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Mitochondria and ferroptosis

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Ferroptosis is a regulated iron-dependent cell death mechanism accompanied by the accumulation of peroxidized phospholipids, particularly phosphatidylethanolamine, in the cell. It occurs due to the disbalance between production and elimination of oxidized phospholipids in response to ferroptotic stimuli. A growing body of recent studies indicates that ferroptosis is involved in the pathogenesis of various human diseases leading to organ/tissue abnormalities. Because of their central role in ATP synthesis, ROS production, iron homeostasis, and redox status, mitochondria have been proposed to mediate ferroptotic signaling pathways. However, precise mechanisms underlying the potential role of mitochondria in ferroptosis remain unrevealed. This review summarizes and discusses previous studies on the contribution of mitochondria to ferroptotic cell death and highlights future directions elucidating the mitochondria as a promising target to prevent cell death through blocking ferroptosis.

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Introduction

Ferroptosis is an iron-dependent nonapoptotic form of regulated cell death. It is triggered by increased levels of oxidized phospholipids (oxPLs) due to activation of non-heme iron-containing lipoxygenases and deficiency of a glutathione peroxidase 4 (GPX4), a selenoprotein and antioxidant enzyme that eliminates oxPLs [1]. Although the term 'ferroptosis' was first invented in 2012, the mechanisms underlying ferroptosis were known earlier. Pioneer studies revealed high sensitivity of human embryonic diploid cells to cysteine deprivation associated with high cell death and depletion of reduced glutathione (GSH) in the cystine-free medium [2]. Likewise, glutamate addition to the culture medium reduced GSH levels leading to oxidative stress and cell death due to inhibition of cystine uptake, in neuroblastoma cells [3]. Notably, key molecules involved in ferroptotic pathways such as lipoxygenase activation, GSH depletion, reactive oxygen species (ROS) accumulation, and changes in Ca^{2+} homeostasis were described in 2001 during oxytosis, programmed cell death induced by oxidative stress, in neuronal cells [4]. Hence, oxytosis and ferroptosis have been recently suggested as two names for the same programmed cell death mechanism [5].

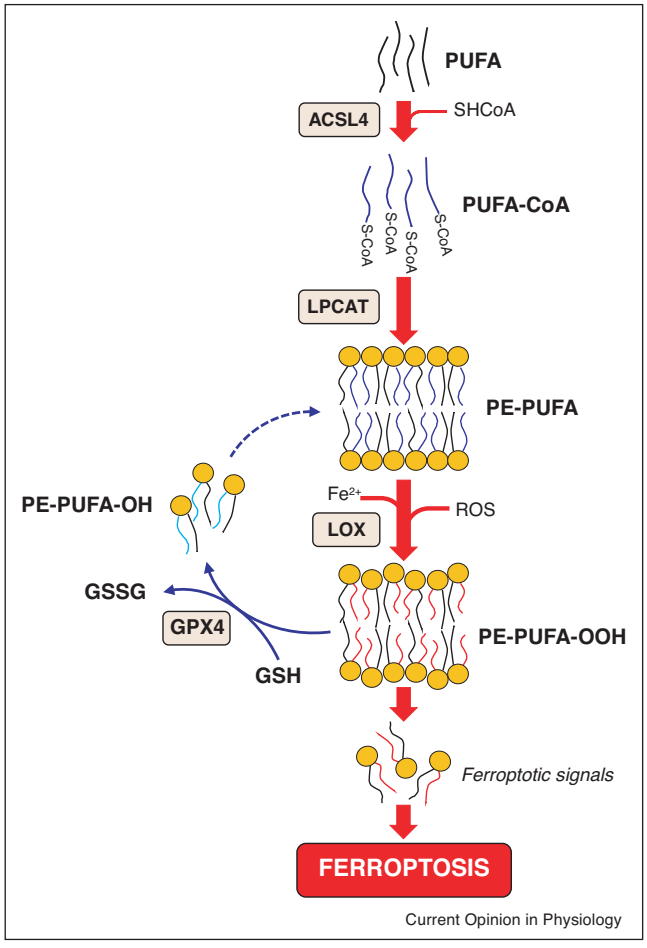
PubMed search for the term 'ferroptosis' revealed roughly 3000 articles, over 75% of which have been published in 2020–2021, highlighting the importance of studies in this field. Ferroptosis was identified in neurodegenerative [6,7] and cardiovascular diseases [8,9••], liver [10] and kidney injury [11], cancer treatment [12], diabetes [13], and sepsis [14]. Ferroptosis is manifested by the specific changes in cell morphology and ferroptotic signaling mediates through modulation of specific pathways. However, the signaling molecules involved in ferroptotic signaling can interact and occur simultaneously with other regulated cell death mechanisms including apoptosis, necroptosis, pyroptosis, and autophagy in response to pathological stimuli.

Mitochondria are involved in the pathogenesis of various human diseases and mediate different cell death mechanisms [15]. Endoplasmic reticulum, lysosomes, and mitochondria were suggested as plausible candidates that can initiate PL oxidation and ferroptotic signaling [16,17,18•]. Recent studies provided strong evidence that mitochondria can produce and mediate ferroptotic signaling. First, LC–MS analysis revealed accumulation of ferroptotic oxPLs in mitochondria of cells exposed to RSL3 [9••]. Second, mitochondria contain the most, if not all, main components of ferroptotic machinery. Third, mitochondria are a major source of ROS in the cell, and mitochondria-targeted ROS scavengers have been shown to protect cells through inhibition of ferroptosis [19–21]. Forth, pro-ferroptotic effects of erastin are mediated through its interaction with VDAC (voltage-dependent anion channel) accompanied by mitochondrial dysfunction [22].

Ferroptosis: main components and signaling pathways

Ferroptosis is manifested by specific signaling pathways and structural features that differ from other programmed cell death mechanisms. Accumulation of oxPLs due to an imbalance between their production and removal is the main process that stimulates ferroptotic signaling (Figure 1). Ferric (Fe^{3+}) and ferrous (Fe^{2+}) ions enhance

Figure 1



The main mechanism of ferroptosis in the cell.
Abbreviations: ACSL4, acyl-CoA synthetase long-chain family member 4; GPX4, glutathione peroxidase 4; GSH, reduced glutathione; GSSG, oxidized glutathione; LOX, lipoxygenases; LPCAT3, lysophosphatidylcholine acyltransferase 3; PE, phosphatidylethanolamine; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SHCoA, coenzyme A.

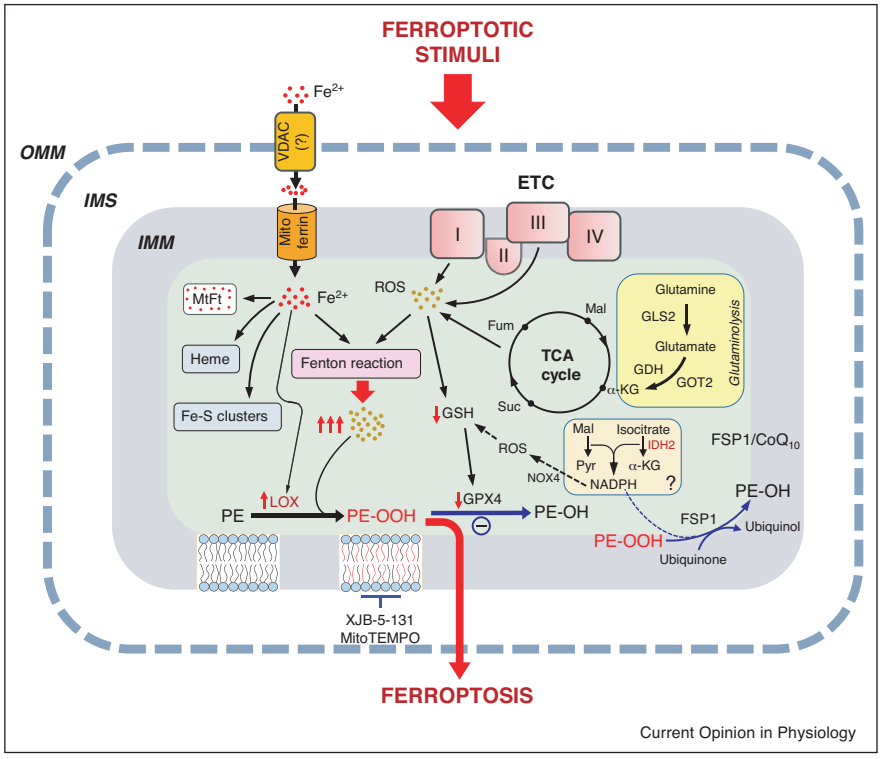
mitochondrial ROS (mtROS) through the Fenton reaction resulting in activation of lipoxygenases, particularly 15-lipoxygenases, which cause oxidation of free polyunsaturated fatty acids esterified into PLs and thus, damage cellular membranes and initiate ferroptotic cell death [1,23,24•,25]. Among thousands of molecular species of oxidizable PLs, only arachidonoyl (AA)-phosphatidylethanolamine (AA-PE) and adrenoyl (AdA)-PE (AdA-PE) were identified as the substrates for 15-lipoxygenase yielding pro-ferroptotic hydroperoxy-PEs (HOO-PE or oxPE) [24•]. Moreover, 15-lipoxygenase forms a complex with a scaffold protein, PE-binding protein 1 (PEBP1), which shifts the substrate preference from free AA to AA-PE and thus, generates the pro-ferroptotic HOO-AA-PE signal [26]. Also, the enzymes of PL biosynthesis, acyl-CoA synthase ligase 4 (ACSL4) and lysophospholipid acyltransferase (LPCAT3) are involved in the formation of oxPEs and ferroptotic signaling [23,24•,26,27]. Most recently, oxPE species were visualized in ferroptotic cardiomyocytes and neurons after traumatic brain injury by the GCIB-SIMS imaging technique [18•]. Under physiological conditions, GPX4 reduces the pro-ferroptotic signals to stable hydroxy-PEs (HO-PEs) at the expense of reduced GSH oxidation and hence, maintenance of GSH levels is essential for GPX4 activity. Inhibition of the cystine/glutamate antiporter responsible for cysteine import (system x_c^- or SLC7A11) by erastin and other inhibitors induces GSH depletion leading to GPX4 inactivation and ferroptosis [25]. Also, RSL3, a broadly accepted ferroptosis inducer, directly inhibits GPX4 activity. It should be noted that system x_c-inhibition or GPX4 depletion can also stimulate other non-ferroptotic cell death mechanisms.

The role of mitochondria in ferroptosis

Mitochondria are the nexus of stress; they are actively involved in different types of regulated cell death mechanisms, including apoptosis, pyroptosis, necroptosis, ferroptosis, and autophagy [28]. Notably, due to their essential role in cell life and cell death, mitochondria can propagate at the same time different death mechanisms and thus, stimulate interaction between death signaling molecules involved in apoptosis, pyroptosis, necroptosis, ferroptosis, and autophagy. Initial studies on Rho0 (mitochondrial DNA depleted) cells found no differences between these cells and their counterparts with undamaged (normal) mitochondria in response to ferroptotic stimuli [1], thus, undermining the role of mitochondria in ferroptosis. The contribution of mitochondria to ferroptosis in mitochondria-depleted HT1080 cells was controversial [17,29••]. However, a large number of studies conducted on different cancer and non-cancer cells demonstrated an important role of mitochondria in the initiation and mediation of ferroptotic signaling in the cell. It should be noted that Rho0 cells are considered not suitable for the evaluation of mitochondrial contribution to cell metabolism because long-term incubation with ethidium bromide for the generation of these cells damages nuclear DNA, thereby, affecting cell metabolism. In addition, various non-cancer and cancer cells, including Rho0 cells, can exhibit different sensitivity to ferroptosis. Most likely, cellular/mitochondrial responses to ferroptosis can vary depending on the cell type, type of ferroptotic insults, and duration and severity of ferroptotic stimuli.

The main mitochondrial pathways engaged in ferroptosis are shown in Figure 2. Mitochondria participate in mtROS production, iron accumulation, metabolism of lipids and amino acids, glutaminolysis, redox status regulation, and the cell's antioxidant capacity. These features

Figure 2



Potential mitochondrial pathways involved in ferroptosis.
Abbreviations: CoQ_{10}, coenzyme Q10; ETC, electron transport chain; FSP1, ferroptosis suppressor protein 1; Fum, fumarate; GDH, glutamate dehydrogenase; GLS2, glutaminase 2; GOT2, glutamic-oxaloacetic transaminase 2; IDH2, isocitrate dehydrogenase 2; IMM, inner mitochondrial membrane; IMS, intermembrane space; α-KG, α-ketoglutarate; Mal, malate; MtFt, mitochondrial ferritin; NOX4, NADPH oxidase 4; OMM, outer mitochondrial membrane; Pyr, pyruvate; Suc, succinate; TCA cycle, tricarboxylic acid cycle; VDAC, voltage-dependent anion channel.

predispose mitochondria to the triggering of ferroptotic pathways. The cardioprotective effects of liproxstatin-1, a lipophilic radical-trapping anti-ferroptotic antioxidant, were associated with the improved structural and functional integrity of mitochondria [30]. Early response of mitochondria to ferroptotic stimuli was observed that demonstrated high levels of oxPE species in cardiomyocytes, and in the heart subjected to ischemia-reperfusion injury [9••], thereby providing direct evidence on the implication of mitochondria in ferroptosis. Ferroptosis induces specific morphological changes in mitochondria that are remarkably different from other types of cell death. Cells exposed to ferroptotic stimuli by erastin or RSL3 contain fragmented, high density, and compact mitochondria, the outer mitochondrial membrane is ruptured, and cristae are mostly lost or disorganized [1,9••,31]. Likewise, *GPX4* silencing increased the number of swollen mitochondria associated with a lamellar architecture and reduced the number of cristae [31]. Ferroptosis affects various aspects of mitochondrial metabolism and quality control mechanisms including mitochondrial biogenesis, dynamics, and mitophagy [32–34].

Mitochondrial redox status and ferroptosis

Mitochondria play a central role in the cellular redox status and are involved in redox-sensitive processes associated with cell survival and death mechanisms. They produce a major part of cellular ROS by ETC complexes, α-ketoglutarate dehydrogenase, NADPH oxidase 4 (NOX4), monoamine oxidase, among others [35]. Several lines of evidence support the role of mtROS in ferroptosis. First, erastin [36] and RSL3 [20] substantially increased mtROS in MEF and HT-22 cells. Second, mitochondria-targeted ROS scavengers such as MitoQ [20], XJB-5-131 [19], and Mito-TEMPO [21] attenuated ferroptosis. Furthermore, cells overexpressed mitochondrial GPX4 were more effective than non-mitochondrial GPX4 against oxidative stress and prevented mitochondrial dysfunctions and cell death by reducing hydroperoxides [37•]. Likewise, breast tumor cells overexpressing mitochondrial GPX4 were highly resistant to cell death induced by cholesterol hydroperoxides [38].

One of the central regulators of cellular redox status is GSH, an essential co-factor for GPX4. GSH exists mainly in the reduced form under physiological conditions; its

concentration is 10–100-folds higher than the oxidized form (GSSG) [39]. The GSH:GSSG ratio is the primary determinant of the cellular and mitochondrial redox state. GSH is synthesized from its constituent amino acids (cysteine, glycine, and glutamate) exclusively in the cytosol, but it also maintains the redox status in the endoplasmic reticulum, nucleus, and mitochondria. Although mitochondria contain 10–15% of total cellular GSH, its concentration in mitochondria is similar to the cytosol [40]. Since the inner mitochondrial membrane (IMM) is impermeable to GSH, which possesses a net negative charge at neutral pH, a specific transport mechanism(s) is required for the transport of GSH across the membrane [41].

Studies in isolated kidney mitochondria demonstrated that over 80% of GSH can be transported through the IMM by the dicarboxylate carrier (DIC, *Slc25a10*) and the oxoglutarate carrier (OGC, *Slc25a11*) [42]. However, other studies questioned the role of these carriers in mitochondrial GSH transport [43]. In mammalian cells, the mitochondrial carrier proteins represent a large *SLC25* family (over 60 proteins) of nuclear-encoded transporters, and only about half of them have been functionally characterized [44]. Only eight carrier proteins are anion carriers that may participate in GSH transport across the IMM [45]. DIC and OGC are two of eight known anion carriers that transport dicarboxylates (malonate, malate, and succinate) across the IMM and are essential for maintaining mitochondrial bioenergetics particularly the TCA cycle. Pharmacological inhibition of DIC and OGC further increased RSL3-induced cell death and reduced mitochondrial GSH in H9c2 cardiomyocytes [9••]. Notably, inhibition of DIC and OGC could diminish mitochondrial GSH levels independently of its transport and be associated with impaired transport of intermediates (dicarboxylates) that are required for the TCA cycle. Thus, the mitochondrial GSH transport mechanisms remain elusive, and the role of DIC and OGC in GSH transport is still controversial; multiple low-affinity IMM carriers that typically transport alternative substrates may be involved in GSH transport in mitochondria.

Mitochondrial iron metabolism and ferroptosis

Cellular iron is mostly sequestered and stored in the cytoplasm by ferritin, a major iron-storage protein. A certain fraction of free iron (Fe^{2+}) is transported across the IMM to the matrix of mitochondria by mitoferrin 1 and mitoferrin 2 [46], although the precise mechanism of the mitochondrial iron transport remains elusive. In the matrix of mitochondria, iron is primarily utilized for biosynthesis of heme and iron–sulfur clusters, the essential co-factors of iron-containing proteins involved in electron transfer through enzymatic redox reactions in mitochondria. In the matrix, the excess of mitochondrial free iron is sequestered in mitochondrial ferritin [47].

Iron overload in rat cardiomyocytes *in vitro* induced mtDNA damage associated with suppressed expression of mitochondrial-encoded ETC subunits and diminished mitochondrial respiration [48]. Likewise, increased free iron levels due to heme degradation during doxorubicin-induced cardiomyopathy and ischemia-reperfusion were associated with enhanced ferroptosis in cardiomyocytes [21]. Interestingly, accumulation of free iron was observed in mitochondria (not in the cytoplasm) that demonstrated lipid peroxidation in mitochondrial membranes. The specific mitochondria-targeted antioxidant Mito-TEMPO, but not TEMPO (a non-specific antioxidant), attenuated lipid peroxidation and ferroptosis. This study suggests that mitochondrial iron accumulation and lipid peroxidation can be used as a target to reduce doxorubicin-induced and IR-induced cardiac dysfunction [21].

Free iron overload in mitochondria could enhance mtROS levels through Fenton reaction and activate mitochondrial NOX4 and 15-lipoxygenase leading to accumulation of oxPLs and ferroptosis. Overexpression of mitochondrial ferritin in neuroblastoma SH-SY5Y cells significantly reduced erastin-induced ferroptosis [49••], most likely, due to improved regulation of mitochondrial iron homeostasis. Genetic inhibition of NFS1 and ABCB7 that regulate biosynthesis and transport of iron–sulfur clusters in mitochondria increased free iron levels and promoted ferroptosis [50]. Downregulation of the CDGSH iron sulfur domain 1 (CISD1) increased iron-mediated lipid peroxidation in mitochondria. In contrast, stabilization of the iron–sulfur cluster of CISD1 inhibited mitochondrial iron uptake and lipid peroxidation and protected against erastin-induced ferroptosis [51]. Altogether, these studies highlight the importance of mitochondrial iron in PL oxidation and ferroptotic signaling.

Mitochondrial bioenergetics and ferroptosis

Mitochondrial glutaminolysis in interaction with the TCA-ETC pathway can potentially participate in ferroptosis through several mechanisms, although the contribution of each mechanism can vary in cancer and non-cancer cells. First, mitochondrial glutaminolysis, a major anaplerosis source, fuels the TCA cycle through the α-ketoglutarate dehydrogenase [44] and plays a critical role in ferroptosis. Glutamine is involved in the biosynthesis of other essential metabolites in the cell; it is crucial for energy metabolism in cancer cells that possess increased metabolic demand and consume high concentrations of glutamine to promote cell growth and proliferation [52,53]. Therefore, cancer cells are more sensitive to the deficiency of glutamine, and thus, to ferroptosis. Glutaminase (GLS) catabolizes glutamine to glutamate, whereas glutamic-oxaloacetic transaminase is responsible

for the conversion of glutamate into α-ketoglutarate. Genetic silencing and pharmacological inhibition of glutaminolysis through the mitochondrial glutaminase 2 (GLS2) and glutamic-oxaloacetic transaminase inhibited erastin-induced ferroptosis [8,54].

Notably, ferroptosis induced by cysteine-deprivation was affected α-ketoglutarate and other intermediates of the TCA cycle such as succinate, malate, and fumarate. Stimulation of ferroptotic cell death in the presence of the TCA cycle intermediates can be explained by enhanced mtROS production by the TCA cycle enzymes (e.g. α-ketoglutarate dehydrogenase) and ETC complexes. These studies demonstrated that inhibitors of ETC complexes were protective against cysteine-deprivation and erastin-induced ferroptosis and significantly reduced cell death and lipid peroxidation [29••]. However, inhibition of ETC complexes and OXPHOS further promoted RSL3-induced ferroptosis in H9c2 cardiomyocytes [9••]. The reason for this discrepancy is not clear but can be related to the differences in cell type, ferroptosis inducers, and exposure time.

Recent studies renamed apoptosis-inducing factor mitochondria-associated 2 (AIFM2), which is known as a mitochondrial apoptotic protein, ferroptosis suppressor protein 1 (FSP1) due to its capability to protect against ferroptosis [55,56]. In response to ferroptotic stimuli, FSP1 translocates from mitochondria to the plasma membrane where it acts as a CoQ oxidoreductase. As a result, it regenerates CoQ_{10} (reduces ubiquinone to ubiquinol) using NADPH, and traps lipid peroxyl radicals that mediate peroxidation of PLs. Importantly, FSP1 eliminates oxPLs and confers protection against ferroptosis independently of the GSH/GPX4 activity [55,56] although the mechanisms underlying the anti-ferroptotic role of FSP1 remain to be elucidated.

Conclusions and future remarks

The role of mitochondria and precise mechanisms of mitochondria-mediated pathways in ferroptosis remain unclear. Mitochondria contain the main ingredients (enzymes, metabolites, and solutes) required for ferroptosis, and most recent studies demonstrated an early response of mitochondria to ferroptotic stimuli and accumulation of ferroptotic oxPL species in mitochondria. The contribution of mitochondria to ferroptosis can be different in cancer and non-cancer cells. Moreover, ferroptotic signals in mitochondria may be occurred independent of the cytoplasmic ferroptotic machinery or may play a causative role in the activation of ferroptotic pathways in the cytoplasm. Also, alterations in mitochondrial metabolism can predispose the cytoplasm and other subcellular organelles to execution of cell death through ferroptosis. Notably, mitochondrial protein expression profiles may be affected differently by ferroptosis and non-ferroptotic cell death mechanisms. The discovery of mitochondria-mediated ferroptotic signaling pathways opens a new avenue for developing new mitochondria-targeted therapeutic strategies. These strategies can include mitochondria-mediated ferroptotic (in cancer) and anti-ferroptotic (in non-cancer diseases) therapies.

Author contribution

Javadov wrote the text, prepared the figures, and approved the final version of the manuscript.

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Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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