IU, Longacre MJ, Kendrick MA, Stoker SW: Discovery of a Genetic Metabolic Cause for Mauriac Syndrome in Type 1 Diabetes. Diabetes 2016;65:2051-2059.

40 Maichele AJ, Burwinkel B, Maire I, Sovik O, Kilimann MW: Mutations in the testis/liver isoform of the phosphorylase kinase gamma subunit (PHKG2) cause autosomal liver glycogenosis in the gsd rat and in humans. Nat Genet 1996;14:337-340.

41

42 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 2016;30:918-930.

43 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 2015;520:57-62.

44 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 U S A 2016;113:E6806-E6812.

45 Shen J, Sheng X, Chang Z, Wu Q, Wang S, Xuan Z, Li D, Wu Y, Shang Y, Kong X, Yu L, Li L, Ruan K, Hu H, Huang Y, Hui L, Xie D, Wang F, Hu R: Iron metabolism regulates p53 signaling through direct heme-p53 interaction and modulation of p53 localization, stability, and function. Cell Rep 2014;7:180-193.

46 Dowdle WE, Nyfeler B, Nagel J, Elling RA, Liu S, Triantafellow E, Menon S, Wang Z, Honda A, Pardee G, Cantwell J, Luu C, Cornella-Taracido I, Harrington E, Fekkes P, Lei H, Fang Q, Digan ME, Burdick D, Powers AF, Helliwell SB, D'Aquin S,

Bastien J, Wang H, Wiederschain D, Kuerth J, Bergman P, Schwalb D, Thomas J, Ugwonali S, Harbinski F, Tallarico J, Wilson CJ, Myer VE, Porter JA, Bussiere DE, Finan PM, Labow MA, Mao X, Hamann LG, Manning BD, Valdez RA, Nicholson T, Schirle M, Knapp MS, Keaney EP, Murphy LO: 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.

47 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.

48 Gao M, Monian P, Pan Q, Zhang W, Xiang J, Jiang X: Ferroptosis is an autophagic cell death process. Cell Res 2016;26:1021-1032.

49 Kroemer G, Marino G, Levine B: Autophagy and the integrated stress response. Mol Cell 2010;40:280-293.

50 Hou W, Xie Y, Song X, Sun X, Lotze MT, Zeh HJ, III, Kang R, Tang D: Autophagy promotes ferroptosis by degradation of ferritin. Autophagy 2016;12:1425-1428.

51 Chance B, GREENSTEIN DS, HIGGINS J, YANG CC: The mechanism of catalase action. II. Electric analog computer studies. Arch Biochem Biophys 1952;37:322-339.

52 Persson HL, Yu Z, Tirosh O, Eaton JW, Brunk UT: Prevention of oxidant-induced cell death by lysosomotropic iron chelators. Free Radic Biol Med 2003;34:1295-1305.

53 Yu Z, Persson HL, Eaton JW, Brunk UT: Intralysosomal iron: a major determinant of oxidant-induced cell death. Free Radic Biol Med 2003;34:1243-1252.

54 Bresgen N, Jaksch H, Bauer HC, Eckl P, Krizbai I, Tempfer H: Astrocytes are more resistant than cerebral endothelial cells toward geno- and cytotoxicity mediated by short-term oxidative stress. J Neurosci Res 2006;84:1821-1828.

55 Bresgen N, Jaksch H, Lacher H, Ohlenschlager I, Uchida K, Eckl PM: Iron-mediated oxidative stress plays an essential role in ferritin-induced cell death. Free Radic Biol Med 2010;48:1347-1357.

56 Pietrangelo A: Iron, friend or foe? "Freedom" makes the difference. J Hepatol 2000;32:862-864.

57 Wang H, An P, Xie E, Wu Q, Fang X, Gao H, Zhang Z, Li Y, Wang X, Zhang J, Li G, Yang L, Liu W, Min J, Wang F: Characterization of Ferroptosis in Murine Models of Hemochromatosis. Hepatology 2017.

58 Yu H, Guo P, Xie X, Wang Y, Chen G: Ferroptosis, a new form of cell death, and its relationships with tumourous diseases. J Cell Mol Med 2016.

Legends

Figure 1. The role of iron in ROS metabolism. Fenton reaction in which ferrous iron reacts with hydrogen peroxide to produce the (A) hydroxyl radical (HO•) or (B) lipid alkoxy (RO•) Ferric ion from the reaction can be reduced back to ferrous iron (a) in the presence of superoxide (Haber–Weiss reaction). All the enzymes involved in these reactions are iron-depenednt except glutathione peroxidase and peroxiredoxin. (Adapted from Dixon SJ, Stockwell BR: The role of iron and reactive oxygen species in cell death. Nat Chem Biol 2014; 10:9-17).

Figure 2. Mechanism of ferroptosis by oxidized phosphatidylethanolamines (PE). Acyl-CoA synthetase long-chain family member 4 (ACSL4) catalyzes the ligation of an arachidonoyl (AA) or adrenoyl (AdA) to produce AA or AdA acyl Co-A derivatives. AA-PE and AdA-PE are esterified to PE by lysophosphatidylcholine acyltransferase 3 (LPCTA3). Subsequently, AA-PE and AdA-PE, are oxidised by 15-lipoxygenase (15-LOX) to generate lipid hydroperoxides, the proximate executors of ferroptosis. (Adapted from D'Herde K, Krysko DV: Ferroptosis: Oxidized PEs trigger death. Nat Chem Biol 2017; 13:4-5).

Figure 3. Role of iron, glutathione and peroxidation in ferroptosis

Ferroptosis occurs as a result of increased ROS levels due to elevated intracellular iron concentration that is possibly accentuated by ferritinophagy, deranged mitochondrial function, and a depletion of antioxidant GSH that could be due to disruption of cysteine transport. Erastin, glutamate and p53 are inhibitors of cysteine transport. Low levels GPX4 activity leads to increased accumulation of ROS that cause lipid peroxidation and consequently to ferroptosis induction. Other inhibitors of ferroptosis are DFO (Fe), BafA1, CQ (Ferritinophagy), and HSPB1 (TfR1 recycling). Liproxstatin-1 and Ferrostatin amongst others are inhibitors of peroxidation. Abbreviations: TF-Fe (Transferrin loaded with iron), deferrioxamine, (DFO) bafilomycin A1 (BafA1), and chloroquine (CQ).

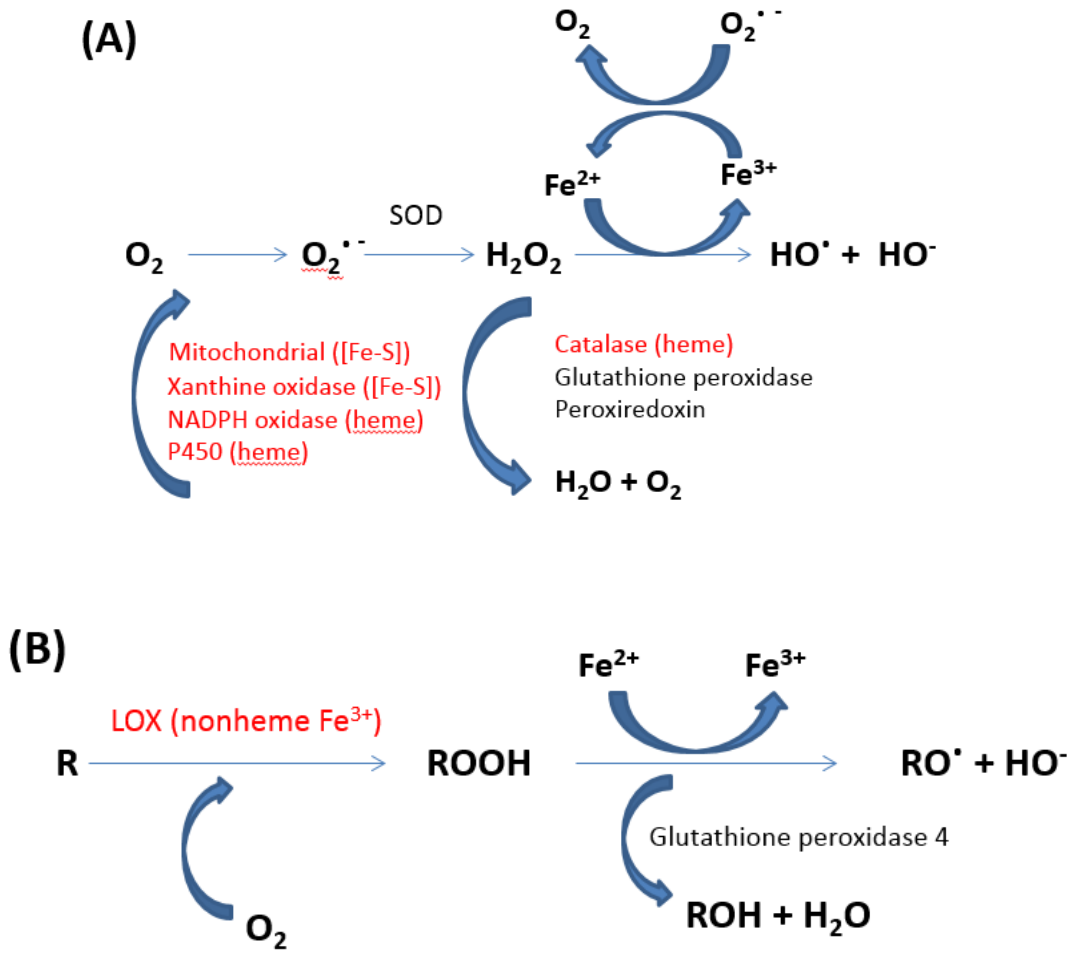
(A)
O_2
$O_2^{\bullet -}$
Fe^{2+}
Fe^{3+}
SOD
O_2
$O_2^{\bullet -}$
H_2O_2
$HO^{\bullet}$ + HO^-
Mitochondrial ([Fe-S])
Xanthine oxidase ([Fe-S])
NADPH oxidase (heme)
P450 (heme)
Catalase (heme)
Glutathione peroxidase
Peroxiredoxin
$H_2O + O_2$
(B)
LOX (nonheme Fe^{3+})
R
ROOH
Fe^{2+}
Fe^{3+}
$RO^{\bullet}$ + HO^-
Glutathione peroxidase 4
O_2
ROH + H_2O

Figure 1

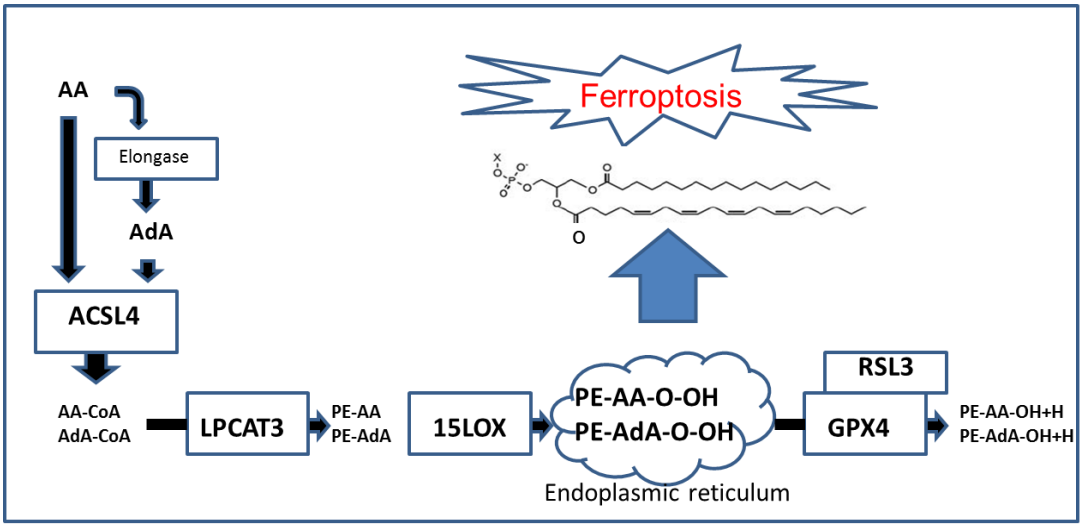
AA
Elongase
AdA
ACSL4
AA-CoA
AdA-CoA
LPCAT3
PE-AA
PE-AdA
15LOX
PE-AA-O-OH
PE-AdA-O-OH
Endoplasmic reticulum
Ferroptosis
RSL3
GPX4
PE-AA-OH+H
PE-AdA-OH+H

Figure 2

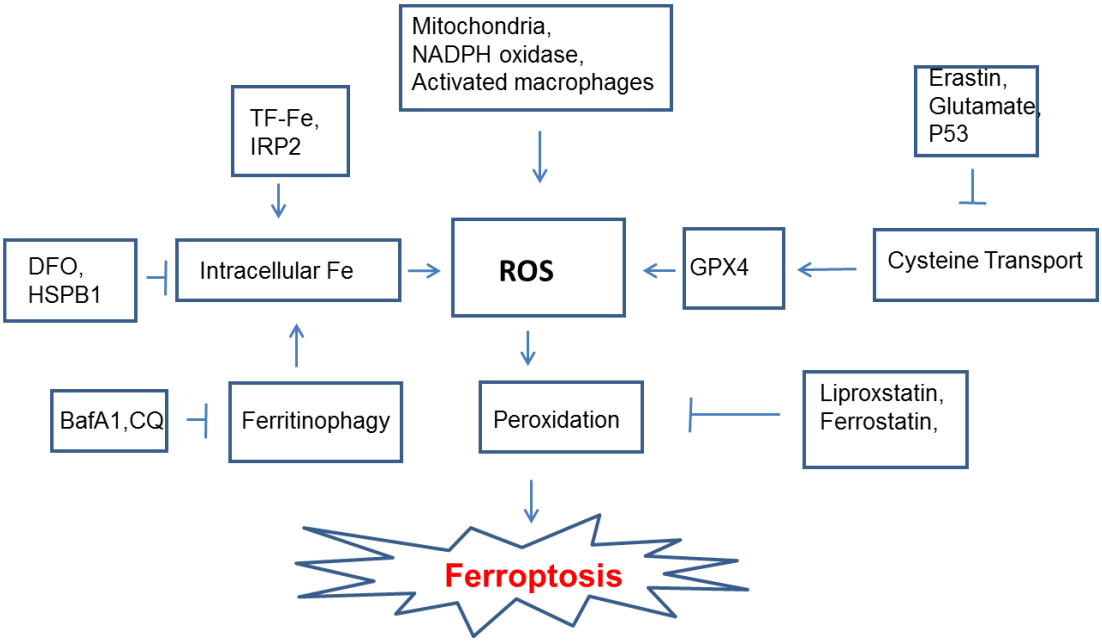
Mitochondria,
NADPH oxidase,
Activated macrophages
TF-Fe,
IRP2
Erastin,
Glutamate,
P53
DFO,
HSPB1
Intracellular Fe
ROS
GPX4
Cysteine Transport
BafA1,CQ
Ferritinophagy
Peroxidation
Liproxstatin,
Ferrostatin,
Ferroptosis

Fig. 3

Table 1. The main features of ferroptosis, apoptosis, necroptosis and autophagy

Cell death	FERROPTOSIS	APOPTOSIS	NECROPTOSIS	AUTOPHAGY
Definition	Iron-mediated, caspase-independent cell death that requires ROS and lipid peroxides	A caspase mediated programmed cell death	RIPK3-dependent regulated necrosis.	Autophagosome and autolysosome catabolic process to maintain nutrients for development and during stress conditions
Morphological features				
Cell membrane	Normal spherical cells	Plasma membrane blebbing of spherical cells	Plasma Membrane rupture	Normal membrane structure
Cytoplasm	Diminutive mitochondria with decreased crista and collapsed and ruptured membrane	Cell shrinkage and loss of cytoplasmic protrusions	Cytoplasmic and organelles swelling	Lysosomal vacuoles exhibit double membranes
Nucleus	Normal	Reduced nuclear size and pronounced nuclear DNA fragmentation and chromatin condensation	Partial chromatin condensation	Normal
Biochemical features	Inhibition of system Xc^- And reduced GSH, inhibition of GPX4. Increased Fe, ROS and lipid peroxidation, mitochondrial membrane	Inter-nucleosomal DNA fragment Caspase activation mitochondrial membrane potential *dissipation*	Receptor-interacting protein kinase 1 is recruited by RIPK3. Increased kinase cytotoxic activity, decreased ATP levels mixed lineage kinase domain-like	Microtubule-associated protein light chain 3-1 conversion to protein light chain 3-11, catabolism of substrates

	potential *dissipation*		protein MLKL	
Core regulators	VDAC2/3, Ras NOX,TFR1, p53 CARS, GPX4 SLC7A11, HSPB1 •NRF2	p53, Bax, Bak, Bcl- 2, Bcl-XL Other pro-apoptotic Bcl-2 family proteins	RIP1, RIP3, MLKL	ATG5 ATG7 Beclin 1
Inducers	System x_ c inhibition Inhibition of GCL Inhibition of GPX4	Extrinsic: Activation of TNF death receptors Recruitment of cytoplasmic adaptor proteins (e.g. FADD and TRADD) Loss of mitochondrial transmembrane potential Mitochondrial outer membrane permeabilization (MOMP) Release of pro-apoptotic proteins into cytosol (e.g. cytochrome c)	TNFR1 activation Recruitment of TRADD and RIPK1	Upregulation of Atg5 and Atg6 Non-selective PI3K inhibitors: 3-ME, LY294002, wortmannin Selective VPS34 inhibitors: PIK-III, compound 31, SAR 405, Vps34-In1 Specific ULK1 inhibitors: MRT68921, MRT67307, SBI-0206965
Inhibitors	Lipophilic antioxidants (e.g. Fer-1, vitamin E) Iron chelators (e.g. DFO, CPX)	Caspase inhibitors	Necrostatins (e.g. Nec-1) Necrosulfonamide	Antioxidants (e.g. vitamin E, idebenone) PD150606, a calpain inhibitor, Autophagy inhibitors (e.g. 3-MA, wortmannin)
Immune features	Pro-inflammatory	Mostly anti-inflammatory	Most often pro-inflammatory	Most often anti-inflammatory

Adapted from Xie *et al* (2) and Yu *et al* (58).