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Review

Ferritinophagy and ferroptosis in the management of metabolic diseases

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Ferroptosis is a form of regulated cell death modality associated with disturbed iron-homeostasis and unrestricted lipid peroxidation. Ample evidence has depicted an essential role for ferroptosis as either the cause or consequence for human diseases, denoting the likely therapeutic promises for targeting ferroptosis in the preservation of human health. Ferritinophagy, a selective form of autophagy, contributes to the initiation of ferroptosis through degradation of ferritin, which triggers labile iron overload (IO), lipid peroxidation, membrane damage, and cell death. In this review, we will delineate the role of ferritinophagy in ferroptosis, and its underlying regulatory mechanisms, to unveil the therapeutic value of ferritinophagy as a target in the combat of ferroptosis to manage metabolic diseases.

Highlights

Ferroptosis is a form of regulated cell death that is driven by iron overload and lipid peroxidation.

Ferroptosis contributes to the onset or progression of various metabolic diseases.

Ferritinophagy is a selective type of autophagy, which induces ferroptosis by degrading ferritin and inducing iron overload.

Ferritinophagy inhibition may ameliorate ferroptosis and ease the management of metabolic diseases.

Role of autophagy in ferroptosis

Macroautophagy (hereafter referred to as autophagy) is a bulk regulated degradation process that sequesters defective organelles, damaged proteins, and cellular components (so-called autophagic cargos) into double-membrane transient compartments, namely phagophores, prior to maturation into autophagosomes and fusion with lysosomes, en route to the ultimate cargo degradation. The breakdown products of autophagy are released into the cytosol to provide energy and/or nutrients for cellular remodeling and homeostasis as part of developmental programming (Figure 1) [1–4]. Although basal autophagy is generally perceived to be cytoprotective, hyperactivated (unchecked) or impaired autophagy contributes to various forms of regulated cell death, including ferroptosis [2,4–6]. Ferroptosis is a highly regulated reactive oxygen species (ROS)-dependent type of cell death, derived from free iron overload (FIO), ROS generation, lipid peroxidation, and, ultimately, membrane damage (Figure 2) [7]. Several morphological hallmarks have been identified for ferroptosis, including impairment in plasma membrane integrity, mild chromatin condensation, swelling of cytoplasm and cytosolic organelles, as well as mitochondrial changes (e.g., enhanced membrane density, outer membrane rupture, and interrupted crista) [8]. Typically, FIO and lipid peroxidation are two biochemical hallmarks of ferroptosis, resulting in pathological conditions and diseases [9]. FIO develops as a consequence of disturbed iron metabolism and accumulation of intracellular or extracellular iron. Mechanistically, FIO may provoke intense ROS production through a reaction, termed the 'Fenton reaction', in subcellular organelles that culminates in DNA oxidative injury, lipid peroxidation, and membrane damage [7,10,11]. Moreover, FIO evokes activation of iron-containing enzymes such as lipoxygenases, which further promotes membrane lipid peroxidation [10]. Lipoxygenases and acyl-CoA synthetase long-chain family member 4 (ACSL4) are two core enzymes promoting lipid peroxidation and ferroptotic cell death. ACSL4 is vital for generation of polyunsaturated fatty acid-containing phospholipids (PUFA-PLs), whereas lipoxygenases catalyze the oxidation of PUFA-PLs, resulting in production of lipid hydroperoxides and reactive aldehydes encompassing 4-hydroxynonenal and malondialdehyde [12]. Likewise, cytochrome P450 oxidoreductase (POR) is also involved in lipid peroxidation of ferroptosis [13].

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To date, a number of selective forms of autophagy, including ferritinophagy, **clockophagy** (see Glossary), **lipophagy**, **mitophagy**, and **chaperone-mediated autophagy**, have been identified to participate in the execution of ferroptotic cell death by degrading antiferroptosis proteins or organelles (Figure 3) [5]. In particular, ferritinophagy refers to selective autophagic degradation of **ferritin**, leading to the buildup of cytosolic irons in the form of ferrous iron (Fe^{2+}), culminating in ferroptosis [7,11]. To this end, regulation of ferritinophagy may be a promising, albeit challenging strategy to halt ferroptosis in metabolic derangement settings. In this review, we will attempt to discuss hitherto identified molecular machinery of ferritinophagy and ferritinophagy-mediated ferroptosis in various metabolic stress settings. We will emphasize potential targets and regulatory pathways in the therapeutic intervention of ferritinophagy to shed light on the combat against ferroptotic cell death in the management of metabolic diseases.

Ferroptosis in metabolic diseases

Ferroptosis in cardiomyopathy

Iron metabolism and homeostasis are of paramount importance for cardiac homeostasis, as evidenced by the culprit role of Fe^{2+} overload in cardiomyocyte ferroptosis and heart failure [14]. In a murine model of sickle cell disease, free iron levels were systemically increased, particularly in cardiac tissues, leading to elevated cardiotoxic markers, including natriuretic peptide A (NPPA), natriuretic peptide B (NPPB), prostaglandin-endoperoxide synthase 2 (PTGS2, a ferroptosis marker), and lipid peroxidation. These findings denote promises of targeting ferroptosis in the alleviation of cardiac damages induced by sickle cell disease [14].

Ferritin stores surplus cytosolic Fe^{2+} in the form of ferric iron (Fe^{3+}) and plays a cardinal role in iron homeostasis [15]. However, an iron-rich diet induces Fe^{2+} overload and ferroptosis, as evidenced by pronounced lipid peroxidation and decreased glutathione (GSH) levels, which is accompanied by exacerbated cardiac injury and hypertrophic cardiomyopathy [16]. These observations suggested that both Fe^{2+} deficiency and Fe^{2+} overload correlate with cardiomyopathy. Ferritin deficiency also induces ferroptosis via downregulation of solute carrier family 7 member 11 (SLC7A11), the inhibition of which triggers ferroptosis [16]. However, SLC7A11 overexpression facilitates GSH synthesis to suppress ferroptosis, suggesting an important role for a ferritin-SLC7A11-GSH axis in the therapy against ferroptosis-mediated heart failure and various cardiomyopathies [16].

Accumulating evidence has noted an important regulatory role for autophagy in ferroptosis and organismal pathologies. For example, data from our group revealed that haploinsufficiency of beclin 1 (BECN1), a core autophagy component, negates cold stress-induced cardiac remodeling and defects through blockade of ferroptosis, apoptosis, excessive autophagy, and mitochondrial injury, suggesting the promises of targeting autophagy and/or ferroptosis in cold stress-induced cardiac anomalies [17].

In murine models of cardiotoxicity, doxorubicin was shown to trigger ferroptosis through nuclear factor, erythroid 2 like 2 (NFE2L2/NRF2)-mediated upregulation of heme oxygenase 1 (HMOX1) [18], leading to heme degradation and FIO in mitochondria, which provokes lipid peroxidation and ferroptosis and, ultimately, cardiomyopathy [19]. Conversely, HMOX1 exhibits antiferroptotic effects in a murine model of acute kidney injury (AKI), and HMOX1-generated free iron does not induce ferroptosis, indicating controversial roles of HMOX1 in mediating ferroptosis in disparate disease settings [20].

Further study examining the role of toll-like receptor 4 (TLR4) and NADPH oxidase 4 (NOX4) in heart failure noted that TLR4 and/or NOX4 knockdown retard overactivated autophagy and

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ferroptosis. In this context, the TLR4-NOX4 axis may serve as a potential target to abrogate autophagy- and/or ferroptosis-mediated cell death [21]. Ferroptosis has also played a role in the coronavirus disease 2019 (COVID-19) pandemic and organ injury. It was reported that COVID-19-induced myocarditis also exhibits signs of ferroptosis, including oxidized phosphatidylcholine and hydroxynonenal in cardiac and renal tissues, which confer deadly ischemia/reperfusion (I/R)-like injuries in COVID-19 patients [22]. It may be concluded that ferritinophagy induces heart failure and ferroptotic death in murine cardiomyocytes, whereas cardiac-specific knockout of nuclear receptor coactivator 4 (NCOA4) alleviates heart failure to preserve cardiac function [23]. Thus, targeting ferroptosis and ferritinophagy-mediated ferroptosis should offer promises in the protection and management against cardiovascular diseases.

Ferroptosis in myocardial I/R injury

Ferroptosis is implicated in the etiology of I/R injury. Subjecting murine hearts to I/R insult noted pronounced ferroptosis in myocardial reperfusion but not the ischemia phase. Nonetheless, inhibition of ferroptosis remarkably attenuated myocardial I/R injury, denoting a vital role for ferroptosis in reperfusion injury and overall myocardial infarction (MI) [24]. Although reperfusion is commonly employed to rescue MI, it may trigger oxidative stress at the same time, leading to production of oxidized and heavily toxic phosphatidylcholines, in particular, PONPC and POVPC (two derivatives of oxidized phosphatidylcholine). It is believed that these intermediates mediate ferroptotic cell death in cardiomyocytes during myocardial I/R injury [25]. These findings were supported by the favorable response of ferroptosis inhibition on I/R injury. Liproxstatin-1, a selective ferroptosis inhibitor, was found to attenuate infarction, sustain mitochondrial integrity and function, reduce mitochondrial ROS generation, and increase glutathione peroxidase 4 (GPX4) level in murine ischemic myocardium, through retarding oligomerization, and level of voltage-dependent anion channel 1 (VDAC1) on the mitochondrial membrane [26]. Furthermore, liproxstatin-1 preserves cristae integrity upon myocardial I/R injury, implying an essential role for mitochondrial integrity and ROS production through manipulating GPX4 activity in ferroptosis inhibition-evoked protection against MI [26].

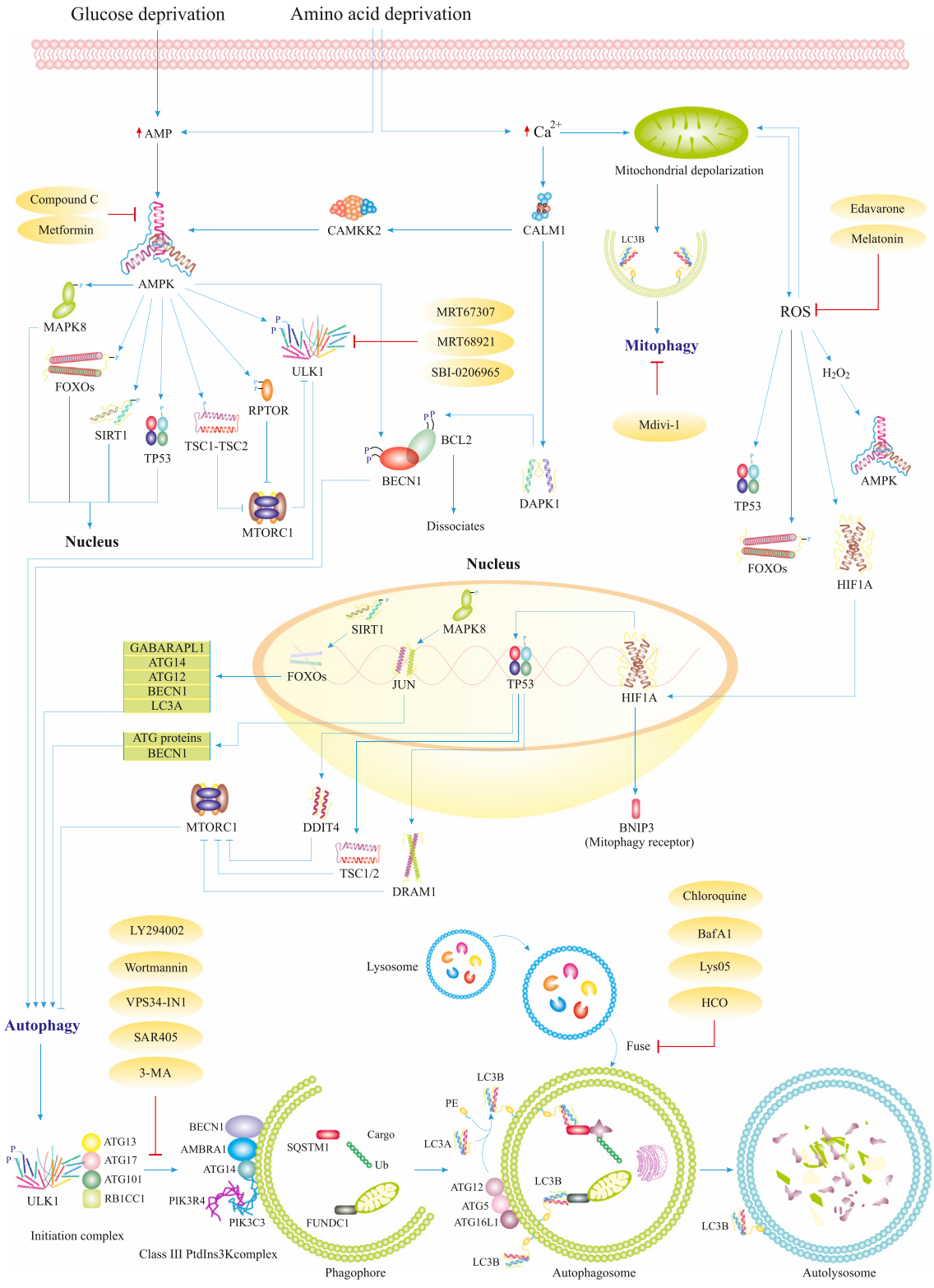
Other than ferroptosis inhibitors, many maneuvers with proven benefit in I/R injury may also work through regulation of ferroptosis. For example, electroacupuncture 'Shenmen' HT7 was shown to inhibit ferroptosis through upregulation of GPX4, ferritin heavy chain 1 (FTH1), and GSH, along with downregulation of FIO, transferrin receptor (TFRC), and ACSL4 in a rat model of acute myocardial ischemia (AMI), suggesting the utility of electroacupuncture in cardiac ferroptosis [27]. Moreover, in the early and chronic phases of MI, GPX4 was found to be downregulated, resulting in ferroptosis in rat neonatal cardiomyocytes, particularly, under cysteine deprivation [28].

Ferroptosis is regulated by noncoding RNAs. For example, mesenchymal stem cell (MSC)-secreted exosomes were shown to downregulate doublesex and mab-3 related transcription factor 1 (DMRT1) through miRNA *Mir23a-3p* and suppress DMRT1-mediated ferroptosis and FIO. Nonetheless, further studies are warranted to unravel the underlying mechanisms behind DMRT1-mediated ferroptosis, a potential therapeutic target for the regulation of ferroptosis. More evidence noted decreased *Mir30d* following MI insult, leading to elevated autophagy-related 5 (ATG5) and excessive autophagy, which might contribute to ferroptosis in H9c2 cells [29]. It is believed that *Mir30d* binds to ATG5 and regulates its expression to avert excessive autophagy-mediated ferroptosis.

There is growing literature suggesting a connection between the ubiquitin-proteasome system (UPS) and ferroptosis in I/R. For instance, exposure of rat hearts to I/R leads to myocardial injury and upregulation of ubiquitin specific peptidase 7 (USP7), tumor protein p53 (TP53), and TFRC, in

Glossary

Chaperone-mediated autophagy: a type of autophagy that specifically selects soluble proteins in the cytosol in a chaperone-dependent manner. The selected proteins are directly translocated to lysosomes for cleavage and degradation.
Clockophagy: refers to a selective type of autophagy that sequesters and degrades ARNTL (a cardinal circadian clock protein).
Ferritin: an intracellular protein for iron storage in the form of Fe^{3+} and its controlled release in form of Fe^{2+} during iron deficiency.
Lipophagy: upon lipophagy, autophagosome selectively engulfs intracellular lipid droplets; thus, fuses with lysosome for ultimate degradation of the cargo content.
Maillard reaction products: products derived from thermal degradation of solutions and foods, which impart flavor to starch-based foods.
Mitophagy: denotes specific autophagy of long-lived or damaged mitochondria.



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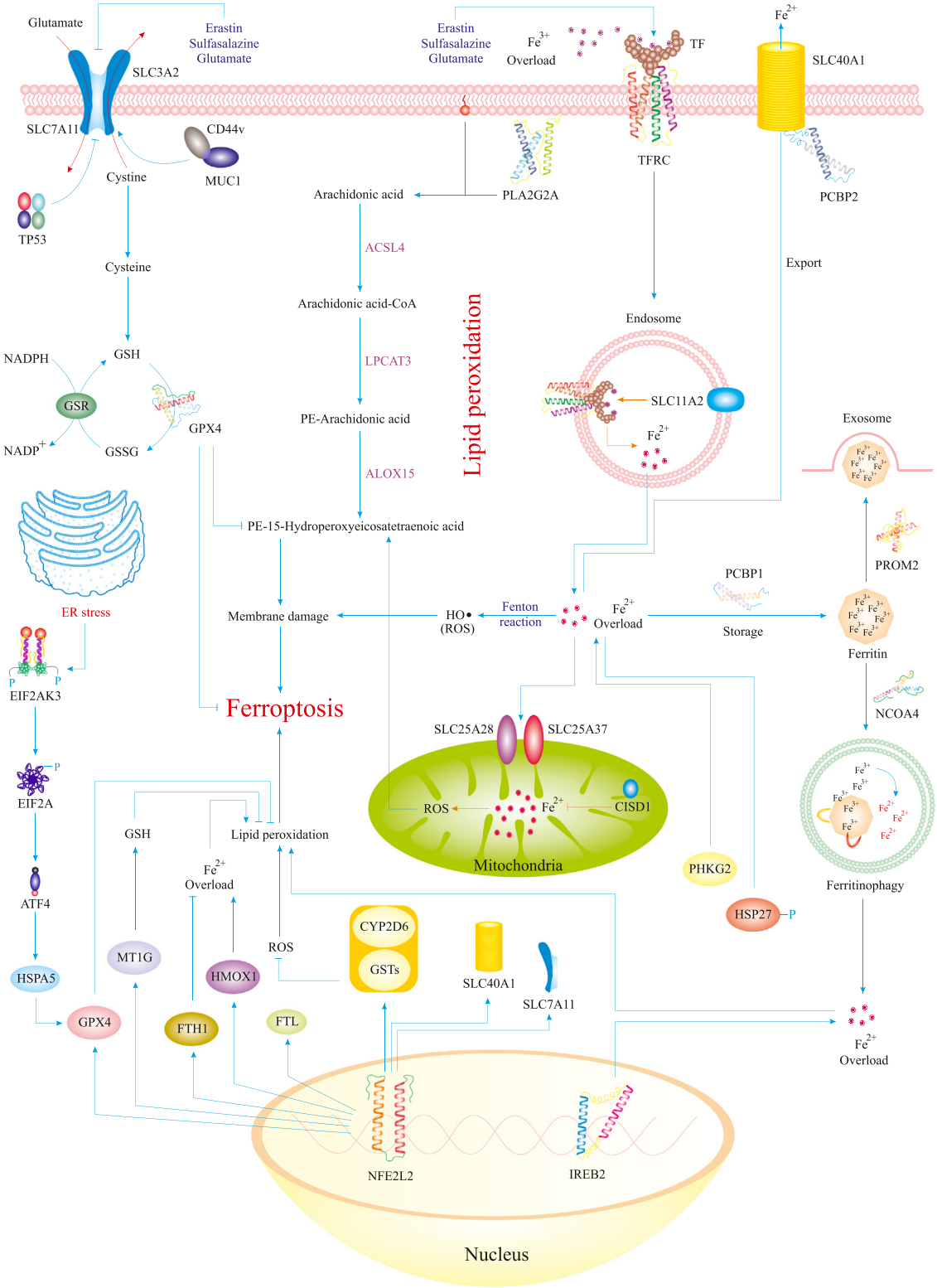
addition to ferroptosis manifested as lipid peroxidation, iron accumulation, and reduced GPX4 function [30]. Interestingly, inhibition of USP7 activates TP53, which in turn, downregulates TFRC, resulting in attenuated ferroptosis and myocardial injury in a rat model of myocardial I/R injury and H9c2 cells subjected to hypoxia/reoxygenation (H/R). Hence, it was concluded that the USP7-TP53-TFRC axis participates in ferroptosis induction upon myocardial I/R injury [30]. Distinct USP subtypes seem to elicit opposite effects on ferroptosis and I/R injury. For example, USP22, sirtuin 1 (SIRT1), and SLC7A11 were downregulated, whereas TP53 was upregulated upon myocardial I/R injury. Overexpression of USP22, SIRT1, and SLC7A11 attenuates infarct size, alleviates cardiac dysfunction, improves cardiomyocyte viability, and suppresses ferroptotic cell death [31]. Thus, interventions against USP22, SLC7A11, and SIRT1 appear to inhibit ferroptosis and alleviate myocardial I/R injury. Taken together, targeting ferroptosis seems to be a reasonable therapeutic approach in the face of myocardial I/R injury.

Ferroptosis in sepsis-mediated cardiac dysfunction

Although ferroptosis induction is implicated in cardiomyopathies, little is unveiled for sepsis-mediated cardiac injury. Septic heart injury is well known to elicit mortality, with a key role for ferroptosis in sepsis-mediated cardiac dysfunction [32]. In particular, sepsis evokes NCOA4-dependent ferritinophagy and increased cytosolic Fe^{2+} content, which upregulates mitochondrial protein sideroflexin 1 (SFXN1), leading to mitochondrial FIO and, subsequently, ferroptosis in lipopolysaccharide-induced cardiac injury (an *in vitro* model for septic heart injury) [32]. However, ferrostatin-1, a ferroptosis inhibitor, and dexrazoxane ameliorated cardiac dysfunction and increased survival rate [32]. Hence, targeting ferritinophagy-induced ferroptosis may prevent cardiac injury in sepsis.

It is noteworthy that conditional depletion of GPX4 in myeloid cells promoted pyroptosis (but not ferroptosis)-related septic mortality in mice, accompanied by tissue damages (including the heart) [33]. These findings suggest the presence of multiple cell death modalities along with ferroptosis in sepsis-evoked organ injury. Overall, ferroptosis is a culprit player in the pathogenesis of sepsis-mediated cardiac dysfunction. Nonetheless, more in-depth effort is needed to explore the precise role of ferroptosis in sepsis and septic complications.

Figure 1. General autophagy pathways and their potential inhibitors. In response to stress such as energy depletion (including glucose and amino acid deprivation), autophagy is triggered. With energy stress, signaling pathways are initiated, ultimately leading to MTORC1 inactivation and, subsequently, phosphorylation/activation of ULK1, which, in turn, mediates autophagy initiation. Glucose and amino acid deprivation culminate in increased levels of Ca^{2+} and AMP in the cytosol, prompting AMPK activation. AMPK phosphorylates/activates (A) proteins that translocate to the nucleus to turn on autophagy genes (*ATG* genes); and (B) proteins that mediate inactivation of MTORC1 and thereby ULK1 activation. However, induction of autophagy may also involve signaling pathways that bypass MTORC1 but still contribute to the initiation of autophagy. For instance, increased Ca^{2+} levels in the cytosol activate DAPK, which phosphorylates BCL2 and mediates its dissociation from BECN1 (a core protein in autophagy). Moreover, Ca^{2+} mediates mitochondrial depolarization, which triggers (A) mitophagy (a specific form of autophagy that sequesters and degrades impaired mitochondria), and (B) ROS generation that activates proteins involved in the autophagy process. Eventually, activated ULK1 and *ATG* gene products form the initiation complex of autophagy, which later recruit other ATG proteins to form phagophores (an incomplete double-membrane structure that starts sequestration of autophagic cargos). Subsequently, the phagophore matures into a complete double-membrane structure, namely the autophagosome, via the ATG12–ATG5-ATG16L1 complex that participates in the conversion of LC3A into its functional form (LC3B), which interacts with autophagy cargo receptors. Autophagosome then fuses with lysosomes for degradation of autophagy cargos into their building blocks. So far, AMPK and ULK1 inhibitors, ROS scavengers, class III PtdIns3K complex inhibitors, which block the formation of phagophores, and inhibitors of lysosome–autophagosome fusion are utilized in preclinical and clinical studies as autophagy inhibitors [1,2,130]. Abbreviations: AMBRA1, autophagy and beclin 1 regulator 1; AMPK, 5′-AMP-activated protein kinase; ATG12, autophagy related 12; ATG16L1, autophagy related 16 like 1; BCL2, BCL2 apoptosis regulator; BECN1, beclin 1; BNIP3, BCL2 interacting protein 3; CALM1, calmodulin 1; CAMKK2, calcium/calmodulin dependent protein kinase 2; DAP1K, death associated protein kinase 1; DDIT4, DNA damage inducible transcript 4; DRAM1, DNA damage regulated autophagy modulator 1; FOXOs, forkhead box O transcription factor family; FUNDC1, FUN14 domain containing 1; GABARAPL1, GABA type A receptor associated protein like 1; HIF1A, hypoxia inducible factor 1 subunit alpha; JUN, Jun proto-oncogene, AP-1 transcription factor subunit; LC3A, solute carrier family 3 member 2; MAPK8, mitogen-activated protein kinase 8; MAP1LC3B/LC3B, microtubule associated protein 1 light chain 3 beta; MTORC1, mechanistic target of rapamycin kinase complex 1; PE, phosphatidylethanolamine; PIK3C3/VPS34, phosphatidylinositol 3-kinase catalytic subunit type 3; PIK3R4/VPS15, phosphoinositide-3-kinase regulatory subunit 4; RB1CC1/FIP200, RB1 inducible coiled-coil 1; ROS, reactive oxygen species; RPTOR, regulatory associated protein of MTOR complex 1; SIRT1, sirtuin 1; TP53, tumor protein p53; SQSTM1, sequestosome 1; TSC1, TSC complex subunit 1; ULK1, unc-51 like autophagy activating kinase 1.



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Ferroptosis in atherosclerosis

Atherosclerosis is a pathological state characterized by altered lipid metabolism, buildup of cholesterol, and sustained inflammation in arterial intima [34]. Ample evidence has depicted a role for ferroptosis in the etiology of atherosclerotic plaque formation, and inhibition of ferroptosis retards lipid peroxidation and atherogenesis and preserves endothelial function both *in vivo* and in cultured mouse aortic endothelial cells [34]. Microvascular leaks in atherosclerotic intima or breaches in the intimal endothelial layer favor extravasation of erythrocytes, a source of iron-rich hemoglobin. An unbiased proteomic examination in human atheroma identified a heme-degrading enzyme, biliverdin reductase, as a prominent protein in atherosclerotic plaques. Extracellular iron deposits can promote local ROS production through the Fenton reaction to augment oxidative stress in plaques [35]. Air pollution has been linked to increased prevalence of atherosclerosis, where ferroptosis appears to play a major role. Defective iron storage and uptake due to altered levels of TFRC, FTH1, and ferritin light chain (FTL), depletion of GSH and NADPH, as well as ferroptosis, were all implicated in PM2.5-mediated endothelial injury [36].

In endothelial cells, *MIR17-92* overexpression averts ferroptotic cell death and ROS generation and upregulates ACSL4 via inhibition of TNF alpha induced protein 3 (TNFAIP3, also known as zinc lipoprotein A20) in primary human umbilical vein endothelial cells (HUVEC). Not surprisingly, *MIR17-92* knockdown reverses these effects [37]. TNFAIP3 is a target of *MIR17-92* in endothelial cells; thus, the *MIR17-92-TNFAIP3*/A20-ACSL4 axis protects against erastin (a ferroptosis inducer)-mediated ferroptosis and provides a new therapeutic avenue for the regulation of ferroptosis in endothelial cell-associated diseases. In sum, the current literature suggests that ferroptosis is implicated in the pathology of atherosclerosis, yet, more studies are needed in this scope.

Ferroptosis in diabetes mellitus

In murine type 2 diabetes, ferroptosis is suggested to contribute to distortion and injury of pancreatic islets, glucose tolerance, iron deposition, and diabetic symptoms, which are recovered following inhibition of ferroptosis using quercetin, suggesting a role for ferroptosis in deteriorated pancreatic β cell function and etiology of diabetes mellitus [38]. Also, ferroptosis is implicated in diabetes-mediated cognitive impairment through altered solute carrier family 40 member 1 (SLC40A1) [39].

Figure 2. Ferroptosis: lipid peroxidation and IO. Upon extracellular IO, Fe^{3+}-bound TF binds to TFRC and mediates formulation of the endosome, inside which Fe^{3+} turns to Fe^{2+} and ultimately causes Fe^{2+} overload in the cytosol (certain intrinsic events can also lead to Fe^{2+} overload in the cytosol). Through the Fenton reaction, IO mediates ROS generation, resulting in lipid peroxidation, a process that also requires enzymes, including ACSL4, LPCAT3, and ALOX15. As a consequence, lipid peroxidation leads to membrane damage and ultimately induces necrosis-like cell death (ferroptosis). Also, cytosolic IO may trigger mitochondrial IO, ROS generation, lipid peroxidation, mitochondrial membrane damage, and ultimately ferroptotic morphology and dysfunction of mitochondria. However, mammalian cells are adapted to resist lipid peroxidation and, thereby, ferroptosis. For instance, cystine/glutamate antiporter systems mediate activation of GPX4, an antioxidant enzyme, which nullifies lipid peroxides, to interrupt ferroptosis. Moreover, accumulated Fe^{2+} can be exported via SLC40A1 or can be stored as ferritin, leading to alleviation of preferroptosis status. Under pathological conditions, these antiferroptotic systems would lose normal function, leaving cells unprotected against ferroptosis and ferroptosis stimulators. Also, basal induction of ferritinophagy replenishes Fe^{2+} via ferritin degradation upon iron deficiency, thus acting as a cytoprotective mechanism. However, under pathological conditions ferritinophagy may contribute to Fe^{2+} accumulation and exacerbation of lipid peroxidation and ferroptosis. In the nucleus, some factors such as IREB2 can trigger IO through manipulation of genes involved in iron metabolism, whereas NFE2L2 is a master regulator of lipid peroxidation and ferroptotic gene expression, contributing to alleviation of ferroptosis [11]. Abbreviations: ACSL4, acyl-CoA synthetase long-chain family member 4; ALOX15, arachidonate 15-lipoxygenase type B; ATF4, activating transcription factor 4; CD44v, variant isoform of CD44 [CD44 molecule (Indian blood group)]; CISD1, CDGSH iron sulfur domain 1; CYP2D6/P450, cytochrome P450 family 2 subfamily D member 6; EIF2A, eukaryotic translation initiation factor 2A; EIF2AK3, eukaryotic translation initiation factor 2 alpha kinase 3; ER, endoplasmic reticulum; FTH1, ferritin heavy chain 1; FTL, ferritin light chain; GPX4, glutathione peroxidase 4; GSH, glutathione; GSR, glutathione-disulfide reductase; GSSG, glutathione disulfide; GSTs, glutathione *S*-transferases; HMOX1, heme oxygenase 1; HSPA5, heat shock protein family A (Hsp70) member 5; HSPB1, heat shock protein family B (small) member 1; IO, free iron overload; IREB2, iron responsive element binding protein 2; LC3A2, solute carrier family 3 member 2; MT1G, metallothionein 1G; MUC1, mucin 1, cell surface associated; NCOA4, nuclear receptor coactivator 4; NFE2L2, nuclear factor, erythroid 2 like 2; PCBP1, poly(rC) binding protein 1; PCBP2, poly(rC) binding protein 2; PE-arachidonic acid, phosphatidylethanolamine-arachidonic acid; PHKG2, phosphorylase kinase catalytic subunit gamma 2; PLA2G2A, phospholipase A2 group IIA; SLC11A2 (solute carrier family 11 member 2), PROM2, prominin 2; ROS, reactive oxygen species; SLC25A28, solute carrier family 25 member 28; SLC25A37, solute carrier family 25 member 37; SLC40A1, solute carrier family 40 member 1; TF, transferrin; TFRC, transferrin receptor; TP53, tumor protein p53.

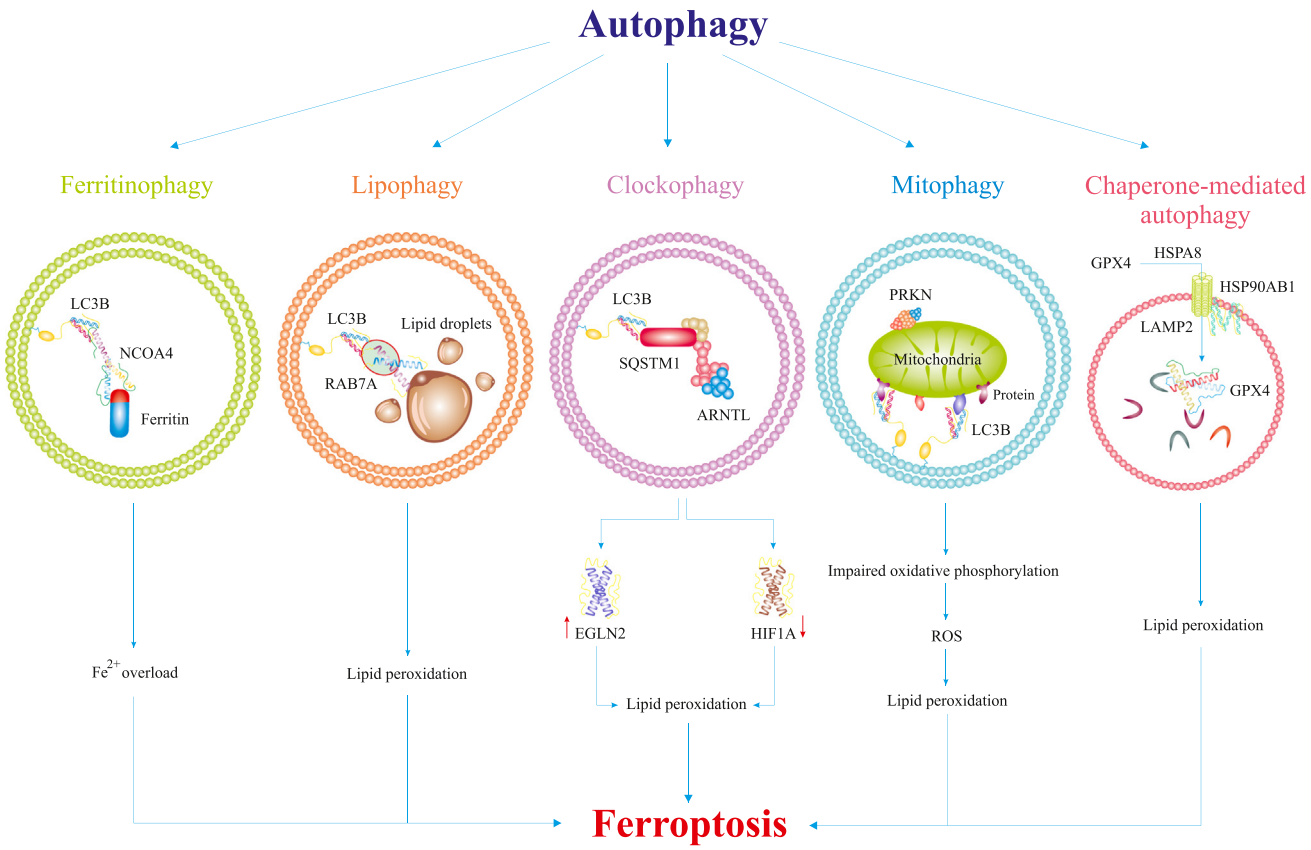

Figure 3. Different types of autophagy can induce ferroptosis. Although basal autophagy rescues mammalian cells against stresses, excessive/maladaptive autophagy can contribute to ferroptotic cell death. Selective degradation of ferritin (ferritinophagy), lipid droplets (lipophagy), ARNTL (clockophagy), mitochondria (mitophagy), and chaperone-mediated autophagy provoke lipid peroxidation and subsequent ferroptosis induction. Thus, not only ferritinophagy but also other types of autophagy can be an avenue for manipulation and regulation of lipid peroxidation and ferroptosis. For instance, ARNTL degradation promotes ferroptosis via inhibiting HIF1A-mediated lipid storage and fatty acid uptake. HIF1A regulates hypoxic response and executes an antiferroptotic role via FABP3 upregulation in the heart and muscles and FABP7 upregulation in the brain for lipid storage [131]. Also, EGLN2 is the target gene of ARNTL, which further regulates HIF1A levels upon ferroptosis [131]. Abbreviations: ARNTL, aryl hydrocarbon receptor nuclear translocator like; EGLN2, egl-9 family hypoxia inducible factor 2; FABP3, fatty acid binding protein 3; GPX4, glutathione peroxidase 4; HIF1A, hypoxia inducible factor 1 subunit alpha; HSPA8, heat shock protein family A (Hsp70) member 8; HSP90AB1, heat shock protein 90 alpha family class B member 1; LAMP2, lysosomal associated membrane protein 2; LC3B, light chain 3 beta; NCOA4, nuclear receptor coactivator 4; PRKN, parkin RBR E3 ubiquitin protein ligase; RAB7A, RAB7A, member RAS oncogene family; ROS, reactive oxygen species; SQSTM1, sequestosome 1.

Furthermore, cryptochlorogenic acid compounds extracted from mulberry leaves exhibited an anti-diabetic property attributed to ferroptosis inhibition both *in vitro* and *in vivo* [40].

From the clinical perspective, it was perceived that diabetic patients suffer from poor ischemic stroke outcomes. Subjecting diabetic mice to thromboembolic middle cerebral artery occlusion (MCAO) followed by deferoxamine (DFO, an iron chelator) treatment showed that ferroptosis inhibition averted poststroke cognitive damage and vasoregression [41]. Following H/R in diabetes, both ferroptosis and necroptosis contributed to cell death in brain microvascular endothelial cell (BMVEC) [42].

Besides, maternal hyperandrogenism and insulin resistance (HAIR) is suggested to trigger fetal loss via ferroptosis induction through mitogen-activated protein kinase 1 (MAPK1)-MAPK3-MAPK14/p38-MAPK8/JNK signaling and upregulation of dipeptidyl peptidase 4

(DPP4) in the placenta and gravid uterus [43,44]. Moreover, Zhang and colleagues reported that maternal HAIR activates ferroptosis in the placenta and gravid uterus through distinct mechanisms. Also, they showed that other cell death mechanisms may be involved in the coordination or compensation of HAIR-induced ferroptosis in the dysfunctional placenta and gravid uterus [45].

Diabetes can aggravate myocardial I/R injury in a manner dependent on ferroptosis and endoplasmic reticulum (ER) stress [46]. In rat diabetic I/R, H/R, and high glucose models, ferroptosis inhibition reduced ER stress and ER stress suppression inhibited ferroptosis, leading to alleviation of myocardial injury [46]. These findings should offer evidence that ferroptosis and ER stress might be intermingled mechanistically, which complicates the manipulation of each process in disease settings [47]. Besides, activation of the TP53-SLC7A11-GSH axis contributes to ferroptosis in endothelial cells under hyperglycemia, suggesting a role for ferroptosis in diabetes-induced endothelial dysfunction [48]. Although limited information is available for the role of ferroptosis in diabetes mellitus, it is well perceived that ferroptosis inhibition may alleviate or prevent diabetes mellitus and associated complications.

Ferroptosis in kidney diseases

Ample evidence has suggested a role for ferroptosis in kidney diseases. Specific deletion of *PANX1* suppresses ferroptotic cell death, lipid peroxidation, and Fe^{2+} overload, thereby ameliorating renal I/R injury in HK-2 cells and mouse models of renal I/R injury [49]. Mechanistically, *PANX1* deletion inhibited ferritinophagy via abrogating the RAS related (RRAS)-Raf-1 proto-oncogene (RAF1)- mitogen-activated protein kinase 1 (MAP2K1)-MAP2K2-MAPK1-MAPK3 signaling cascade [49]. Therefore, the pannexin 1 (PANX1)-RRAS-RAF1-MAP2K1-MAP2K2-MAPK1-MAPK3 axis is expected to regulate ferritinophagy, global autophagy, and ferroptosis, thus denoting a role for ferritinophagy and ferroptosis in AKI in the perioperative period.

In a renal I/R injury model, prolonged mechanical ventilation exacerbated renal I/R injury due to ferroptosis induction, manifested by aberrant mitochondrial morphology [50]. As reported, a combination of ferrostatin-1 and TIMP metallopeptidase inhibitor 2 (TIMP2) knockout or knockdown reduces ferroptotic cell death in a lethal renal I/R injury model *in vivo* and *in vitro*, leading to alleviation of renal failure. Mechanistically, TIMP2 participates in ferroptosis and kidney injury through activation of TIMP2-NFE2L2-SLC7A11. In this context, the *TIMP2* gene can be a potential target for the inhibition of ferroptosis [51].

Furthermore, murine *GPX4* knockout model displays acute renal failure, indicating a role for ferroptosis inhibition in the prevention against I/R-induced hepatic damage [52]. Moreover, overexpression of the renal proximal tubule-residing myo-inositol oxygenase (MIOX) aggravates cellular redox damage and ferroptosis by promoting ferritinophagy and suppressing GPX4 activity, NADPH, and GSH levels in HK-2 cells treated with cisplatin (an *in vitro* model of AKI), and CD1 mice (an *in vivo* model of nephropathy) [53]. Therefore, MIOX may be a potential target for intervening in ferroptosis and AKI.

Ferroptosis is involved in vascular calcification, typically associated with end-stage renal disease (ESRD). In a calcified model of vascular smooth muscle cells (VSMCs), ferroptosis was involved through SIRT2 downregulation and, thereby, mitochondrial carrier 1 (MTCH1) upregulation [53]. However, further studies are needed to elucidate how ferroptosis can modulate gene expression of *SIRT2* and *MTCH1*. Also, in a murine model of AKI induced by folic acid, *NR1D1* and *NR1D2* knockout attenuated ferroptosis and AKI [54]. That is because *NR1D1* and *NR1D2* promote ferroptosis via suppression of SLC7A11 and HMOX1, indicating a role for circadian clock elements

[nuclear receptor subfamily 1, group D, members 1 and 2 (NR1D1 and NR1D2)] in ferroptosis upon AKI. Additionally, SR8278 (an antagonist of NR1D1 and NR1D2) endow similar effects and block ferroptosis by targeting NR1D1 and NR1D2 [53].

Furthermore, severe acute pancreatitis-induced AKI is accompanied by upregulation of ferroptotic genes and shrunken mitochondria [55]. Upregulation of *Mir378a-3p* and *Mir182-5p* contribute to ferroptosis through downregulation of GPX4 and SLC7A11 through direct binding to SLC7A11 mRNA and the 3′ untranslated region of GPX4 in a rat model of renal I/R injury, revealing one of the mechanisms through which I/R induces ferroptosis in renal cells, and silencing of these miRNAs might alleviate I/R-induced ferroptosis [56]. Conditional depletion of GPX4 in kidneys led to acute renal failure in mice in a ferroptosis-dependent manner [57], highlighting the importance of lipid peroxidation in the pathogenesis of kidney damage.

Overall, ferroptosis is a maladaptive process that contributes to the initiation or progression of metabolic diseases (Box 1), including the aforementioned pathological settings. Studies have shown that metabolic stress activates distinct preferroptosis signalings, which would converge at the induction of cytosolic iron overload (IO) and, subsequently, lipid peroxidation, ultimately ferroptosis. Although the therapeutic intervention of ferroptosis should be considered in a context-dependent manner, here we only delve into ferritinophagy and its manipulation targets, as one of the potential avenues for the regulation of ferroptosis.

Ferritinophagy-mediated ferroptosis

A quantitative proteomic assay has unveiled a role for NCOA4 as a ferritinophagy receptor, which interacts with an arginine residue in the C terminal domain of FTH1, accounting for selective sequestration and degradation of ferritin and elevated Fe^{2+} bioavailability in the cytosol [58]. Increased levels of Fe^{2+} negatively regulates the abundance of NCOA4 through two independent mechanisms, including: (i) mediating its ubiquitination via interacting with HECT and RLD domain containing E3 ubiquitin protein ligase 2 (HERC2), or (ii) mediating autophagic degradation of NCOA4 through an unknown mechanism [59].

Given that NCOA4 enhances Fe^{2+} bioavailability in the cytosol, a rather simple negative feedback inhibition mechanism for NCOA4 may also be possible. Upon excessive induction of ferritinophagy, Fe^{2+} overload contributes to the peroxidation of lipids, particularly PUFAs, resulting in plasma membrane damage and hitherto unidentified downstream signaling cascades that mediate ferroptotic cell death. Therefore, inhibition or partial downregulation of ferritinophagy can alleviate Fe^{2+} overload and, subsequently, lipid peroxidation and ferroptosis.

Besides, whether metabolic diseases impair ferritinophagy homeostasis proceeding evident changes in global metabolism or vice versa remains unclear. However, as aforementioned, MIOX hyperactivation elicits metabolic disorder that culminates in ferroptosis via different mechanisms, including the reduction of NADPH and GPX4 activity in HK-2 cells [53]. Thus, it can be postulated that metabolic disorders may proceed change global metabolism much sooner than the impairment of ferritinophagy homeostasis. Although evidence is still lacking for the regulatory role of ferritinophagy on metabolic enzymes, it may be speculated that ferritinophagy may directly influence the function of Fe^{2+}-containing metabolic enzymes, such as phenylalanine hydroxylase, via releasing Fe^{2+} to the cytosol. Overall, the potential adverse effect of ferritinophagy regulation on metabolic homeostasis is that ferritinophagy activation can induce ferroptosis, whereas its inhibition can inhibit ferroptosis. In the next section, we delineate signaling pathways implicated in ferritinophagy to summarize potential therapeutic targets for manipulation/inhibition of ferritinophagy as a potential avenue for the regulation of ferroptosis.

Box 1. Ferroptosis in other metabolic diseases

Ferroptosis in hepatic diseases

It was revealed that Fe^{2+} overload-induced ferroptosis contributes to hepatic I/R injury after liver transplantation in a mouse model of hepatic I/R injury [106]. In response to erastin-induced ferroptosis, the NFE2L2-sestrin 2 (SESN2) axis is activated, leading to SESN2 upregulation, which inhibits ROS generation, GSH depletion, and ferroptotic cell death in injured liver [107]. Thus, it is thought that SESN2 plays a protective role against ferroptosis and therapeutic strategies based on SESN2 manipulation might be beneficial. Intestine-specific SIRT1 was proven to be detrimental in alcoholic liver injury. However, intestinal *SIRT1* knockout mitigates hepatic ferroptosis, increases GSH levels, reduces lipid peroxidation, and downregulates ferroptotic genes [108].

Ferroptosis in lung diseases

NFE2L2 primarily participates in the regulation of lipid peroxidation and cellular oxidation status, although its role in ferroptosis upon acute lung injury (ALI) is largely unknown. It was found that NFE2L2 knockdown significantly downregulates SLC7A11 and HMOX1 and inhibits ferroptosis in pulmonary epithelial cells upon intestinal I/R-induced ALI [109]. Therefore, targeting the NFE2L2-HMOX1-SLC7A11 axis might be a strategy to inhibit ferroptotic cell death. Moreover, NFE2L2 activation using dimethyl fumarate enhances cell viability and GSH, depletes lipid peroxidation, increases GPX4 and FTH1 expression, and preserves the integrity of mitochondrial membrane upon seawater drowning-triggered ALI, indicating the therapeutic utility of NFE2L2 activation to exhaust ferroptosis processes by reinvigorating redox balance in cells [110]. Furthermore, protein phosphatase 1 regulatory subunit 13 like (PPP1R13L) inhibits ferroptosis in a manner dependent on the NFE2L2-hypoxia inducible factor 1 subunit alpha (HIF1A)-transferrin (TF) pathway in lung epithelial type 2 cells and mice subjected to intestinal I/R-induced ALI [111]. Although it is still a long journey to establish the clinical utility of PPP1R13L, and PPP1R13L-NFE2L2-HIF1A-TF signaling cascade upon intestinal I/R-induced ALI, it might be a potential therapeutic target to block ferroptosis. Also, inhibition of ferroptosis using liproxstatin-1 remarkably reduces inflammatory elements, including C-X-C motif chemokine ligand 1 (CXCL1) and interleukin 6 (IL6) in lung I/R injury in both *in vivo* and *in vitro* models [112]. Therefore, a maladaptive axis of ferroptosis-inflammation is one of the underlying causes of lung I/R-induced damages, suggesting the importance of targeting ferroptosis to ameliorate inflammation.

Ferroptosis in cerebrovascular diseases

Cerebral ischemia is the most prevalent type of cerebral injury. Ferritin overexpression reverses cerebral I/R-induced microtubule-associated protein tau (MAPT) hyperphosphorylation, ROS generation, and GSH consumption and downregulates SLC7A11 and TP53 in an Irish rat model subjected to MCAO. In this regard, it was suggested that cerebral I/R induces ferritin reduction, leading to ferroptosis in hippocampal neurons in a manner dependent on TP53 and SLC7A11 upregulation, suggesting that regulating ferritin dynamic status is of significant importance for the regulation of FIO, as well as signaling pathways associated with ferroptosis [113]. Similarly, subjecting cultured neurons to hemoglobin and hemin [for simulation of intracerebral hemorrhage (ICH)] revealed that lysed blood cells activate ferroptotic and necroptotic signaling cascades leading to cell death upon brain hemorrhage, suggesting that tremendous efforts should be given towards hemin-induced ferroptosis and necroptosis, as well as their targeting, as part of a new therapeutic era in the context of ICH and stroke [114]. Rats with sepsis-associated encephalopathy develop cognitive impairment and display increased ferroptosis, whereas using DFO reverses these effects via activation of the NFE2L2-GPX4 axis [115]. Moreover, ferroptosis is induced in a murine model of traumatic brain injury, although administration of Fer-1 attenuates injury lesions and neuronal degeneration, as well as improves long-term cognitive function [116]. Targeting ferroptosis is also a therapeutic strategy for spinal cord injury, given that DFO inhibits ferroptosis and endorses protection against spinal cord injury in rat models [117]. Besides, arsenite-induced neurotoxicity was attributed to ferroptosis induction via ferritinophagy activation both *in vitro* and *in vivo* [118].

Ferroptosis in intestinal diseases

The role of ferroptosis in intestinal I/R injury remains largely unknown. It was revealed that inhibition of ACSL4, a key regulator of lipid composition, blocks ferroptotic cell death prior to reperfusion upon intestinal I/R injury. Also, Sp1 transcription factor (SP1) binds to the promoter region of *ACSL4* and upregulates it, suggesting that the SP1-ACSL4 axis participates in ferroptosis induction during intestinal I/R injury. In this context, both SP1 and ACSL4 could be potential therapeutic targets [119]. Besides, the heterozygous deletion of GPX4 in intestinal epithelial cells mediates the etiology of Western-style diet-related inflammatory bowel disease in mice [120].

Manipulation of ferritinophagy: signaling pathways and molecular targets

It is believed that inhibition especially towards ferritinophagy may interrupt ferroptosis in metabolic diseases. For instance, NCOA4 deficiency abolishes ferritinophagy and confers protection against erastin-induced ferroptosis, while its overexpression retards RAS-selective lethal (RSL3)-triggered ferroptotic cell death [60]. Besides, knockdown of the *NCOA4* gene suppresses erastin-induced ferroptosis, whereas its overexpression sufficiently evokes ferroptosis [5]. Furthermore, NCOA4 silencing downregulates SFXN1 and blocks SFXN1-mediated mitochondrial Fe^{2+} overload,

which alleviates cardiomyocyte hypertrophy upon apelin-13 treatment, suggesting inhibition of ferroptosis with genetic knockdown of ferritinophagy-specific elements, especially NCOA4 [61].

Scrutiny of the effect of hypoxia on ferroptosis in primary human macrophages revealed that hypoxia activates MAPK8, which, in turn, turns on *MIR6862-5p*. Subsequently, *MIR6862-5p* mediates *NCOA4* mRNA degradation. Hence, findings from this study suggest an inhibitory role for the MAPK8-*MIR6862-5p*-*NCOA4* axis on NCOA4-mediated ferritinophagy and IO, and also elevated mitochondrial ferritin (FTMT) to protect against ferroptosis [62]. Moreover, HERC2-mediated ubiquitination and autophagic degradation of NCOA4 can block ferritinophagy and, thereby, ferroptosis [59]. However, therapeutic approaches that boost NCOA4 autophagic degradation or its ubiquitination via HERC2 remain challenging in inhibition of NCOA4-mediated ferritinophagy.

In HepG2 cells, formosanin C-induced ferritinophagy and ferroptosis are more pronounced in cells expressing high levels of NCOA4 and low levels of FTH1 [63]. Moreover, FTH1 overexpression was reported to attenuate ferritinophagy, downregulate NCOA4 and microtubule associated protein 1 light chain 3 alpha (MAP1LC3A/LC3A), and inhibit ferroptosis in a PC-12 cell line and rat Parkinson disease (PD) model [64]. Thus, levels of FTH1 may dramatically impact levels of other genes in ferritinophagy and autophagy, making genetic interventions a potential option for ferritinophagy and ferroptosis inhibition.

Suppression of cargo (ferritin) degradation in autolysosomes leads to ferritinophagy inhibition. In line with this notion, synuclein alpha (SNCA) inhibits ferritinophagy through disrupting hydrolases trafficking to lysosomes, resulting in ferritin accumulation in the outer retina and, subsequently, retinal degeneration in PD *in vivo* and *in vitro* [65]. However, RAB1A overexpression reverses SNCA-mediated ferritinophagy inhibition. Hence, therapeutic maneuvering of RAB1A and SNCA can attenuate ferritinophagy, ferritin degradation, FIO, and ferroptosis [65]. Nonetheless, SNCA does not specifically suppress ferritinophagy and also affects general autophagy, resulting in autophagosome accumulation to contribute to the pathogenesis of certain diseases [66]. This observation should denote the importance of therapeutic strategies in targeting ferritinophagy to minimize the off-target effects of general autophagy and accumulation of autophagosomes.

In hepatic stellate cells, ELAV like RNA binding protein 1 (ELAVL1) upregulation promotes autophagy, autophagosome accumulation, ferritinophagy, and ferroptosis through binding to *BECN1* mRNA (3′-untranslated region) and elevated *BECN1* mRNA stability, indicating a role for the ELAVL1-BECN1 axis in the inhibition of ferritinophagy and autophagosome accumulation [67]. Although more evidence has recently surfaced denoting a role for ferritinophagy in human diseases, more in-depth studies are warranted to elucidate the cellular and molecular regulatory machineries of ferritinophagy.

Manipulation of ferritinophagy: impact on the general autophagy machinery

Given that ferritinophagy represents a type of selective autophagy, manipulation of general autophagy pathways might regulate ferritinophagy and, thereby, ferroptosis. In *ATG5* knockout mice, iron deposition level is decreased, whereas GSH level and GPX4 activity are augmented in early brain injury, indicating blockade of ferroptosis by inhibition of autophagy via attenuated ferritinophagy and ferritin degradation [68]. Also, di-2-pyridyl ketone dithiocarbamate (DpdtC; an iron chelator) induces ferritinophagy and ferritin degradation, leading to ROS generation, which upregulates TP53 and consequently downregulates the AKT serine/threonine kinase 1 (AKT1)- mechanistic target of rapamycin kinase complex 1 (MTORC1) axis, suggesting a role for the ROS-TP53-AKT-MTORC1 axis in the regulation of general autophagy and ferritinophagy

[67]. In this regard, targeting general autophagy might potentially abolish ferritinophagy and ferroptosis.

For autophagy inhibition, pharmacological agents can be utilized, which are categorized into four groups, including: (i) phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3/VPS34) complex blockers; (ii) AAA ATPases blockers; (iii) V-type ATPase blockers; and (D) lysosomal alkalizers [1,4]. For instance, spautin-1 targets deubiquitinating enzymes, including USP10 and USP13, thus mediating degradation of PIK3C3 complexes, leading to inhibited autophagy [69]. Xanthohumol also binds to valosin containing protein (VCP, involved in autophagosome maturation and a member of the AAA ATPase family) and suppresses autophagy [70]. Concanamycin A and bafilomycin A1 also block V-type ATPases, resulting in lysosomal hydrolase dysfunction and, consequently, inhibition of autophagic flux [2,4]. Moreover, monensin, matrine, and chloroquine, which are under preclinical and clinical examination, are lysosomal alkalizers that avert lysosomal acidification and autophagic degradation [71,72]. Figure 1 summarizes potential autophagy inhibitors and their possible signaling targets. Moreover, Box 2 represents new trends in autophagy intervention using nanotechnology and drug delivery systems.

Nonetheless, inhibition of general autophagy also dampens basal autophagy flux, essential for the proper function of mammalian cells. Thus, future studies should be geared towards the development of chemicals and drugs on ferritinophagy with minimal effects on general autophagy. To accomplish this goal, we like to speculate on the existence of 'ferritinophagy-specific molecules' other than NCOA4, which specifically recruit ATG proteins and autophagy elements around the ferritin during ferritinophagy, thus differentiating it from general and other types of autophagy. For instance, high mobility group box 1 (HMGB1) plays a role in ferritinophagy of renal tubules and its inhibition (using isoliquiritigenin) is known to ameliorate lipopolysaccharide-induced AKI due to the inhibition of ferritinophagy and ferroptosis. Therefore, we believe in the existence of ferritinophagy-specific molecules that can differentiate ferritinophagy from general pathways of autophagy. To this end, emphasis should be given to the exclusive targeting of ferritinophagy. Given that ferritinophagy-specific molecules are still at large (except NCOA4 and possibly HMGB1), we propose two main research areas, including: (i) ferritin recognition process during ferritinophagy, and (ii) recruitment process of ATG proteins during autophagosome formation around ferritin, to foster future studies to unravel ferritinophagy-specific molecules. Undoubtedly, identification of such molecules would enable us to specifically target ferritinophagy while leaving basal autophagy intact (Box 3). Such maneuvers should drastically change the translational capacity of ferritinophagy in the realm of metabolic disease management.

General targeting of ferroptosis: a complement for ferritinophagy manipulation

Effective therapeutic regulation of ferroptosis may require the implementation of both ferritinophagy manipulation and other general strategies. Because Fe^{2+} overload is an essential arm of ferroptosis induction, chelating Fe^{2+} using iron chelators has been described in the literature to halt the Fenton reaction and ferroptosis [19,73]. Genetic knockdown of ACSL4 or its inhibition using

Box 2. Autophagy inhibition using nano-drug delivery systems

Using nano-drug delivery systems can optimize inhibitory measures targeting autophagy. For instance, loading metformin to polyethylene glycol–poly lactic acid-co-glycolic acid (PEG-PLGA) nanoparticles as a nano delivery system boosted its bioavailability, enhanced half-life, and increased anticancer capacity [121]. Besides, chitosan nanoparticles can be used for modifying metformin as a targeted drug delivery system due to their remarkable biodegradability and biocompatibility [122]. Moreover, using the polyol method, polymeric iron nano-chloroquine phosphate was constructed, which rendered delayed-release and reduced chloroquine toxicity [123]. Therefore, it is recommended to modify autophagy inhibitors using nanotechnology to embellish their specificity and efficacy.

Box 3. Risks of ferritinophagy inhibition

Although ferritinophagy inhibition is a preferable form of manipulation in human diseases, ferritinophagy activation is beneficial under certain circumstances.

Endothelial PAS domain protein 1 (EPAS1/HIF2A) regulates intestinal ferritinophagy through inducing NCOA4 upregulation during high iron demands. Thus, EPAS1-NCOA4-mediated ferritinophagy is cardinal for systemic intestinal iron homeostasis and absorption [124].

EPAS1 could be a new target for maneuvering ferritinophagy and NCOA4 expression for the management of ferroptosis and iron homeostasis. Moreover, NCOA4-dependent ferritinophagy promotes the survival of murine HT22 hippocampal neuronal cells upon iron deficiency [125].

Due to low iron bioavailability in the cytosol, *ncoa4* knockout mice develop mild anemia and microcytosis [126].

The olamine salt of ciclopirox (CPX-O) induces NCOA4-mediated ferritinophagy and ferritin degradation, resulting in the inhibition of cyst growth and the amelioration of murine polycystic kidney disease [127].

Ferritinophagy inhibition induces iron deficiency, which mediates ER stress [128] through upregulation of endoplasmic reticulum to nucleus signaling 1 (ERN1/IRE1α), leading to hepatic insulin resistance in mice and HepG2 cells in the face of high-fat diet or palmitate challenge [129].

Interrupted ferritinophagy flux due to high-fat diet intake also induces ER stress. High-fat diet or palmitate diminishes the labile Fe^{2+} pool, causes accumulation of sequestosome 1 (SQSTM1/p62), as well as disturbs NCOA4 and ferritin expression, leading to ER stress and aggravation of hepatic insulin resistance in mice and HepG2 cells. To tackle these issues, further studies are warranted to reveal a molecular connection between ferritinophagy flux and ER stress induction [129].

thiazolidinediones (commonly used for type 2 diabetes treatment), such as pioglitazone and rosiglitazone, also remarkably averted ferroptosis [74].

Liproxstatin-1 and ferrostatin-1, two common ferroptosis inhibitors, are widely utilized in various disease contexts. These compounds act as electron donors to radical lipid species in a manner reminiscent of vitamin E and natural flavonoids [8–10]. Ferrostatin-1, as a scavenger of lipid peroxides, blocks butyrate-induced ferroptosis in periodontal ligament fibroblasts [8–10]. Table 1 lists the current utilized therapeutic inhibitors of ferroptosis in metabolic disease settings. However, it is still somewhat remote to optimize these inhibitors for effective utilization in clinical settings.

Given that human lipoxygenases possess six distinct isoforms, isoform specificity remains a paramount challenge for the safe application of lipoxygenase inhibitors (a group of ferroptosis inhibitors such as Zileuton and PD146176) [75]. Risks and adverse effects of lipoxygenase inhibitors can be averted by manufacturing isoform-specific inhibitors. Moreover, the antioxidant activity of ferrostatin-1, liproxstatin-1, and vitamin E may be context-dependent and may not be translated from bench-to-bedside. Thus, it is still inconclusive with regards to the beneficial effects of antioxidants [76]. Importantly, ACSL4 inhibitors such as rosiglitazone might influence the activity of other imperative arachidonic acid processes [74]. Therefore, risk assessment must be given prior to the application of ACSL4 inhibitors.

Of note, implementing a healthy lifestyle undoubtedly decreases the risk of ferroptosis and associated diseases. For example, cigarette smoke extract (constituting methyl vinyl ketone, acrolein) upregulates PTGS2, depletes GSH, and induces ferroptosis in VSMCs, resulting in loss of medial VSMCs in *ex vivo* aorta. However, ferrostatin-1 treatment may reverse these unfordable effects [77]. More evidence suggested that cigarette smoking triggered ferroptosis via NCOA4-mediated ferritinophagy, which contributed to chronic obstructive pulmonary disease using *in vitro* models [78]. Therefore, smoking cessation should help to alleviate ferroptosis.

Table 1. Compounds utilized for ferroptosis inhibition in human diseases[a]

Compound	Description	Function	Refs
Carvacrol	Found in many aromatic plants	Exhibits neuroprotection against cerebral ischemia by attenuation of lipid peroxidation and inhibition of ferroptosis via upregulation of GPX4 *in vivo* and *in vitro*	[82]
(+)-Clausenamide	An alkaloid extracted from *Clausena lansium* leaves	Blocks drug (acetaminophen)-induced liver ferroptosis *in vitro* and *in vivo*, reduces pathological damage to liver, lipid peroxidation, and PTGS2 levels, and increases GPX4 expression, also reacts with KEAP1 and releases NFE2L2 to prompt increased antioxidant capacity	[83]
Compound 51	Promethazine (an antihistamine) derivative	Novel ferroptosis blocker that exhibits therapeutic potential and pharmacokinetic characteristics such as considerable permeability to penetrate the blood–brain barrier in the MCAO model of ischemic stroke	[84]
Dexmedetomidine	An agonist of ADRA2A	Blocks sepsis-mediated ferroptosis and heart failure through upregulation of GPX4 and SOD, increases GSH, downregulates HMOX1, TFRC, NOS2, and GSDMD, and reduces cleaved CASP3 and iron levels in male C57BL/6 mice	[85]
Ferrostatin-1	Ferrostatin-1	Protects brain and promotes neurological function by attenuating lipid peroxidation and *PTGS2* gene expression and inhibiting ferroptosis in a collagenase-induced murine model of ICH	[86]
		Upregulates SLC40A1, reduces iron content and lipid peroxidation, thus ameliorating early brain injury	[87]
		Protects against lipopolysaccharide-induced ALI by targeting ferroptosis	[88]
		Attenuates HFD-induced pathology, fibrosis, inflammation, and cytokine expression by suppressing obesity-triggered ferroptosis in renal and liver tissues	[89]
		Ferrostatin-1 analog (UAMC-3203) has superior *in vivo* efficacy, more soluble and stable	[90]
Galangin	A type of flavonoid	Attenuates lipid peroxidation, upregulates GPX4 and SLC7A11, and protects hippocampal neurons against a gerbil model of cerebral I/R injury	[91]
Irisin	A myokine	Enhances mitochondrial function and GPX4 expression, suppresses inflammation and ferroptosis in septic mice liver	[92]
Melatonin	A pineal gland hormone	Inhibits ferroptosis and provides cerebroprotection upon traumatic brain injury	[93]
Myrrh extract		Mitigates neurological deficits, histopathological injury, and infarct volume by attenuating cerebral inflammation and ROS-mediated ferroptosis via downregulation of the TXNIP-NLRP3 axis in MCAO rats and neurons subjected to OGD	[94]
Naotaifang	A Chinese herbal medicine-derived extract	Confers neuroprotection against experimental acute cerebral ischemia via downregulation of TFRC and DMRT1, reduction of MDA, free iron accumulation, and ROS levels, upregulation of GPX4 and SLC7A11, and enhancement of GSH, which culminate in ferroptosis inhibition	[95]
N-acetylcysteine	An antioxidant	Neutralizes toxic lipids and protects neuronal cells against ferroptotic cell death and boosts functional recovery in rodent hemorrhagic stroke model	[96]
		Blocks ferroptosis in I/R-induced lung injury in rat models	[97]

(continued on next page)

Table 1. (continued)

Compound	Description	Function	Refs
Pachymic acid	A natural steroid derived from *Poria cocos*	Ameliorates renal pathological injury via upregulation of GPX4, SLC7A11, HMOX1, and NFE2L2 in a murine model of I/R-induced AKI	[98]
Proanthocyanidins	Polyphenols found in blueberry	Decreases iron level, ACSL4, TBARS, and ALOX15B, while increasing GSH, NFE2L2, and GPX4, thus blocking ferroptosis in spinal cord injury	[99]
Puerarin	One of the isoflavones	Inhibits ferroptosis via upregulation of NOX4 and downregulation of GPX4 and FTH1, exerts cardioprotective effects in H9c2 myocytes and a rat model of heart failure	[100]
Quercetin	A natural flavonoid found in fruits and vegetables	Inhibits ferroptosis by downregulation of ATF3, which increases GPX4 and SLC7A11, thus ameliorating I/R-induced AKI	[101]
Selenium	Se	Boosts GPX4 expression via activation of TFAP2C and SP1, leading to improved behavior and neuronal protection in a murine model of hemorrhagic or ischemic stroke	[102]
SRS 16-86	A third-generation small chemical compound	Upregulates antiferroptosis elements, including GPX4 and SLC7A11, reduces lipid peroxidation product 4HNE and inflammatory cytokine, leading to neuronal survival	[103]
Tocilizumab mimotope	Immunosuppressant	Blocks ferroptosis, FIO, and lipid peroxidation in fibrotic kidney models	[104]
XJB-5-131	An antioxidant with a strong affinity for tubular epithelial cells	Diminishes renal I/R injury via specific suppression of ferroptosis upon tubular epithelial cells injury	[105]

[a]Abbreviations: ADRA2A, adrenoceptor alpha 2A; ALOX15B, arachidonate 15-lipoxygenase type B; ATF3, activating transcription factor 3; CASP3, caspase 3; GSDMD, gasdermin D; HFD, high-fat diet; 4HNE, 4-hydroxynonenal; ICH, intracerebral hemorrhage; MDA, malonaldehyde; NLRP3, NLR family pyrin domain containing 3; NOS2, nitric oxide synthase 2; OGD, oxygen-glucose deprivation; SOD, superoxide dismutase; SP1, Sp1 transcription factor; TBARS, thiobarbituric acid reactive substances; TFAP2C, transcription factor AP-2 gamma; TXNIP, thioredoxin interacting protein.

Moreover, nutrients and minerals possess antiferroptotic effects. For instance, vitamin E remarkably enhances the survival of murine hippocampal neurons following irradiation through a ferroptosis inhibition-mediated mechanism [79]. Conversely, **Maillard reaction products** such as furosine induce ferroptosis in kidney cells via its furan ring and aldehyde reductase targeting [80]. Besides, fatty acids, particularly arachidonic acid, induces ferroptosis and mediate Crohn's disease [81]. Taken together, excessive attention should be paid towards iron chelators, healthy lifestyle, and ferroptosis inhibitors, as strategies along with ferritinophagy manipulation to counter ferroptic cell death in patients afflicted with metabolic diseases.

Concluding remarks

To date, ample evidence has implicated a vital role of ferroptosis as the underlying cause for various chronic diseases, whereas targeting ferroptosis may ameliorate the pathogenesis of these comorbidities. Many potential targets and general strategies have recently surfaced for the regulation of ferroptosis, yet therapeutic manipulation of ferritinophagy could herald a new era for ferroptosis regulation. We believe that future breakthroughs might enable us to specifically inhibit ferritinophagy and apply relevant targeting drugs to halt ferroptosis and metabolic diseases (see Outstanding questions).

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Outstanding questions

Although much has been done to decipher the process of ferroptosis and its underlying upstream signaling, much needs to be understood. What are the downstream signals ensuing lipid peroxidation and membrane damage that ultimately lead to ferroptotic cell death?

Although disease-specific underlying mechanisms of ferroptosis have been unraveled, no potential therapeutic interventions have been implemented to tackle ferroptosis in these diseases. Therefore, what therapeutics should be applied to effectively target ferroptosis and these mechanisms in a context-dependent manner?

Although inhibiting ferritinophagy may alleviate ferroptosis induction, specific inhibition of ferritinophagy is still lacking. Therefore, what are the hallmarks of ferritinophagy that differentiate it from general autophagy pathways? What smart therapeutics should be designed for specific suppression of ferritinophagy without damaging general autophagy?

Given that ferritinophagy functions as a mechanism to replenish cytosolic Fe^{2+} in iron deficiency, inhibiting ferritinophagy might also dampen its cytoprotective function; thus, ferritinophagy inhibition is a therapeutic strategy that is only suitable under IO and ferroptotic conditions. Therefore, what biomarkers or mathematical and analytical approaches can be developed to notify the preferroptotic status of certain tissue in a real-time manner to guarantee the safety of ferritinophagy inhibition?

Declaration of interests

No interests are declared.

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