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The molecular and metabolic landscape of iron and ferroptosis in cardiovascular disease

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Abstract | The maintenance of iron homeostasis is essential for proper cardiac function. A growing body of evidence suggests that iron imbalance is the common denominator in many subtypes of cardiovascular disease. In the past 10 years, ferroptosis, an iron-dependent form of regulated cell death, has become increasingly recognized as an important process that mediates the pathogenesis and progression of numerous cardiovascular diseases, including atherosclerosis, drug-induced heart failure, myocardial ischaemia–reperfusion injury, sepsis-induced cardiomyopathy, arrhythmia and diabetic cardiomyopathy. Therefore, a thorough understanding of the mechanisms involved in the regulation of iron metabolism and ferroptosis in cardiomyocytes might lead to improvements in disease management. In this Review, we summarize the relationship between the metabolic and molecular pathways of iron signalling and ferroptosis in the context of cardiovascular disease. We also discuss the potential targets of ferroptosis in the treatment of cardiovascular disease and describe the current limitations and future directions of these novel treatment targets.

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Various forms of regulated cell death, such as apoptosis, necroptosis, pyroptosis and autophagy, have been implicated in the pathogenesis of cardiovascular diseases[1]. In the past 10 years, a growing number of studies support the notion that ferroptosis — an iron-dependent form of non-apoptotic cell death that involves the accumulation of lipid hydroperoxides — has a pathophysiological role in the development of cardiovascular diseases, including doxorubicin-induced cardiomyopathy, myocardial ischaemia–reperfusion injury, myocardial infarction and heart failure[1,2].

As an essential trace element that is present in nearly all forms of life, iron is involved in many biological processes, including energy metabolism and nucleotide synthesis and repair[3]. In humans, iron deficiency is the most prevalent malnutrition-related condition and affects up to 75% of patients with heart failure[4]. Conversely, both primary and secondary forms of iron overload can cause heart disease via oxidative damage, but the exact mechanisms underlying this process are not clear[5]. Excess iron in cardiomyocytes has been shown to directly induce ferroptosis via the accumulation of phospholipid hydroperoxides in the cell membrane[6].

In addition to altered iron homeostasis, excessive production of reactive oxygen species (ROS) or reactive nitrogen species can also directly induce ferroptosis in cardiomyocytes by catalysing the oxidation of phospholipids in the cell membrane. Indeed, oxidative stress has been linked to the development of cardiovascular disease, and targeting the molecular and metabolic pathways that regulate cellular defence against oxidative stress — particularly the glutathione-dependent antioxidant system — has been shown to prevent cardiomyopathy in animal models[7,8].

The evidence to date suggests that the development of many forms of cardiovascular disease is driven by ferroptosis. For example, high levels of ferroptosis mediated by distinct signalling and metabolic pathways can contribute to ischaemic heart disease, cardiac injury, heart failure and cardiomyopathy. In this Review, we summarize the mechanisms involved in the regulation of iron homeostasis, glutathione synthesis and lipid metabolism in cardiomyocytes; we discuss newly identified putative targets of ferroptosis in heart disease; and we provide critical perspectives on the potential of new clinical therapies that target ferroptosis in the heart.

Discovery of ferroptosis
Although ferroptosis was first reported in 2012 as a novel form of cell death that could be inhibited by the iron-chelating agent deferoxamine[2], various forms of cell death involving iron and oxidative stress had already been known for decades[9]. The concept of ferroptosis might have been derived from our knowledge

of cysteine depletion-induced cancer cell death and glutamate-induced cytotoxicity[10,11]. Interestingly, oxytosis was first reported in neurons as far back as 2001, and is characterized by oxidative stress-induced non-apoptotic and non-excitotoxic pathways that promote glutamate-induced cell death, although the underlying mechanism remains largely unknown[12]. Since its initial identification, ferroptosis has been defined as an iron-dependent form of regulated cell death that involves the iron-catalysed accumulation of lethal lipid peroxides[13].

Molecular and metabolic drivers of ferroptosis

Cells that undergo ferroptosis have genetic, biochemical, morphological and metabolic features that are distinct from those of other known forms of cell death, including apoptosis, necroptosis and pyroptosis[14]. Interestingly, unlike other forms of cell death identified to date, ferroptosis can propagate rapidly through cell populations in a wave-like manner[15,16]. In terms of morphological changes, cells undergoing ferroptosis have mitochondrial abnormalities that can be visualized using electron microscopy, including swelling, changes in density and rupture of the outer membrane[2,17,18]. Finally, the pathways involved in iron, glutathione and lipid metabolism converge to control the initiation and execution of ferroptosis, particularly in cardiomyocytes (FIG. 1). The roles of these metabolic pathways in mediating ferroptosis and cardiovascular disease are discussed below.

Iron metabolism and ferroptosis in the heart

Regulation of iron homeostasis in the cardiovascular system. The cellular uptake of iron is mediated by the binding of iron-bound transferrin (which contains two ferric iron molecules) to its receptor (transferrin receptor protein 1 (TFR1)), which triggers clathrin-dependent endocytosis of the entire holo-complex[19]. The endosome is then acidified by vacuolar ATPase, leading to the reduction of ferric iron to ferrous iron by the STEAP (six-transmembrane epithelial antigen of prostate) family of metalloreductases[19]. Ferrous iron is then released from the endosome into the cytoplasm via natural resistance-associated macrophage protein 2 (NRAMP2; also known as DMT1), and apo-transferrin and TFR1 are shuttled back to the cell surface to be reused by the cell[20]. Mice lacking *Tfr1* in the heart have severe cardiomyopathy with cardiac iron deficiency and

die in the second week of life[21]. Non-transferrin-bound iron was thought to be transported into cardiomyocytes by voltage-dependent calcium channels[20,21], but a study published in 2021 raised doubts about the role of calcium channels in mediating the cardiac uptake of non-transferrin-bound iron[22].

In the cytoplasm, ferrous iron is oxidized to its ferric state by cytoplasmic ferritin, and the resulting ferritin-bound iron can be either degraded for use in enzymatic reactions or stored for later use[23]. Iron-saturated ferritin is degraded by nuclear receptor coactivator 4 (NCOA4)-mediated autophagy — a process known as ferritinophagy[24] — which leads to the degradation of lysosomal ferritin, with subsequent release of its iron content[25] and its export to the cytosol via lysosomal NRAMP2 (REF.[26]). Cardiac-specific deletion of *Fth1* (encoding ferritin heavy chain) leads to iron dysregulation and increased oxidative stress in the heart, resulting in increased susceptibility to iron overload-induced tissue injury[27]. Conversely, deletion of *Ncoa4* in mouse hearts improved cardiac function and attenuated ferritinophagy-mediated ferritin degradation that was induced by pressure overload[28].

Ferroportin is the only known iron exporter in vertebrate cells, and cardiac iron overload, impaired heart function and a shortened lifespan have been observed in mice with early cardiomyocyte-specific deletion of the gene encoding ferroportin (*Fpn*)[29,30]. Hepcidin, a peptide hormone primarily synthesized in the liver, inhibits ferroportin via E3 ubiquitin protein ligase RNF217-mediated ubiquitination in the gut and spleen, which regulates iron absorption and iron recycling, respectively[31,32]. Genetic hepcidin deficiency causes the most severe form of systemic iron overload in both mice and humans[33,34]. However, loss of hepcidin specifically in cardiomyocytes results in a fatal cardiomyopathy as a consequence of cardiac iron deficiency, highlighting a cell-autonomous role of the hepcidin–ferroportin axis in cardiac iron homeostasis[35] (FIG. 2a).

Cellular iron levels are regulated at the post-transcriptional level by iron regulatory protein 1 (IRP1) and IRP2 (REFS.[36,37]). These cytoplasmic proteins bind to the 3′-untranslated region (UTR) of target transcripts such as *TFR1* mRNA, stabilizing the transcripts and increasing translation. By contrast, binding of IRP1 or IRP2 to the 5′-UTR of target mRNAs such as *FPN* and *FTH1* blocks ribosomal entry and prevents their translation[36,37]. Holo-IRP1 attached to iron–sulfur (Fe–S) clusters functions as a cytosolic aconitase and cannot bind to mRNA molecules. However, when cellular iron levels decrease, the Fe–S clusters dissociate from IRP1, which can then bind to the ribosome entry sites. IRP2 is constitutively active; however, when cellular iron levels are sufficient, IRP2 is ubiquitinated and degraded via a process that requires F-box/LRR-repeat protein 5 (REFS.[36,37]). Therefore, when iron levels are sufficient, IRP1 contains an Fe–S cluster and IRP2 is degraded, thereby inhibiting the IRP system.

When the IRP system fails to maintain adequate cellular iron levels during severe iron deficiency, another iron regulatory pathway can be activated. This pathway is known as the 'iron conservation pathway' and is

distinct from the 'iron acquisition pathway' mediated by the IRP system[38]. During a state of iron deficiency, levels of the mRNA-binding protein tristetraprolin increase, paradoxically reducing TFR1 levels to conserve cellular iron. Tristetraprolin degrades its target mRNAs by binding to the CNOT1 (CCR4–NOT transcription complex subunit 1) deadenylase complex[39]. The induction of tristetraprolin during iron deficiency is crucial for cell survival, because tristetraprolin-deficient mice develop cardiac dysfunction in response to iron deficiency[40], and depletion of tristetraprolin in vitro causes cell death

under low iron conditions[38]. In the heart, tristetraprolin binds to and causes the degradation of mRNA transcripts that encode proteins involved in several processes, including Fe–S cluster-containing proteins in the mitochondrial electron transport chain. Consequently, under iron-deficient conditions, excess numbers of apo-proteins that would otherwise bind to iron are not synthesized, allowing the limited amount of iron to be used by essential proteins[40].

Iron metabolism in ferroptosis. Iron availability is a crucial factor in driving ferroptosis. Mice fed a high-iron diet, as well as haemojuvelin-deficient mice and SMAD family member 4-deficient mice, have severe iron overload and increased hepatic ferroptosis[41]. By contrast, mice lacking *Hfe* (encoding hereditary haemochromatosis protein homologue) have only moderate iron overload without hepatic ferroptosis[41]. Of note, the accumulated cellular iron — particularly labile ferrous iron — can react directly with cellular oxidants to produce cytotoxic hydroxyl radicals via the Fenton reaction, which in turn promotes ferroptosis. By contrast, the binding of free ionic iron to proteins such as transferrin or ferritin can have a protective effect against the Fenton reaction and ferroptosis[27,42]. In addition, peroxidation of polyunsaturated fatty acids (PUFAs) by lipoxygenases via the phosphorylase kinase G2-dependent iron pool is required for initiating ferroptosis[43]. Therefore, numerous proteins involved in regulating cellular iron homeostasis can affect the sensitivity of the cell to ferroptosis[44] (TABLE 1).

Iron uptake via TFR1, solute carrier family 39 member 14 (SLC39A14; also known as metal cation symporter ZIP14) and/or NRAMP2 can dictate cellular sensitivity to ferroptosis[42,45–47]. Cytosolic ferritin confers resistance to ferroptosis by controlling iron availability[27,48,49]; therefore, selective autophagy of ferritin via NCOA4 can increase the susceptibility of the cell to ferroptosis[50,51]. The iron chaperone poly(rC)-binding protein 1 (PCBP1) binds and delivers Fe^{2+} to ferritin[52]. Moreover, *Pcbp1*-knockout mouse hepatocytes have increased levels of labile iron and lipid peroxidation, suggesting that PCBP1 might have a key role in preventing ferroptosis-related disease[52]. By contrast, knocking down the expression of iron or ferritin exporters such as ferroportin and prominin 2 has been shown to promote ferroptosis[53,54]. Caeruloplasmin helps ferroportin to leave cells via its ferroxidase activity, and loss of ceruloplasmin promotes both erastin-induced and transcription factor RSL3-induced ferroptosis, whereas overexpression of caeruloplasmin suppresses ferroptosis in tumour cells[55,56].

The maintenance of mitochondrial iron homeostasis also has an important role in preventing ferroptosis. Mitoferrin 1 (also known as SLC25A37) and mitoferrin 2 (also known as SLC25A28) are key mitochondrial iron importers involved in haem and Fe–S biogenesis[57]. Deletion of mitoferrin 2 reduces erastin-induced cell death, whereas overexpression of mitoferrin 2 increases ferroptosis[58]. Activation of haem oxygenase 1 (HO1), a mitochondrial enzyme that catalyses the degradation of haem to produce ferrous iron, causes mitochondrial iron overload and increases ferroptosis both in vitro and in vivo[6,59,60]. However, mild upregulation of HO1 might
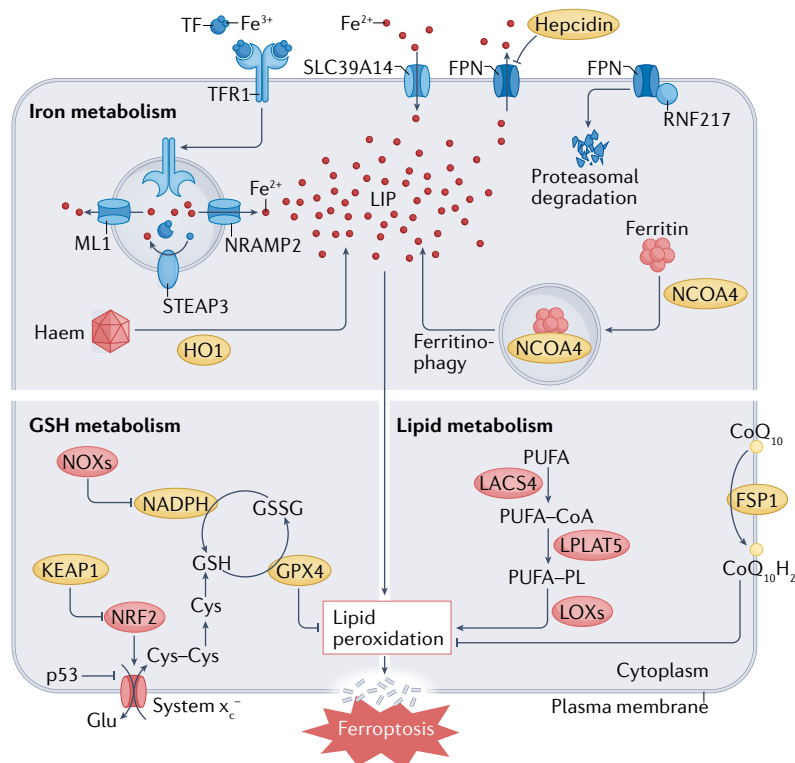
Fig. 1 | **Metabolic pathways implicated in ferroptosis.** At the cellular level, ferroptosis is driven primarily by iron-dependent lipid peroxidation. Many aspects of iron metabolism such as the absorption, storage and utilization of iron have important roles in regulating ferroptosis. In addition, the activation of long-chain fatty-acid CoA ligase 4 (LACS4), lysophospholipid acyltransferase 5 (LPLAT5), lipoxygenase (LOX) or NADPH oxidase (NOX) in the lipid metabolic pathway promotes lipid peroxidation and ferroptosis. The canonical ferroptosis-suppressing pathway involves the uptake of cystine (Cys) via the cystine–glutamate antiporter (system x_c^-), which results in glutathione (GSH) biosynthesis. Using GSH as a cofactor, the glutathione peroxidase 4 (GPX4) reduces phospholipid hydroperoxides to their corresponding alcohols. The peroxidation of phospholipids is also kept in check by the ferroptosis suppressor protein 1 (FSP1)–coenzyme Q_{10} (CoQ_{10}) system. Ferroptosis is also regulated by the iron metabolism pathway that involves iron absorption, transport, storage and utilization. At the cellular level, non-haem iron is transported into cells by either transferrin receptor protein 1 (TFR1)-mediated, transferrin (TF)-bound iron uptake or metal transporter solute carrier family 39 member 14 (SLC39A14; also known as metal cation symporter ZIP14)-mediated, non-TF-bound iron uptake. In addition, haem degradation and nuclear receptor coactivator 4 (NCOA4)-mediated ferritinophagy can increase the labile iron pool (LIP), thereby sensitizing cells to ferroptosis via the Fenton reaction. FPN, ferroportin; Glu, glutamate; GSSG, glutathione disulfide; HO1, haem oxygenase 1; KEAP1, Kelch-like ECH-associated protein 1; ML1, mucolipin 1; NRAMP2, natural resistance-associated macrophage protein 2; NRF2, nuclear factor-erythroid 2-related factor 2; PUFA, polyunsaturated fatty acid; PUFA–CoA, coenzyme A-activated polyunsaturated fatty acid; PUFA–PL, polyunsaturated fatty acid-containing phospholipid; RNF217, E3 ubiquitin protein ligase RNF217; STEAP3, metalloreductase STEAP3.

actually be cytoprotective[61]. Like cytosolic ferritin, mitochondrial ferritin has a protective role against ferroptosis. For example, *Drosophila* and cells overexpressing mitochondrial ferritin are resistant to erastin-induced ferroptosis[62].

Several Fe–S proteins have a role in lipid peroxidation during ferroptosis. For example, suppressing NFS1 (cysteine desulfurase, mitochondrial), which uses sulfur from cysteine to synthesize Fe–S clusters, sensitizes cancer cells to ferroptosis[63]. In addition, the Fe–S-binding proteins mitoNEET (also known as CISD1) and NAF1 (also known as CISD2) have been shown to participate in mitochondrial iron transportation, increasing the tolerance of cancer cells to ROS-induced cell death[61,62]. Increased expression of mitoNEET prevented erastin-induced ferroptosis in human hepatocellular carcinoma cells[64], and NAF1 overexpression similarly conferred resistance to sulfasalazine-induced ferroptosis in a mouse tumour xenograft model[65].

Iron overload-associated cardiac disorders. Iron overload occurs when the body stores excess iron. The heart is particularly susceptible to damage induced by accumulation of iron[66]. Two general types of iron overload have been described. Primary iron overload is caused by genetic disorders that cause dysregulated absorption of dietary iron, whereas secondary iron overload develops as a result of repeated blood transfusions, drug-induced toxicity or excess consumption of iron[67]. However, given the lack of robust biomarkers for measuring ferroptosis in patients, the link between ferroptosis and these diseases remains poorly understood.

Hereditary haemochromatosis is one of the most common inherited diseases among white populations[68]. A wide range of mutations affecting either the production or function of the iron-regulating peptide hepcidin can cause various degrees of iron overload in a number of organs, including the heart. Therefore, heart failure is a common complication among patients with hereditary haemochromatosis and is more prevalent in those with juvenile forms of the disease[69]. Carriers of the C282Y mutation in *HFE* (encoding hereditary haemochromatosis protein) have a higher risk of acute myocardial infarction and cardiovascular death than non-carriers[70,71]. Similarly, impaired endothelial function and increased intima–media thickness have been associated with altered iron status in patients with hereditary haemochromatosis; iron depletion therapy can therefore reduce the risk of cardiovascular events in these patients[72].

Friedreich ataxia is an autosomal recessive neurodegenerative disease that affects both the nervous system and non-neural tissues, including the heart and pancreas[73]. Friedreich ataxia is caused by a homozygous expansion of the GAA triplet repeat in the first intron of the *FXN* gene, which encodes the protein frataxin (also known as mitochondrial frataxin)[74]. Frataxin is essential for mitochondrial function owing to its role in the biogenesis of Fe–S clusters and antioxidant defence[75]. Frataxin deficiency causes mitochondrial iron accumulation, excess production of ROS and increased lipid peroxidation, leading to the development of Friedreich ataxia[76]. In addition to the typical neurological symptoms, the clinical manifestations of Friedreich ataxia include severe cardiomyopathy, which is the most common cause of death among these patients[77,78]. The severe neurological and cardiac symptoms are to be expected, given that the heart and the brain contain a large number of mitochondria. Interestingly, mice with a cardiomyocyte-specific *Fxn* deletion have increased mitochondrial iron in the heart owing to
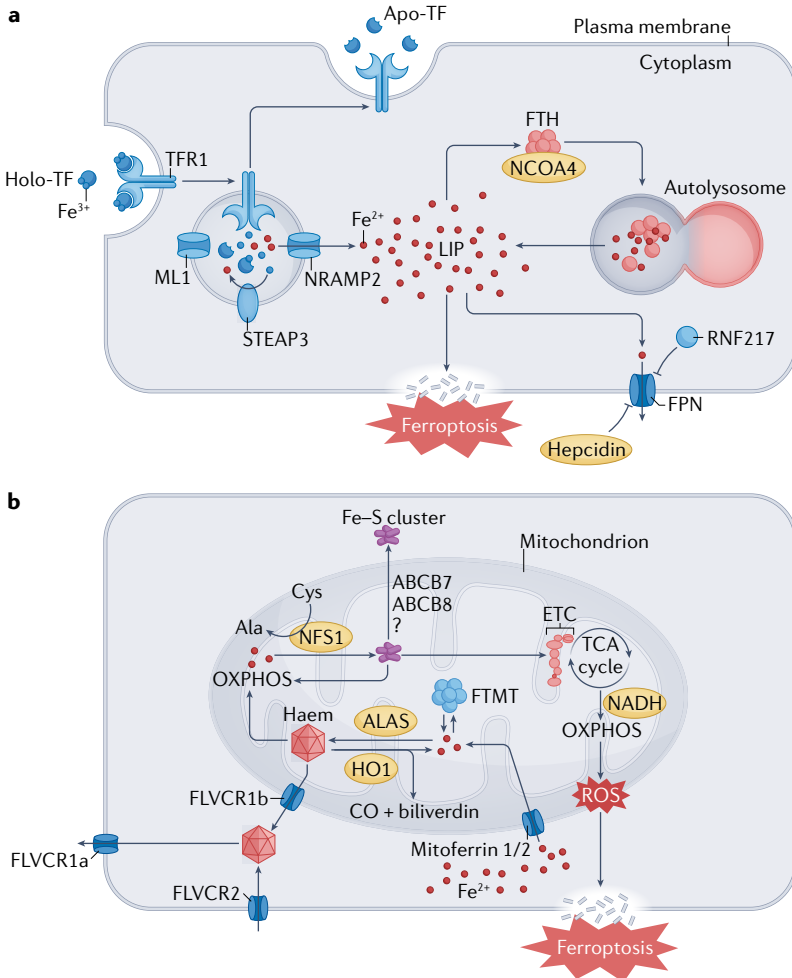
Fig. 2 | **The metabolism of cardiac iron and haem regulates ferroptosis. a** | Iron uptake in cardiomyocytes is dependent on the endocytosis of diferric transferrin (TF) bound to its receptor transferrin receptor protein 1 (TFR1). To maintain the levels of iron in the cytoplasm, iron can be released from TF in endolysosomes and exported to the cytoplasm by natural resistance-associated macrophage protein 2 (NRAMP2) after a metalloreductase STEAP3-mediated reduction. Excess iron is either bound to ferritin heavy chain (FTH) or exported by ferroportin (FPN), the only iron exporter. In addition, iron can be released from FTH via nuclear receptor coactivator 4 (NCOA4)-mediated autophagic degradation of ferritin, a process known as ferritinophagy. **b** | Mitoferrin 1 (also known as SLC25A37) and mitoferrin 2 (also known as SLC25A28) mediate the transport of iron across the mitochondrial membrane. Iron is primarily used to synthesize iron–sulfur (Fe–S) clusters and haem in the mitochondria. Excess iron can be stored in the mitochondria-specific form of ferritin (FTMT). FLVCR1B (feline leukaemia virus subgroup C receptor-related protein 1B) promotes haem efflux into the cytoplasm, whereas the export of Fe–S clusters into the cytoplasm might require iron–sulfur clusters transporter ABCB7, mitochondrial (ABCB7) and ABCB8 (also known as mitochondrial potassium channel ATP-binding subunit). Ala, 5-aminolevulinate; ALAS, aminolevulinic acid synthase; Apo-TF, apo-transferrin; CO, carbon monoxide; Cys, cysteine; ETC, electron transport chain; HO1, haem oxygenase 1; Holo-TF, holo-transferrin; LIP, labile iron pool; ML1, mucolipin 1; NFS1, cysteine desulfurase, mitochondrial; OXPHOS, oxidative phosphorylation; RNF217, E3 ubiquitin protein ligase RNF217; ROS, reactive oxygen species; TCA, tricarboxylic acid.

Table 1 | **Principal modulators of iron metabolism that are involved in ferroptosis**

Gene	Protein	Function	Effects of genetic deletion or overexpression	Refs.
ACO1	Cytoplasmic aconitate hydratase	Iron–sulfur protein that converts citrate to isocitrate	Deletion suppresses cystine starvation-induced ferroptosis	[51]
CISD1	CDGSH iron–sulfur domain-containing protein 1 (also known as mitoNEET)	Regulates mitochondrial iron uptake	Deletion promotes erastin-induced ferroptosis	[64]
CISD2	CDGSH iron–sulfur domain-containing protein 2 (also known as NAF1)	Regulates mitochondrial iron uptake	Deletion promotes sulfasalazine-induced ferroptosis	[65]
CP	Ceruloplasmin	Converts Fe^{2+} to Fe^{3+}	Infusion prevents ferroptotic damage after ischaemic stroke and deletion promotes erastin-induced or transcription factor RSL3-induced ferroptosis	[55,56]
SLC11A2	Natural resistance-associated macrophage protein 2	Iron importer	Deletion suppresses hypoxia–reoxygenation-induced ferroptosis	[47]
FTH1	Ferritin heavy chain	Iron storage	Deletion promotes iron-induced cardiac ferroptosis, increases ferroptosis in *Drosophila* and promotes erastin-induced ferroptosis	[27,48,49]
FTMT	Mitochondrial ferritin	Mitochondrial iron storage	Overexpression suppresses erastin-induced ferroptosis	[62]
HMOX1	Haem oxygenase 1	Degrades haem into biliverdin, carbon monoxide and Fe^{2+}	Inhibition suppresses doxorubicin-induced cardiac ferroptosis and erastin-induced ferroptosis, whereas deletion suppresses ferroptosis induced by BAY 11-7085 (an inhibitor of nuclear factor-κB) or erastin	[6,56–58]
IREB2	Iron-responsive element-binding protein 2	Regulates the translation and stability of iron-related microRNAs	Deletion suppresses erastin-induced ferroptosis and cystine starvation-induced ferroptosis	[2,49]
NCOA4	Nuclear receptor coactivator 4	Regulates ferritinophagy	Deletion suppresses erastin-induced ferroptosis and cystine starvation-induced ferroptosis	[49,50]
NFS1	Cysteine desulfurase, mitochondrial	Iron–sulfur cluster biosynthetic enzyme	Deletion promotes ferroptosis	[63]
PCBP1	Poly(rC)-binding protein 1	Iron chaperone	Deletion increases hepatic ferroptosis	[52]
PHKG2	Phosphorylase b kinase γ-catalytic chain, liver/testis isoform	Regulates iron availability to lipoxygenases	Deletion suppresses erastin-induced ferroptosis	[43]
PROM2	Prominin 2	Regulates ferritin export	Deletion promotes ferroptosis	[53]
SFXN1	Sideroflexin 1	Regulates mitochondrial iron uptake	Deletion suppresses lipopolysaccharide-induced ferroptosis	[208]
SLC25A28	Mitoferrin 2	Regulates mitochondrial iron uptake	Deletion suppresses erastin-induced ferroptosis	[58]
SLC39A14	Solute carrier family 39 member 14 (also known as metal cation symporter ZIP14)	Iron importer	Deletion suppresses iron-induced hepatic ferroptosis	[42]
SLC40A1	Solute carrier family 40 member 1	Iron exporter	Deletion promotes erastin-induced ferroptosis	[54]
TF	Serotransferrin	Iron carrier	Deletion promotes hepatic ferroptosis	[42]
TFR1	Transferrin receptor protein 1	Iron importer	Deletion suppresses erastin-induced ferroptosis and amino acid deprivation-induced ferroptosis	[46]

pronounced changes in *Tfr1* expression and alterations in iron transport from the cytosol to the mitochondria[79]. Co-treating patients with Friedreich ataxia with both the iron chelator deferiprone and the coenzyme Q_{10} analogue idebenone results in the attenuation of cardiac hypertrophy[80]. Other studies have also indicated that treatment with deferiprone can improve heart function, but not the neurological symptoms[81,82]. Nevertheless, the clinical benefits of iron chelation are currently unclear and long-term, large-scale trials are warranted.

Ineffective erythropoiesis and transfusion-induced iron overload. Iron overload is a common complication among patients with thalassaemia, a group of hereditary disorders caused by impaired haemoglobin synthesis[83]. Ineffective erythropoiesis, in which an increase in the production of erythroid cells is not matched by a corresponding increase in mature red blood cells, is a major cause of iron overload in organs[84]. In addition, repeated blood transfusions, a common treatment for thalassaemia, is another source of excess iron that can lead to iron overload[85].

Iron overload can also lead to cardiomyopathy, the primary cause of morbidity and mortality in patients with thalassaemia[86–88]. Although the clinical presentation of thalassaemia-associated cardiomyopathy

is both variable and complex, the majority of patients present with left-sided heart failure and reduced ejection fraction[89]. Another major symptom is cardiac arrhythmia, which can directly lead to sudden cardiac death[89,90]. To manage the deleterious effects of iron overload, chelation therapy was first used to treat patients with iron overload-related, thalassaemia-associated cardiomyopathy in the 1970s; today, iron chelation is globally accepted as the most effective treatment for this condition[91,92].

Ineffective erythropoiesis and transfusion-induced iron overload can also be caused by other inherited blood disorders such as sickle cell disease (discussed below), myelodysplastic syndromes, pure red cell aplasia and leukaemia. The management of iron overload-induced cardiac conditions can be improved by monitoring tissue iron levels more precisely, developing new iron chelators and modulating hepcidin levels to reduce iron loading and cardiotoxicity[93,94].

Anthracycline-induced cardiotoxicity. Since the late 1960s, anthracyclines — a class of drugs that includes doxorubicin, daunorubicin, epirubicin and idarubicin — have been widely used to treat breast cancer, leukaemia and many other types of malignancies[95]. Despite their powerful anticancer effects, the clinical use of anthracyclines is severely limited owing to the risk of cardiotoxicity[96]. Indeed, >25% of patients who received a cumulative dose of 550 mg/m^2 of doxorubicin developed congestive heart failure[97]. Although the mechanism underlying doxorubicin-induced cardiomyopathy is unclear, a growing body of evidence suggests that several risk factors are involved, including iron overload. Systemic iron accumulation in mice mediated by a high iron diet or genetic modification significantly increased susceptibility to doxorubicin-induced cardiotoxicity[98,99]. Conversely, mice fed an iron-deficient diet had a lower risk of doxorubicin-induced cardiotoxicity and increased survival compared with control mice, indicating that the targeting of metabolic pathways that regulate cardiac iron levels might be a clinically effective strategy for the treatment of chemotherapy-related cardiomyopathy[6].

In terms of how doxorubicin affects iron metabolism in the heart, one study found that iron accumulates specifically in the mitochondria of doxorubicin-treated cardiomyocytes owing to suppression of the mitochondrial iron exporter ABCB8 (also known as mitochondrial potassium channel ATP-binding subunit)[100]. Overexpression of ABCB8 or direct chelation of mitochondrial iron using dexrazoxane protects against doxorubicin-induced cardiotoxicity. In addition, doxorubicin treatment in mice has been shown to induce cardiac mitochondrial ferritin expression, and genetic deletion of mitochondrial ferritin increased the sensitivity of cardiomyocytes to doxorubicin-induced iron toxicity[101].

Using RNA sequencing (RNA-seq) analysis, we showed that HO1 is upregulated via nuclear factor-erythroid 2-related factor 2 (NRF2) activation during doxorubicin-induced cardiomyopathy in mice, causing haem degradation and the release of free iron in mitochondria[6]. In addition, we found that treating mice with the competitive HO1 inhibitor zinc protoporphyrin IX

protects against doxorubicin-induced cardiomyopathy[6]. A study published in 2021 found that the E3 ubiquitin protein ligase TRIM21 negatively regulates the NRF2-mediated antioxidant pathway and that *Trim21*-knockout mice are protected against doxorubicin-induced cardiotoxicity and death[102]. Additional studies examining the clinical applicability of these compounds and their molecular targets should lead to improved therapeutic strategies that are designed to reduce anthracycline-induced heart injury.

Dietary iron overload. In a typical human diet, the two forms of iron (haem iron and non-haem iron) are derived from distinct dietary sources and have different absorption mechanisms and metabolic pathways[103]. Haem iron, which constitutes approximately 15% of the total iron intake of a typical diet, is present exclusively in the haemoglobin and myoglobin in red meat, fish and poultry, whereas non-haem iron is present in cereals, fruits and vegetables. Given that haem iron is buried and protected within the porphyrin complex, its absorption rate is fivefold to tenfold higher than that of non-haem iron[104].

To date, the population-based studies that have examined the putative association between dietary iron intake and the risk of heart disease have found inconsistent findings[105–116] (TABLE 2). Nonetheless, meta-analyses of these prospective cohort studies suggest that a high intake of dietary haem iron, irrespective of non-haem iron intake, is significantly correlated with an increased risk of heart disease and cardiovascular death in the general population[117,118]. Therefore, a reduction in the consumption of foods that are high in haem iron might help to prevent heart disease.

Glutathione metabolism and ferroptosis in the heart
In addition to iron metabolism, cysteine or cysteine deficiency, glutathione depletion and inactivation of the enzyme phospholipid hydroperoxide glutathione peroxidase 4 (GPX4) have also been shown to promote ferroptosis[13]. Produced primarily in the liver, the master antioxidant glutathione is a tripeptide composed of the amino acids cysteine, glutamic acid and glycine. Among these three amino acids, cysteine is the rate-limiting precursor in glutathione synthesis. Although intracellular cysteine can be produced by either de novo biosynthesis or protein catabolism, most cells obtain cysteine primarily from the cystine–glutamate antiporter system x_c^- (also known as xCT), which consists of the solute carrier family 7A member 11 subunit (SLC7A11; also known as cystine–glutamate transporter) and the SLC3A2 subunit (also known as 4F2HC)[119]. The SLC7A11 subunit contains 12 transmembrane domains and primarily mediates the transporter activity of the protein complex, whereas SLC3A2 is a chaperone protein that helps to stabilize SLC7A11 and ensures the appropriate membrane localization of the complex. System x_c^- exports intracellular glutamate and imports extracellular cystine at a 1:1 ratio; the newly imported cystine is then converted to cysteine in the cytosol via an NADPH-consuming reduction reaction[120]. The structure of human system x_c^-, which was resolved in 2020, clearly demonstrates

the interaction between the two subunits at the extra-cellular interface and in the transmembrane region[121–123]. In addition, a well-resolved non-protein density was found in the intracellular vestibule of SCL7A11, to which an erastin molecule can bind[123]. Therefore, inhibiting system x_c^- with erastin or its analogues can lead to the depletion of intracellular glutathione and trigger ferroptosis, suggesting a potentially viable strategy for antitumour therapies[2,124].

The expression of SLC7A11 is positively regulated by the transcription factor NRF2 under conditions of cellular stress[125]. Moreover, both the genetic deletion of *NRF2* and overexpression of KEAP1 (Kelch-like ECH-associated protein 1, which binds to and facilitates the ubiquitination and proteasomal degradation of NRF2) have been shown to promote ferroptosis in cancer cells[126]. Of note, the KEAP1–NRF2 axis regulates a wide range of genes involved in glutathione biosynthesis and iron metabolism, which can also affect the susceptibility of cells to ferroptosis. Conversely, the tumour suppressor protein p53 represses *SLC7A11* transcription, thereby promoting ferroptosis[127]. The cAMP-dependent transcription factor ATF3 has also been shown to promote ferroptosis by binding to the *SLC7A11* promoter and repressing its expression in a p53-independent manner[128]. Moreover, our research group has shown that overexpressing *Slc7a11* selectively in cardiomyocytes increases cellular glutathione levels and prevents ferritin H deficiency-mediated cardiac ferroptosis, providing the first evidence that SLC7A11 has an anti-ferroptotic role in the heart[27]. In addition, knocking out *Slc7a11* aggravates cardiac hypertrophy and dysfunction in mice, both of which can be reversed by inhibiting ferroptosis[129].

The enzyme GPX4, together with glutathione as an essential cofactor, scavenge the harmful by-products of iron-dependent lipid peroxidation, thereby protecting the cell membrane from damage. Using a chemoproteomics assay, investigators have determined that overexpression of GPX4 in cancer cells can inhibit ferroptosis that is mediated by transcription factor RSL3, whereas deletion of GPX4 increases sensitivity to ferroptosis[130]. The synthesis and activity of GPX4, which is a selenoprotein, are affected by the concentration of the essential trace mineral selenium[124,125]. Selenium is required for GPX4 function, and its supplementation can increase the expression of GPX4 and protect cells against ferroptosis[131,132]. Interestingly, selenium deficiency in humans is thought to cause Keshan disease, an endemic cardiomyopathy present in children and pregnant women residing in the Keshan region of China where selenium levels in food are low[133]. Patients with Keshan disease develop dilated cardiomyopathy

Table 2 | **Prospective cohort studies of dietary iron intake in cardiovascular disease**

Study	Year	Location	Number of patients	Age (years)	Sex	Follow-up (years)	Disease setting	Findings	Ref.
HPFS	1994	USA	44,933	40–75	Male	4	MI	Increased risk of MI with higher intake of haem iron, but not total iron	[105]
NHANES-I	1994	USA	4,237	40–74	Both	13	CHD	No link between dietary total iron intake and risk of CHD	[106]
Rotterdam Study	1999	Netherlands	4,802	>55	Both	4	MI	Increased risk of MI with higher intake of haem iron, but not total iron	[107]
NSNS	2002	Canada	2,198	18–74	Both	8	MI	No link between dietary intake of either total or haem iron and risk of MI	[108]
IWHS	2005	USA	34,492	55–69	Female	15	CVD	Increased cardiovascular risk with higher intake of haem iron in patients with alcoholic use disorder	[110]
Prospect-EPIC	2005	Netherlands	16,136	49–70	Female	4.3	CHD	Increased risk of CHD with higher intake of haem iron, but not total or non-haem iron	[109]
NHS	2007	USA	6,161	30–55	Female	8.8	CHD	Increased risk of CHD with higher intake of haem iron, but not total iron	[111]
LEOGRA	2011	Italy	906	61.1±17.1	Female	10	CVD	No link between dietary total, non-haem or haem iron and cardiovascular risk	[112]
MESA	2012	USA	5,285	45–84	Both	6.2	CVD	No link between dietary non-haem or haem iron and cardiovascular risk	[113]
JACC	2013	Japan	58,615	40–79	Both	14.7	CVD	Increased cardiovascular risk with higher intake of total iron, but not haem or non-haem iron	[114]
COSM	2014	Sweden	36,882	45–79	Male	11.7	MI	Increased risk of MI-related death with higher intake of haem iron, but not non-haem iron	[115]
NHANES	2020	USA	14,826	>18	Both	9.3	CVD	Increased cardiovascular risk with higher intake of haem iron, but not total or non-haem iron	[116]

CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction.

and heart failure. High serum levels of selenium have also been independently associated with both reduced mortality and fewer cases of new-onset heart failure in a population-based cohort study[134]. Therefore, well-powered interventional studies designed to further evaluate the potential benefits of high selenium levels in humans are warranted.

Given the wide range of physiological functions of glutathione, its role in the pathogenesis and development of heart disease is not surprising. In a Japanese population-based study, plasma glutathione levels were found to be significantly lower in all patients with heart disease than in healthy controls[135]. In a subsequent study, circulating glutathione levels were reduced by 21% and 40% in patients with asymptomatic and symptomatic heart disease, respectively[136]. Importantly, the investigators also measured glutathione concentrations in atrial tissue and found direct evidence that cardiac glutathione deficiency is closely associated with heart disease[136]. Glutathione deficiency has also been observed in patients with hypertension[137].

Mice deficient in the glycoprotein apolipoprotein E (apoE), a component of all lipoproteins except LDL, are the most commonly used preclinical model of atherosclerosis[138]. Glutathione levels are significantly reduced in the atheroma-prone aortic arch of male apoE-deficient mice compared with age-matched, wild-type controls[139]. The study investigators proposed that glutathione deficiency is central to the suppression of intracellular antioxidant defences in these animals and therefore has a causal role in the pathogenesis of atherosclerosis[139], an intriguing hypothesis that is supported by subsequent studies. For example, liposomal-coated glutathione was shown to significantly reduce oxidative stress and correlate with decreased levels of either lipid peroxides or oxidized LDL[140]. Furthermore, other studies have shown that increased glutathione levels significantly reduced liver and plasma cholesterol levels in mice fed a diet rich in saturated fats[141,142].

Given that the heart has a very high energy demand and is particularly susceptible to oxidative damage, cardiomyocytes are thought to require a specialized and enhanced antioxidant system to avoid ferroptosis. Despite the lack of direct evidence that ferroptosis is involved in heart disease in humans, cardiomyocyte-specific animal disease models with genetically modified ferroptosis-related genes (such as *SLC7A11* and *GPX4*) and studies on ferroptosis inducers or inhibitors in animal models have provided compelling preclinical evidence that the glutathione pathway has a protective role against cardiac ferroptosis.

Lipid metabolism and ferroptosis in the heart
In mammalian cells, the peroxidation of PUFA-containing phospholipids in cell membranes is an essential step in ferroptosis[143]. The enzyme long-chain fatty acid CoA ligase 4 (LACS4) converts PUFAs to the acylated form and is considered to be a specific driver of ferroptosis, as its upregulation increases PUFA content in phospholipids and renders the cell more susceptible to ferroptosis[144,145]. Although an in-depth study into the role of LACS4

in cardiac muscle has not yet been conducted, this enzyme has been shown to be a novel therapeutic target for limiting skeletal muscle cell death and preventing rhabdomyolysis[146].

By contrast, LACS3 catalyses the conversion of exogenous monounsaturated fatty acids to fatty acyl-CoAs, thereby displacing PUFAs and inhibiting ferroptosis[147]. Conversely, the enzyme lysophosphatidylcholine acyltransferase 3 catalyses the insertion of these acylated PUFAs into membrane phospholipids; therefore, deleting this enzyme increases cellular resistance to ferroptosis[145,148].

Lipoxygenases are a family of iron-containing enzymes that directly oxygenate PUFAs and PUFA-containing lipids in cellular membranes. Lipoxygenases are thought to have an important role in lipid peroxidation and ferroptosis[149]. In addition, the scaffold protein phosphatidylethanolamine-binding protein 1, which inhibits protein kinase cascades, has been shown to bind to and direct 15-lipoxygenase to PUFAs in the membrane, thereby promoting ferroptosis[150].

The heart is highly susceptible to oxidative damage, and lipid peroxidation is an important contributor to ROS-induced heart injury. Cardiac tissue has several potential sources of endogenous ROS, including the mitochondrial electron transport chain, NADPH oxidase, xanthine oxidoreductase, nitric oxide synthases and cytochrome P450 (REF.[151]). In addition, several exogenous factors and chemicals can cause oxidative damage. An accumulation of lipid peroxides that originate from the oxidation of PUFA-containing membrane phospholipids suggests that ROS production cannot always be effectively balanced by the antioxidant system; as such, lipid peroxidation is one of the most pronounced manifestations of oxidative stress in the heart. Electrophilic reactive aldehydes, such as malondialdehyde, 4-hydroxyhexenal and 4-hydroxynonenal, are major end-products of PUFA oxidation, and are commonly used as markers of lipid peroxidation[152]. In patients with chronic heart failure, high serum levels of malondialdehyde were found to be an independent predictor of death and a combined clinical end point in a 1-year follow-up study[153]. These results were consistent with those of previous studies[154,155], and support the hypothesis that lipid peroxidation has a role in both the development and severity of heart disease. Of note, lipid peroxidation is a driver of ferroptosis through its effects on damaging cellular membranes[156]. The scavenging of lipid peroxides protects against lipid peroxidation-induced damage to membranous structures in cardiomyocytes and results in inhibition of ferroptosis[27,157,158].

Mitochondria and cardiac ferroptosis
As the energy powerhouse in eukaryotic cells, mitochondria coordinate essential metabolic processes such as oxidative phosphorylation. Given that the production of haem and Fe–S clusters occurs primarily in the mitochondria, this organelle contains large amounts of iron[159] (FIG. 2b). Iron is taken up by mitochondria via the proteins mitoferrin 1, which is expressed in the erythroid lineage, and mitoferrin 2, which is ubiquitously expressed[160]. Mitochondrial iron binds to mitochondrial ferritin

(a ferroxidase enzyme encoded by *FTMT*) to prevent ROS production, and mutations in mitochondrial ferritin can cause mitochondrial iron overload and cytoplasmic iron deficiency[161]. The mechanism by which iron is exported from the mitochondria is currently unknown; whether exported mitochondrial iron is conjugated to glutathione or is incorporated into Fe–S clusters or haem molecules is unclear[159]. Deletion of the mitochondrial protein ABCB8 selectively in the heart has been shown to cause mitochondrial iron overload, increased ROS production, defects in the cytosolic maturation of Fe–S clusters and cardiomyopathy[162]. Furthermore, iron–sulfur clusters transporter ABCB7, mitochondrial (ABCB7) has a role in mitochondrial iron homeostasis[163], and ATP-binding cassette subfamily B member 10, mitochondrial (ABCB10) has been shown to regulate the early steps of haem synthesis in mitochondria[164] and can also export biliverdin from mitochondria[165].

In the mitochondria, iron is also used to synthesize haem, which functions as a cofactor in mediating catalysis and electron transfer[166]. The production of haem is a multistep process that requires eight different enzymes, with aminolevulinic acid synthase (ALAS; also known as ALAS-H) involved in the rate-limiting first step[167]. Excess haem is either exported to the cytoplasm via feline leukaemia virus subgroup C receptor-related protein 1B (FLVCR1B) or catabolized via the HO1 pathway into equimolar amounts of Fe^{2+}, CO and biliverdin[168]. HO1 expression is induced ubiquitously in response to oxidative stress, whereas HO2 is constitutively expressed and not inducible[169]. In mammals, haem efflux is mediated primarily via the plasma membrane exporter FLVCR1A[170]. Conversely, FLVCR2 has been identified as a plasma membrane haem importer[171]. Other haem transporters such as haem transporter HRG1 and ATP-binding cassette subfamily C member 5 (ABCC5; also known as MRP5) are thought to deliver haem from various organelles, but their functions are poorly understood[172].

Given that the mitochondrial respiratory chain is a major source of ROS in most mammalian cells[173], mitochondria have been hypothesized to have a central role in the regulation of erastin-induced cell death since the discovery of ferroptosis in 2012. Substantial morphological changes in the mitochondria of ferroptotic cells have been observed using transmission electron microscopy, including reduced mitochondrial volume and increased mitochondrial membrane density[2]. In addition, certain nitroxide-based lipid peroxidation mitigators have been designed to specifically target mitochondria and are protective against erastin-induced and RSL3-induced ferroptosis[174].

By contrast, evidence also exists against the link between mitochondria and ferroptosis. For example, an early report suggested that a mitochondrial DNA-depleted cancer cell line is just as sensitive to ferroptosis as its parental cell line[2]. A subsequent study showed that mitochondria-deficient cells can still undergo ferroptosis and can be rescued by treatment with ferrostatins and iron chelators[175]. Nevertheless, these findings are highly debatable. In a separate study, cysteine depletion was shown to mediate

hyperpolarization of mitochondrial membranes and promote lipid peroxide accumulation, whereas inhibition of the tricarboxylic acid cycle or the electron transfer chain suppressed mitochondrial ferroptosis[176]. However, the investigators in this study also concluded that mitochondrial function is not required for ferroptosis induced by GPX4 inhibition, suggesting that the role of mitochondria in ferroptosis is context-dependent, consistent with observations in *Gpx4*^{−/−} primary cells[18].

Of note, *Gpx4* knockout in mice is embryonically lethal, whereas conditional *Gpx4* deletion promotes disorders in the brain, liver, endothelium, haematopoietic system and immune system[177]. In mammalian cells, several isoforms of GPX4, including cytosolic and mitochondrial isoforms, are encoded by a single gene. The mitochondrial isoform of GPX4 is believed to localize in mitochondria owing to the presence of a mitochondrial targeting signal at its amino terminus[178,179]. However, this isoform might not be expressed endogenously in the majority of cell types, with the exception of sperm cells. The cytosolic GPX4 isoform can cross the mitochondrial outer membrane and accumulate in the intermembrane space, where it helps to suppress mitochondrial lipid peroxidation[178]. Interestingly, overexpressing cytosolic GPX4, but not the mitochondrial isoform, prevented the death of *Gpx4*-knockout mouse embryonic fibroblasts[180]. Taken together, these findings indicate that the expression of cytosolic GPX4 is sufficient to prevent ferroptosis.

In vivo evidence also supports the notion that mitochondria have an essential role in ferroptosis. The mitochondria-targeted antioxidant MitoTEMPO has been shown to protect against ferroptosis-induced cardiac injury[6]. In addition, mice lacking mitochondrial ferritin develop more severe brain damage and neurological deficits after cerebral injury, as well as more typical features of ferroptosis, such as increased lipid peroxidation and disturbed glutathione antioxidative defence, whereas overexpression of mitochondrial ferritin inhibits ferroptosis and prevents these pathological changes[181].

A study published in 2021 described a novel mitochondrial defence mechanism against ferroptosis[182]. By analysing global metabolomics data, the researchers linked the enzyme dihydroorotate dehydrogenase (quinone), mitochondrial (DHOH) to ferroptosis in vitro. DHODH is localized to the inner mitochondrial membrane, where it catalyses the rate-limiting fourth step in the de novo pyrimidine synthesis pathway, converting dihydroorotate to orotate[183]. Interestingly, deleting DHODH in cancer cells with low expression of GPX4 has been shown to markedly increase mitochondrial lipid peroxidation and ferroptosis[182]. In addition, delivering GPX4 to mitochondria rescues RSL3-induced ferroptosis in DHODH-deficient cells[182]. SLC25A39 has been identified as a mitochondrial glutathione importer, highlighting its potential functional role in regulating mitochondrial lipid peroxidation and ferroptotic cell death[184]. Nevertheless, additional studies are needed to determine the in vivo function of DHODH and identify other ferroptosis-regulating mitochondrial enzymes, particularly in the context of cardioprotection (FIG. 3).

Other pathways that regulate ferroptosis

Ferroptosis suppressor protein 1 (FSP1) has been identified by two separate studies as a glutathione-independent, anti-ferroptosis factor[185,186]. Upon myristoylation, FSP1 is recruited to the plasma membrane, where it functions as an oxidoreductase to catalyse the production of coenzyme Q_{10} using NADPH[185,186]. Coenzyme Q_{10} functions as an endogenous inhibitor of ferroptosis through its antioxidative properties in the cell membrane, and depletion of coenzyme Q_{10} via the squalene synthase-mevalonate pathway explains, at least in part, the mechanism by which the type 3 ferroptosis inducer Fin56 promotes ferroptosis[187]. Although a previous study in mice indicated that doxorubicin can activate FSP1 translocation via lipid peroxidation products in the heart[188], the precise function of FSP1 in the heart remains poorly understood. Therefore, mice with a conditional *Fsp1* knockout might be a potential tool to provide further mechanistic insight.

Ferroptosis in cardiovascular disease

Before the discovery in 2014 that GPX4 is a key regulator of ferroptosis[18,130], numerous studies provided preliminary evidence on the role of altered GPX4 in a non-apoptotic form of cell death. For example, global *Gpx4* knockout in mice is lethal by embryonic day 7.5

Fig. 3 | **The regulatory role of mitochondria in ferroptosis.** Mitochondria host a wide range of key metabolic processes (such as the tricarboxylic acid (TCA) cycle) and are a major source of reactive oxygen species (ROS). Separate mitochondria-localized defence systems have evolved to prevent mitochondrial lipid peroxidation and ferroptosis. For example, either the mitochondrial version of phospholipid hydroperoxide glutathione peroxidase 4 (GPX4) or dihydroorotate dehydrogenase (quinone), mitochondrial (DHODH) can specifically detoxify mitochondrial lipid peroxides. Moreover, the mitochondria-specific form of ferritin (FTMT) protects mitochondria from iron overload-induced oxidative injury, and mitoNEET (also known as CISD1) suppresses ferroptosis by limiting mitochondrial iron uptake. CoQ_{10}, coenzyme Q_{10}; FPN, ferroportin; FSP1, ferroptosis suppressor protein 1; GSH, glutathione; GSSG, glutathione disulfide; HO1, haem oxygenase 1; LIP, labile iron pool; PL-PUFA-OOH, polyunsaturated fatty acid-containing phospholipid hydroperoxides; PLOO·, phospholipid peroxyl radical; RNF217, E3 ubiquitin protein ligase RNF217; SLC25A39, probable mitochondrial glutathione transporter SLC25A39; SLC39A14, solute carrier family 39 member 14; TF, transferrin; TFR1, transferrin receptor protein 1.

(REFS.[189,190]), whereas an inducible knockout of *Gpx4* in adult mice causes a rapid reduction in body weight and death within 2 weeks[191]. Moreover, mice with a conditional *Gpx4* knockout in endothelial cells generally die within 3 weeks, owing to thromboembolic events when deprived of dietary vitamin E[192]. This phenotype resulting from the combination of GPX4 and vitamin E deficiency indicates an important role of GPX4 in cardiovascular physiology. However, there remains a lack of evidence to support the existence of ferroptosis in the *Gpx4*-knockout animal models.
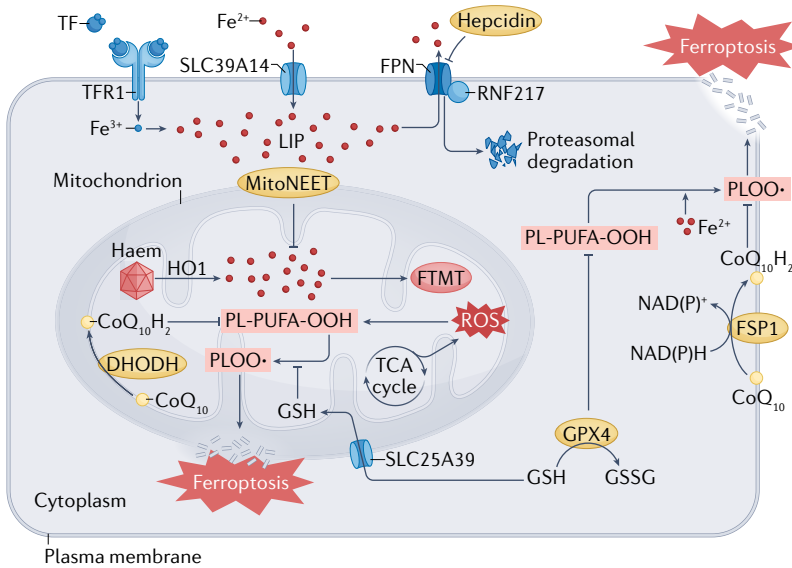
Myocardial ischaemia–reperfusion injury

Ischaemia–reperfusion injury is a fairly common, life-threatening clinical complication that can occur in nearly any organ, including the heart, liver, kidneys and brain. Mitochondria-specific overexpression of GPX4 was reported to be cardioprotective after ischaemia–reperfusion injury long before the concept of ferroptosis was first established[193]. In 2014, two studies found that ferroptosis is a primary driver of renal and hepatic ischaemia–reperfusion injury[18,194]. A subsequent study demonstrated that suppressing ferroptosis by inhibiting glutaminolysis reduced ischaemia–reperfusion injury in an ex vivo heart model[45]. Further in vivo data provided additional evidence indicating that either inhibiting ferroptosis or chelating iron during both acute and chronic myocardial ischaemia–reperfusion injury can provide cardioprotective benefits, highlighting the potential of targeting ferroptosis as a promising novel therapeutic strategy for ischaemia–reperfusion injury[6,195,196].

Ischaemia–reperfusion injury after heart transplantation can lead to serious complications such as primary graft dysfunction and increased risk of death[197]. In addition to the direct loss of cardiomyocytes, the release of endogenous substances during ischaemia–reperfusion induced by ferroptosis can trigger a harmful inflammatory response in the donor heart by promoting the adhesion of neutrophils to coronary vascular endothelial cells via a Toll-like receptor 4-dependent signalling pathway[157]. Importantly, oxidized phosphatidylethanolamine was also identified as a specific product of ferroptosis by analysing oxidative lipidomics, providing direct evidence of ferroptosis in the heart[157]. An accumulation of ferroptotic oxidized phosphatidylethanolamine species was also found in mitochondria isolated from hearts with ischaemia–reperfusion injury, further highlighting the role of cardiac mitochondria in the production of lipid peroxides and in ferroptotic signalling[198]. In addition, a new imaging protocol was developed in 2021 that can directly detect the presence and distribution of oxidized phosphatidylethanolamine in specific cells and tissues[199]. Application of this protocol in preclinical studies will facilitate the detection of peroxidized lipids in disease conditions, including cardiovascular disease.

Anthracycline cardiotoxicity

As discussed above, the use of doxorubicin for the treatment of malignancies is limited by its cardiotoxic effects. Our research group has examined the relative contributions of various forms of regulated cell death

in doxorubicin-induced cardiotoxicity by measuring the effect of the respective inhibitors of cell death on survival in doxorubicin-treated mice and shown that inhibition of ferroptosis is cardioprotective[6]. In addition to inducing heart injury, doxorubicin treatment causes a robust increase in cardiac levels of iron, lipid-derived ROS and ferroptosis biomarkers. Together, these findings suggest that ferroptosis has a major role in doxorubicin-induced cardiomyopathy and death[6]. Furthermore, at the subcellular level, we found that mitochondria are the target of HO1-mediated release of free iron, which causes lipid peroxidation in the mitochondrial membrane.

A subsequent study published in 2020 confirmed that mitochondria-dependent ferroptosis has a major pathogenic role in doxorubicin-induced cardiotoxicity[200]. Specifically, the study showed that doxorubicin treatment can downregulate GPX4 expression in the heart, leading to excessive lipid peroxidation. Moreover, overexpression of GPX4 in mice prevented doxorubicin-induced cardiomyopathy, whereas knocking down *Gpx4* exacerbated doxorubicin-induced cardiomyopathy[200]. However, the mechanism by which doxorubicin treatment can downregulate GPX4 requires further elucidation.

Finally, it is important to note that caspase-dependent apoptosis, but not ferroptosis, drives doxorubicin-induced cardiotoxicity in cultured cardiomyocytes[6,200]. This apparent discrepancy between in vivo and in vitro findings reflects the complexity of the mechanism of action of doxorubicin in vivo. For example, the HO1 pathway is differentially regulated in cultured cells compared with the in vivo setting, thereby affecting both iron accumulation and lipid peroxidation[6,201].

Diabetic cardiomyopathy

Diabetes mellitus is a common comorbidity in patients with cardiovascular disease and can increase the susceptibility of the heart to ischaemia–reperfusion injury[202]. Consequently, patients with diabetes have a poorer prognosis after acute myocardial infarction than patients without diabetes[202]. Diabetes can aggravate myocardial ischaemia–reperfusion injury by activating the NADPH oxidase pathway in an AMPK-dependent manner, subsequently inducing various forms of programmed cell death, including ferroptosis[203]. Ferroptosis has been shown to have a pathogenic role in mediating myocardial ischaemia–reperfusion injury in a streptozotocin mouse model of type 1 diabetes[204]. Moreover, hyperglycaemia-induced endoplasmic reticulum stress seems to be involved in cardiomyocyte damage mediated by ferroptosis[204].

Patients with diabetes are also at increased risk of developing myocardial dysfunction that is independent of coronary artery disease and hypertension — a phenomenon known as diabetic cardiomyopathy[205]. Indeed, several pathogenic factors, including oxidative stress, have been shown to contribute to the structural and functional changes that characterize the diabetic heart[205]. Ferroptosis was reported for the first time in the heart of diabetic mice in a study published in 2022, in which NRF2 activation was shown to prevent ferroptosis by upregulating ferritin and SLC7A11 levels[158].

Sepsis-induced cardiac injury

In patients, sepsis-induced cardiac injury and dysfunction correlate with increased mortality[206]. Caecal ligation and puncture is currently the most commonly used animal model for studying sepsis and involves the perforation of the caecum to allow the release of faecal material into the peritoneal cavity, which triggers an immune response mediated by polymicrobial infection[207]. Caecal ligation and puncture has been shown to increase cardiac iron content and lipid peroxidation levels, as well as to reduce cardiac glutathione content and GPX4 expression, suggesting that the development of sepsis-induced heart injury might involve ferroptosis[207]. In addition, ferroptosis has been shown to have a role in a lipopolysaccharide-induced model of septic cardiomyopathy[208]. A lipopolysaccharide-induced increase in sideroflexin 1 expression in the cardiac mitochondrial membrane can increase the production of mitochondrial ROS, leading to ferroptosis[208].

Hypertrophic cardiomyopathy

In the heart, pressure overload (owing to pulmonary hypertension and/or systemic hypertension) can lead to cardiac hypertrophy, cardiac fibrosis and eventual heart failure. Increased cardiac NADPH oxidase 4 expression and decreased cardiac GPX4 activity in animal models after either aortic banding or isoprenaline administration suggest that ROS production and ferroptosis might participate in the progression from compensated hypertrophy to heart failure[209,210]. In mice, genetic deletion of the key ferroptosis regulator SLC7A11 was found to exacerbate angiotensin II-mediated cardiac fibrosis, hypertrophy and dysfunction, providing genetic evidence of the involvement of ferroptosis in hypertrophic cardiomyopathy[129].

Interestingly, genetic deletion of *Ncoa4* specifically in cardiomyocytes has been shown to attenuate transverse aortic constriction (TAC)-induced heart failure by suppressing ferritinophagy[28]. Moreover, ferroptosis inhibitors attenuated cardiac remodelling in wild-type mice that had undergone TAC, but did not provide additional protection in *Ncoa4*-knockout mice, implicating cardiac ferroptosis as the downstream consequence of NCOA4-mediated ferritinophagy[28]. In addition, in a TAC mouse model, the enzyme mixed lineage kinase 3 (also known as MAP3K11) has been shown to induce pyroptosis and ferroptosis, which are essential for the development of TAC-mediated myocardial fibrosis[211]. Nevertheless, the mechanisms underlying the involvement of various forms of cell death in the pathogenesis of hypertrophic cardiomyopathy remain to be determined.

Putative link between ferroptosis and arrhythmia

Cardiac arrhythmia is common among patients with heart failure[212]. Findings from a preclinical study suggest a possible link between ferroptosis and arrhythmia. In mice, frequent excessive alcohol consumption triggered ferroptosis and increased the inducibility of atrial fibrillation[213]. Ferroptosis inhibitors could partially or completely reverse most of the adverse change induced by excessive alcohol intake.

As mentioned above, ferroptosis can propagate to adjacent cells in a wave-like manner, a phenomenon that has not been reported to occur with other forms of regulated cell death[15,194]. However, the mechanism underlying this seemingly directed pattern of propagation remains unknown. Cardiac tissue is a functional syncytium for coordinated muscle contraction, mediated via cell-to-cell electrical and chemical coupling through gap junction channels that determine the rhythm of the heart and might contribute to the wave-like propagation of ferroptosis. Therefore, cardiac muscle might be an ideal model for studying the propagation of cell death. Interestingly, myocardial damage such as ischaemia–reperfusion injury often results in arrhythmia as well as the formation of a necrotic zone, which has been suggested to account for the cell-to-cell propagation of ferroptosis[214]. Further investigation of this unique feature of ferroptosis is warranted.

Other cardiovascular-related diseases

Sickle cell disease is a group of inherited red blood cell disorders characterized by the presence of haemolysis that lead to organ ischaemia and cardiovascular complications[215]. Increased haem levels in mice with sickle cell disease resulted in an upregulation of cardiac HO1 levels, which then promoted iron overload, lipid peroxidation and ferroptosis in the heart. Furthermore, inhibiting and inducing ferroptosis attenuated and exacerbated, respectively, the cardiomyopathy that was associated with sickle cell disease[216].

Currently, the global population is in the midst of the coronavirus disease 2019 (COVID-19) pandemic, a respiratory tract infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[217]. The cardiovascular complications associated with COVID-19 are well recognized[218]. Iron might also have an important role in the pathogenesis of COVID-19, given that an estimated 90% of patients admitted to hospital present with abnormal serum iron levels, and these levels correlate with disease severity[219]. On the basis of these observations, ferroptosis has been suggested as a potential target for the treatment of COVID-19 (REF.[220]). Primary pacemaker cells in the heart have been shown to develop ferroptosis-associated cardiac dysfunction after infection with SARS-CoV-2 (REF.[221]). Moreover, a high-throughput chemical screen showed that the tyrosine kinase inhibitor imatinib and the iron-chelating agent deferoxamine can block SARS-CoV-2 infection and associated ferroptosis[221].

Ferroptosis as a promising treatment target

Given its role in the pathogenesis of heart disease, ferroptosis is a highly promising therapeutic target for the treatment and prevention of cardiovascular disease. In this section, we summarize the various small molecules that inhibit the ferroptosis pathway and discuss the use of these molecules in various models of heart disease (TABLE 3).

Ferrostatin 1, liproxstatin 1 and antioxidants. Ferrostatin 1 is a first-generation inhibitor of ferroptosis[2]. Since the anti-ferroptotic effects of this small-molecule compound were first reported, ferrostatin 1 has been tested in a wide range of diseases, including cardiovascular disease. Our group pioneered the use of ferrostatin 1 in mice with experimental cardiomyopathy and found that it can protect against doxorubicin-induced cardiac damage without affecting iron levels[6]. A subsequent study showed that ferrostatin 1 can also prevent doxorubicin-induced cell death in cultured cardiomyocytes[200]. In addition, ferrostatin 1 has been shown to improve cardiac function in animal models of acute or chronic myocardial ischaemia–reperfusion injury[6,157], as well as in diabetic mice[204]. In a mouse model of heart transplantation, ferrostatin 1 treatment blocked the recruitment of neutrophils after cardiomyocyte cell death, suggesting a potential strategy for improving clinical outcomes in patients receiving a heart transplantation[157]. Moreover, ferrostatin 1 treatment has also been reported to attenuate sepsis-induced cardiomyopathy and atherosclerosis in mice[208,222]. Of note, some researchers have indicated that the in vivo function of ferrostatin 1 is weaker than its function in vitro, given its low stability in plasma[223]. To address this issue, a soluble ferrostatin analogue called UAMC-3203 was developed as a more stable and potent inhibitor of ferroptosis than ferrostatin 1. In animals, UAMC-3203 outperformed ferrostatin 1 in preventing ferroptosis-driven multiorgan dysfunction and might therefore be a suitable candidate for clinical testing[224].

Liproxstatin 1, a spiroquinoxalinamine derivative, was first identified as a specific inhibitor of ferroptosis from high-throughput screening of $Gpx4^{-/-}$ cells[18]. Liproxstatin 1 is not as well studied as ferrostatin 1 in the context of cardiovascular pathophysiology, but a study in mice has shown that liproxstatin 1 can protect the myocardium against ischaemia–reperfusion injury by reducing mitochondrial ROS production and maintaining GPX4 activity[225]. A subsequent study found that liproxstatin 1 significantly reduces palmitic acid-induced cardiac injury, with similar protective effects exerted by ferrostatin 1 (REF.[226]).

Other commonly studied antioxidants in the context of cardiovascular disease are 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO) and MitoTEMPO, a mitochondria-targeted version of TEMPO that was developed to scavenge mitochondrial superoxides[227]. We have previously demonstrated that MitoTEMPO can potently prevent doxorubicin-induced lipid peroxidation, cardiac ferroptosis and cardiac dysfunction in mice, whereas TEMPO only mildly reduces these doxorubicin-induced effects[6]. These findings strongly support the notion that mitochondrial lipid peroxidation and cardiac ferroptosis work in a coordinated fashion to mediate doxorubicin-induced cardiomyopathy.

Given that ferroptosis is only one pathway that contributes to the death of cardiomyocytes[228], a combination strategy targeting both ferroptosis and necroptosis might be more effective at managing heart disease. Nec-1f, a dual inhibitor that targets both receptor-interacting serine/threonine protein kinase 1 and ferroptosis primarily in kidney cells and kidney tubules, significantly increased survival in a mouse model of heart transplantation and a mouse model of renal ischaemia–reperfusion injury[229].

Table 3 | **Summary of small-molecule modulators in ferroptosis-related diseases**

Drug	Mechanism	Targets	Refs.
Ferrostatin 1	Inhibits lipid peroxidation	Doxorubicin-induced cardiomyopathy, myocardial I–R injury, TAC-induced cardiomyopathy, heart transplantation, atherosclerosis, sepsis-induced cardiomyopathy, palmitic acid-induced myocardial injury, atrial fibrillation	[6,28,157,200, 204,208,213, 222,226]
Liproxstatin 1	Inhibits lipid peroxidation	Myocardial I–R injury, palmitic acid-induced myocardial injury, diabetic cardiomyopathy	[158,225,226]
Nec-1f	Inhibits necroptosis and ferroptosis	Heart transplantation	[229]
UAMC-3203	Inhibits lipid peroxidation	Multiorgan dysfunction	[224]
Dexrazoxane	Inhibits iron overload	Doxorubicin-induced cardiomyopathy, myocardial I–R injury, sepsis-induced cardiomyopathy	[6,208]
Deferoxamine	Inhibits iron overload	Myocardial I–R injury	[45,232]
Deferiprone	Inhibits iron overload	Sepsis-induced cardiomyopathy	[208]
Zinc protoporphyrin IX	Inhibits HO1	Doxorubicin-induced cardiomyopathy	[6]
MitoTEMPO	Scavenges mitochondrial ROS	Doxorubicin-induced cardiomyopathy	[6]
Mito-FerroGreen	Inhibits mitochondrial iron overload	Doxorubicin-induced cardiomyopathy	[200]
Compound 968	Inhibits glutaminolysis	Myocardial I–R injury	[45]
N-Acetyl cysteine	Glutathione precursor	Myocardial I–R injury in diabetes	[203]
Vas2870	Inhibits NOX2	Myocardial I–R injury in diabetes	[203]
Acadesine	Activates AMPK	Myocardial I–R injury in diabetes	[203]
Sulforaphane	Activates NRF2	Diabetic cardiomyopathy	[158]
Dexmedetomidine	Activates GPX4	Sepsis-induced cardiomyopathy	[207]
Salubrinal	Inhibits ER stress	Myocardial I–R injury in diabetes	[204]
P22077	Inhibits the p53–TFR1 pathway	Myocardial I–R injury	[236]
Puerarin	Unknown	Heart failure	[210]
Atorvastatin	Inhibits ferritinophagy	Isoprenaline-induced cardiomyopathy	[237]

AMPK, AMP-activated protein kinase; ER, endoplasmic reticulum; GPX4, glutathione peroxidase 4; HO1, haem oxygenase 1; I–R, ischaemia–reperfusion; NOX2, NADPH oxidase 2; NRF2, nuclear factor-erythroid 2-related factor 2; ROS, reactive oxygen species; TAC, transverse aortic constriction; TFR1, transferrin receptor 1.

Iron chelators and iron chelation therapy. Given that ferroptosis is an iron-dependent form of programmed cell death, it is no surprise that ferroptosis can be inhibited by iron chelation[2]. The iron chelator dexrazoxane is currently the only FDA-approved drug for preventing doxorubicin-induced cardiotoxicity in patients with cancer. Dexrazoxane is a cyclic derivative of ethylenediaminetetraacetic acid that readily crosses cell membranes and chelates intracellular free iron[230]. A study showed that inhibition of ferroptosis is the predominant mechanism by which dexrazoxane exerts its cardioprotective effect[6]. However, this finding raises the question of why other iron chelators do not seem to be effective against doxorubicin-induced heart injury[231], and researchers have suggested that dexrazoxane can directly enter mitochondria in cardiomyocytes and reduce iron accumulation, whereas other iron chelators cannot enter the mitochondria[100]. This notion is further supported by the finding that Mito-FerroGreen, a novel mitochondria-specific iron chelator, provides cardioprotection in mice treated with doxorubicin[200]. In addition, other iron chelators such as deferiprone and deferoxamine have been shown to alleviate both myocardial ischaemia–reperfusion injury and sepsis-related cardiac damage by blocking ferroptosis[45,208,232]. Of note, however, the finding that an iron chelator can protect tissue from damage does not necessarily indicate that it does so by inhibiting ferroptosis.

GSH precursors. Cysteine, the reduced form of cystine imported via system x_c^-, is the rate-limiting precursor in glutathione biosynthesis, and the addition of cystine or cysteine to cell culture media in vitro has been shown to inhibit ferroptosis[45]. The antioxidant N-acetyl cysteine (NAC) was developed to improve the bioavailability of cysteine and has been shown to have beneficial effects on cardiovascular function[233,234]. The anti-ferroptosis effect of NAC has been demonstrated in the context of cysteine depletion and system x_c^- inhibition-induced ferroptosis[2,235]. NAC treatment has also been shown to reduce myocardial ischaemia–reperfusion injury in diabetic rats, suggesting that this compound might have clinical applications in heart disease[203].

Other compounds that can affect cardiac ferroptosis. In addition to the aforementioned drugs, other compounds can also inhibit cardiac ferroptosis by acting on other targets. For example, zinc protoporphyrin IX, a competitive inhibitor of HO1, reduces doxorubicin-induced cardiac iron accumulation and ferroptosis in mice by blocking haem degradation and the resulting release of free iron[6]. Glutaminolysis is required for cysteine deprivation-induced ferroptosis, and compound 968, a cell-permeable, small-molecule inhibitor of glutaminolysis, has been shown attenuate myocardial ischaemia–reperfusion injury ex vivo by limiting glutamine levels[45]. Moreover, P22077, an inhibitor of ubiquitin-specific protease 7, has been shown to suppress ferroptosis by activating p53 and reducing TFR1 levels, providing protection against myocardial ischaemia–reperfusion injury[236]. Interestingly, dexmedetomidine, an α_2-adrenergic receptor agonist used clinically to sedate patients, has been shown to alleviate sepsis-induced myocardial injury via the ferroptosis suppressor GPX4 (REF.[207]). In addition, puerarin, an isoflavone extracted from the kudzu root, has been shown to protect against erastin-induced and isoprenaline-mediated ferroptosis in cultured cardiomyocytes and can mitigate heart failure induced by pressure overload in rats[210]. Furthermore, atorvastatin has also been shown to ameliorate isoprenaline-induced cardiac dysfunction and remodelling in mice by inhibiting ferritinophagy-mediated ferroptosis, thereby providing another potential therapeutic strategy for the prevention of hypertrophic cardiomyopathy[237].

Finally, commonly prescribed heart medications might have previously unidentified anti-ferroptotic activity. For example, carvedilol, which is widely used to treat hypertension and heart failure, has been shown to block ferroptosis independent of its effect on β-adrenergic receptors[238,239], and the underlying mechanism might contribute to its capacity to scavenge lipid peroxides and chelate iron[240].

Limitations

A growing body of evidence supports the role of ferroptosis in the initiation and progression of various cardiovascular diseases. However, a number of questions need to be addressed before the therapeutic potential of ferroptosis-targeted agents can be clinically evaluated. First, what are the crucial safeguarding mechanisms against cardiac ferroptosis? Second, can we identify reliable biomarkers for predicting ferroptosis in cardiovascular disease? The ferroptosis biomarkers currently used in preclinical studies are non-specific and present in other types of cell death and certain pathological conditions. In this fast-growing field, the lack of ferroptosis-specific biomarkers has been a long-standing bottleneck limiting the development of ferroptosis-targeted clinical applications. Third, is it possible to design effective ferroptosis-targeted strategies for preventing and treating ferroptosis-related cardiovascular disease? Finally, when and how do other forms of cell death occur together with ferroptosis in the development of cardiovascular disease? Although selective inhibition of ferroptosis has been shown to substantially improve cardiac function in a variety of animal models, no clinical trials have so far been performed using ferroptosis-specific inhibitors to treat cardiovascular disease. Additional population-based data are urgently needed to determine whether selectively blocking ferroptosis can improve cardiovascular outcomes in the clinical setting.

Conclusions

The link between iron and cardiovascular disease was first proposed nearly three decades ago, but the mechanistic pathways underlying this relationship remained elusive until the discovery of ferroptosis, an iron-dependent form of regulated cell death, just 10 years ago. Ferroptosis is induced by the activation of iron-dependent lipid peroxidation, but the key effector molecules involved in this process are unclear. Excess levels of free reactive iron can cause tissue damage, and iron chelation therapy has been widely recommended for the treatment of patients with iron overload-related cardiomyopathy. Preclinical studies are paving the way to the development of effective ferroptosis-specific antagonists for clinical testing.

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