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Ferroptosis: Can Iron Be the Downfall of a Cell?

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Abstract

Ferroptosis is one of the forms of programmed cell death. Besides being a necessary micronutrient, iron is the key element that initiates ferroptosis in the cell. Intracellular unstable iron accumulation increases the amount of intracellular ROS, especially by the peroxidation of unsaturated membrane phospholipids. Insufficient antioxidant capacity and decreased glutathione levels play an important role in this process. The research reveals that an imbalance between unoxidized polyunsaturated fatty acids (PUFAs) and oxidized PUFAs, particularly oxidized arachidonic acid, accelerates ferroptosis. These oxidative reactions change the permeability of lysosomal and cellular membranes and cell death occurs. Iron chelators, lipophilic antioxidants, and specific inhibitors prevent ferroptosis. In addition to being accepted as a physiological process, it seems to be associated with tissue reperfusion damage, ischemic, neurodegenerative diseases, hematological and nephrological disorders. Ferroptosis is also being explored as a treatment option where it may offer a treatment option for some types of cancer. In this section, the brief history of ferroptosis, its morphological, molecular, and pathophysiological features are mentioned. Ferroptosis seems to be a rich field of research as a treatment option for many diseases in the future.

Keywords: ferroptosis, iron, RCD (regulated cell death), ROS (reactive oxygen species), lipid peroxidation (LP)

1. Introduction

Iron is an essential micronutrient for all living cells. Microorganisms, plant, animal, and human cells need iron to sustain their vital reactions. However, iron overload can cause various metabolic problems and even cell death. Ferroptosis, which has been revealed in the past years, is a form of regulated cell death that develops depending on the increase in the iron load in the cell [1, 2].

Recently, many new cell death modalities have been described. All cell death is considered primarily “regulated cell death” (RCD) and “accidental cell death” (ACD). The accidental cell death occurs in the cell exposed to chemical and physical attacks, independent of genetic coding and molecular pathways. Accidental cell death is a biologically uncontrolled process. Whereas regulated cell death requires signaling cascades and biologically effector molecules. RCD includes apoptosis, necroptosis, autophagy, ferroptosis, pyroptosis, entotic cell death, netotic cell death, parthanatos, lysosome-dependent cell death, alkaliptosis and oxeiptosis [1].

RCD was first observed in the dying cells of frogs by Karl Vogt in 1842 [1]. Kerr coined the term “apoptosis” for the first time in 1972. Kerr et al. defined apoptosis as a form of programmed cell death (PCD) with morphological changes that differ from necrosis [2]. A new milestone was the identification of CED9 (also known as BCL2 in mammalian cells) and CED4 (also known as apoptotic peptidase activating factor 1 [APAF1] in mammalian cells) from *Caenorhabditis elegans* development studies in the 1990s [1, 3–5]. RCD is also known as PCD when it occurs in physiological conditions [6]. Apoptosis is considered one of the main reference forms when examining cell death models. Thus, studies on apoptosis accelerated and it was revealed that it can develop in two different ways as intrinsic and extrinsic apoptosis. In recent years, many research results have been revealed about other sub-titles of RCD. The current classification system of cell death has been updated by the Nomenclature Committee on Cell Death (NCCD), which formulates guidelines for the definition and interpretation of all aspects of cell death since 2005 [7].

Ferroptosis is a new type of programmed cell death. In 2003, Dolma et al. discovered the molecule erastin (ST), which has a selective lethal effect on cancer cells that express the RAS family of small GTPases (HRAS, NRAS, and KRAS) protein. The pattern of cell death induced by erastin was different from the previous ones. This new form of cell death did not show any nuclear morphological changes, DNA fragmentation, or caspase activation. In addition, this process was irreversible with caspase inhibitors [8]. Yang and Yagoda found the RSL3 (Ras select and lethal 3) component that inhibits this cellular death pattern and revealed that this cell death process can be stopped by iron chelators [8–10]. Erastin and RSL3 treatment do not induce morphological changes consistent with apoptosis, such as cleavage of ADP-ribose polymerase (PARP). The mechanism of cell death induced by erastin and RSL3 is not attenuated by deletion of the intrinsic apoptotic effectors BCL-2-associated X protein (BAX) and a small molecule inhibitor of BCL-2 antagonist/killer 1 (BAK). These differences distinguish the newly described cell death mechanism from apoptosis, autophagy, and necroptosis. Furthermore, neither mitochondrial ROS production nor Ca^{+2} influx is required for cell death in ferroptosis to occur. Erastin has also been found to cause mitochondrial dysfunction by affecting voltage-dependent anion channels (VDAC) [10]. The term ferroptosis was first used by Dixon et al. For cell death in cancer cells with RAS mutations in 2012. This newly recognized form of cell death can be initiated by iron accumulation and prevented by iron-binding chelators. That's why it's called ferroptosis [8–11].

2. Morphological features of ferroptosis

Ferroptosis is characterized as a cell death model defined as morphologically, reduced mitochondrial volume, decreased or completely absent mitochondrial cristae, increased bilayer membrane density, while the cell membrane is intact, the nucleus remains normal in size, and there is no increase in chromatin density [10, 11]. On electron microscopy, it looks similar to the typical dysmorphic mitochondrial appearance caused by Erastin treatment [12]. Biochemically, intracellular glutathione (GSH) depletion, decreased activity of glutathione peroxidase 4 (GPX4) enzyme, inability to metabolize lipid peroxides, and accumulation of large amounts of ROS (Reactive Oxygen Space) due to iron initiate a lethal process similar to Fenton's reaction and genetically regulated by many genes that have not yet been elucidated [13]. Cancer cells with highly active RAS-RAF-MEK (Receptor Tyrosine Kinases) pathways are susceptible to ferroptosis. The genetic mechanisms that regulate ferroptosis may be related to iron homeostasis and lipid peroxidation [14]. Ferroptosis shows similarities to pathways

in other RDC types. Iron-dependent lipid peroxide accumulation is considered to be the basis of the ferroptosis mechanism. It is thought to be a physiological process in mammals rather than a disease or pathological process [15, 16].

3. Accumulation of lipid peroxidase

Mainly phosphatidylethanolamine-OOH (PE-OOH), lipid peroxides are reduced to appropriate lipid alcohols (PE-OOH) by antioxidant reductase mechanisms in the cell under physiological conditions. The effect that will initiate ferroptosis is either by increasing lipid peroxides or by inhibiting the reduction pathway. Glutathione (GSH), the cofactor of glutathione peroxidase (GPX4), is important for the conversion of toxic lipid peroxides to nontoxic lipid alcohols. Glutathione is a tripeptide containing selenocysteine, glutamine, tryptophan. GPX4 catalyzes the following reaction:

This reaction occurs in selenocysteine within the catalytic center of GPX4. During the catalytic cycle of GPX4, active selenol ($-\text{SeH}$) is oxidized by peroxides to selenic acid ($-\text{SeOH}$) and then reduced by glutathione (GSH) to an intermediate selenodisulfide ($-\text{Se-SG}$). GPX4 is eventually reactivated by a second glutathione molecule and glutathione disulfide (GS-SG) is released [17, 18]. GPX4 contains eight neutrophilic amino acids. One of them is selenocysteine and seven cysteines. Selenium, together with cysteine, is essential for the function of GPX4. Inactivation of GPX4 is the most important factor in increasing intracellular lipid ROS and initiating ferroptosis [17–19]. Firstly, in the 1950s, Harry Eagle et al. reported that amino acids, vitamins, and other nutrients are required to protect against oxidative stress in cell culture [20, 21]. Among the molecules reported to be essential was cystine, the oxidized form of cysteine-containing thiol groups [21]. Banni et al., despite glutathione deficiency in human diploid fibroblast cell culture, were able to induce cell growth with α -tocopherol (vitamin E), a lipophilic antioxidant [22].

Compounds that stimulate ferroptosis via GPX4 are divided into four groups (i.e., erastin, RSL3, FIN56, FINO2).

3.1 Erastin

The first group includes erastin (ST). ST inhibits Xc (System Xc-cystine/glutamate antiporter) and decreases intracellular glutathione (GSH) levels. System Xc is an amino acid anti-transporter commonly found in phospholipid bilayer phospholipid membranes. It is an important part of the antioxidant system in cells. It has a heterodimer structure and consists of two subunits, SLC7A11 and SLC3A2, linked to each other by disulfide bonds. System Xc operates on a sodium-free, chlorine-dependent basis. It exchanges cysteine and glutamate in a 1/1 ratio dependent on ATP [21]. Inhibition of the Xc system reduces the uptake of cystine, the oxidized form of cysteine [23]. Cysteine is used in intracellular glutathione synthesis [24]. In cells, GSH synthase and glutamate cysteine synthase synthesize GSH with glutamate, glycine, and cysteine, which is reduced from cystine in the cell as substrates [25]. Glutathione reduces the increased load of ROS and the amount of reactive nitrogen decreased cystine causes a decrease in cysteine and depletion of GSH which uses it as a cofactor, to convert lipid peroxides into suitable lipid alcohols, and an increased intracellular oxidant load [25]. Glutathione reduction and

decreased glutathione peroxidase activity increase ROS accumulation, oxidative damage, and ultimately ferroptosis [19, 24, 25]. Erastin's blocking of the Xc system and disrupting the intracellular lipid ROS balance damages all intracellular organic substances (e.g. proteins, lipids and nucleic acids), particularly lipid peroxidation initiates ferroptosis. It has been shown that erastin, a prototype compound that inhibits GPX4 via system Xc, also causes ferroptosis by affecting voltage-dependent anion channels. Early chemoproteomic studies showed that voltage-dependent mitochondrial voltage-dependent anion channels 2 and 3 (VDAC2, VDAC3) are direct targets for erastin blockade. VDAC2, purified and reconstituted as artificial liposomes, has been shown to be the target of erastin and modulates transport flow [10, 26]. However, it is accepted that erastin initiates ferroptosis mainly by blocking the Xc system cystine/glutamate antitransporter [27]. Also, butionine sulfoximine (BSO), sorafenib, and artesunate induce ferroptosis by depletion of GSH [14, 28, 29]. Reagents or treatments that increase the intracellular amount of cystine/cysteine can reverse erastin-induced ferroptosis, such as β -mercaptoethanol (β -ME), transsulfuration, and processes that increase cysteine synthesis [30–32].

3.2 RSL3

RAS-selective lethal 3 (RSL3), contains an electrophilic moiety and a chloroacetamide moiety and reacts with selenocysteine in the nucleophilic eight amino acid moiety of GPX4, and the enzyme is blocked [33]. Altretamine, which is thought to have a mechanism similar to RSL3, has been defined by the FDA as an anti-cancer drug, but the mechanism of altretamine GPX4 resistance has not been clarified yet [34].

3.3 FIN56

It was named CIL 56, which causes death by ferroptosis in RAS cells while caspases 3 and 7 have no activity. The effect of CIL 56 causing cell death could only be eliminated with low doses of anti-oxidants and iron chelators. At high doses, the lethal effect was irreversible. Later found a CIL56 analog FIN56 (ferroptosis inducing 56) which preserves ferroptosis selectivity in RAS cells. The toxic small-molecule FIN56 is required for mevalonate pathway-mediated ferroptosis. FIN56 can activate its own target protein SQS besides inducing ferroptosis by decreasing the abundance of GPX4 [35]. FIN56 can cause ferroptosis in two ways, either by causing degradation of GPX4 or by reducing the amount of CoQ (i.e., an antioxidant in the cell). The enzymatic activity of acetyl-CoA carboxylase (ACC) is required for FIN56 to degrade GPX4. Therefore, the mechanism of FIN56-induced ferroptosis involves two distinct pathways in association with the mevalonate pathway and fatty acid synthesis. FIN56-mediated mevalonate pathway reduces CoQ. FIN56 binds and activates SQS, the enzyme that converts farnesyl pyrophosphate (FPP) to squalene, which ultimately reduces the level of coenzyme Q10' by reducing the FPP pool available for protein prenylation and metabolite synthesis [35].

3.4 FINO2

FINO2 (endoperoxide-containing 1,2-dioxolane) is a 1,2-dioxolane with both an endoperoxide moiety and a hydroxyl head, capable of inducing ferroptosis. Although its mechanism has not been fully resolved, it indirectly reduces the activity of GPX4. It also provides lipid peroxidation by forming oxygen-centered radicals, similar to the Fenton reaction. Ferroptosis initiated by both FIN56 and FINO2 is partially reversible by β -mercaptoethanol (β -ME) [36, 37].

4. Lipid peroxides and ROS increase

Lipids are important organic molecules because they provide energy for the cell and participate in the structure of cell membranes. Oxygenation of phospholipid (PL) (e.g. PE, phosphatidylcholine, cardiolipin) facilitates ferroptosis. Lipid peroxides are produced in three different ways, each requiring an iron molecule [24, 33, 38]. 1. Lipid ROS produced non-enzymatically by the Fenton reaction with the iron molecule. 2. Lipid peroxides formed by esterification and oxidation of polyunsaturated fatty acids (PUFAs) [24, 33, 39, 40]. 3. Lipid peroxides are formed by catalyzing the iron molecule by lipid auto-oxidation [41]. Fenton reaction is an inorganic reaction that occurs commonly in nature. However, although not fully resolved, (PUFAs) are likely to be the reaction that most contributes to the ferroptosis process [12].

Kagan et al. used RSL3, known as a selective inhibitor, to induce GPX4 inhibition in mouse embryogenic fibroblast cells. RSL3 caused a marked decrease in the chemical activity of GPX4. Among the eight different forms of GPX, GPX4 is the only one that reduces PL-OOH (phospholipid hydroxyls) and PUFA (polyunsaturated fatty acid hydroxyls) in membranes. Kagan et al. screened 350 species of PLs (phospholipids) and identified oxidized AA-containing PE (acyls-arachidonoyl phosphatidyl ethanol) as a ferroptotic cell death signal. AA is a type of PUFAs that can be elongated into adrenoyl (AdA) by elongase [24]. Accumulation of oxidized AA-PE and AdA-PE causes ferroptosis in cells.

It was revealed that the molecule that induces ferroptosis is AA-OOH-PE rather than PL-OOH. The formation of AA-OOH-PE from AA in the cell requires three types of enzymes: 1. lipoxygenases (LOXs), 2. acyl-CoA synthetase long-chain family 4 (ACSL4), and 3. lysophosphatidylcholine acyltransferase 3 (LPCAT3) [11, 17, 24, 39, 40]. In this process, AA is first converted to AA-CoA by being catalyzed by ACSL4, then esterified with LPCAT3 to AA-PE, and finally to AA-OOH-PE with AA-PE LOXs. Generally accepted views 1. Lipid autooxidation is definitely associated with ferroptosis. 2. Lipid oxidation is associated with ferroptosis rather than lipid peroxidation and is a continuation of lipid peroxidation that cannot be prevented from continuing. 3. Lipid peroxidation initiates lipid autooxidation, while lipid autooxidation causes cell death [38, 41]. In cells undergoing ferroptosis, arachidonic acid (AA) is the most affected by autooxidation. Abundant AA residues were observed in the supernatant of mouse embryo fibroblast (MEFs) with GPX4 depletion. Acyl-CoA synthetase long-chain family4 (ACSL4) and lysophosphatidylcholine acyltransferase3 (LPCAT3) encode enzymes involved in the insertion of AA into membrane phospholipids [42, 43]. ACLSs are composed of proteins expressed on the outer membrane of the endoplasmic reticulum and mitochondria. ACLSs are responsible for the formation of acyl-Cos from fatty acids. There are 5 isoforms: ACLS1, ACSL3, ACSL4, ACSL5, ACSL6 [41]. It has been reported that ACSL4 correlates with ferroptosis. ACSL4 is required for ferroptosis to occur in cells with GPX4 knockout or cells with GPX4 [39]. ACSL4 is not the only enzyme that can activate AA arachidonic acid (arachidonic acid) and ADA, but very high concentrations of AA and AdA are required for ACSL3 to activate, for example. However, AA is normally present in the cell in lower amounts than other fatty acids. Thus, ACSL4 is considered a major regulator for AA and ferroptosis [39]. The mentioned 3 enzymes (ACSL4, LPCAT3, LOX) are active in the initial phase of ferroptosis. As a result of these reactions, the amount of intracellular LOOH increases. Increased intracellular LOOH levels and low valent metals (Fe^{+2}) initiate lipid autooxidation, which is essential for ferroptosis. Lipid autooxidation is the specific and final stage of ferroptosis. Lipid autooxidation can only be reversed by radical-trapping antioxidants (RTAs). Lipid autooxidation, rather than LOXs-directed lipid peroxidation, is considered to be the final phase

of ferroptosis that causes cell death [38]. It is accepted that intracellular RTA and LOXs levels determine the sensitivity of cells to ferroptosis. According to this assumption, sensitivity to ferroptosis is a physiological process that is affected by many variables such as cell type, physiological conditions and environmental factors [44].

5. Iron and ROS

Iron is an essential element for almost all living organisms. An adult human body contains about 3–5 g of iron. Iron in erythrocytes accounts for 80% of the total iron, and less than 20% is stored in macrophages and hepatocytes. The iron in the macrophage comes from aged red blood cells and is reused, providing 90% of the daily required iron. Approximately 1 g of iron from the diet per day is absorbed through the gastrointestinal tract as ‘new iron’. Daily iron loss occurs mostly with the desquamation of epithelial cells in the skin and gastrointestinal tract. In women, menstruation and labor bleeding can cause large amounts of iron losses. The excessive increase of iron in the human body causes hemochromatosis, and less than an adequate amount causes anemia [45, 46]. Inside the cell, iron exists in two forms, Fe^{+2} and Fe^{+3} . The Fe^{+2} form is more functional because of its ability to transfer electrons and have high solubility. The Fe^{+3} form is more stable chemically, so this form is suitable for storage and transportation. Fe^{+2} plays an important role in oxidation-reduction reactions. Fe^{+2} reacts with H_2O_2 to form hydroxyl reagent and Fe^{+3} . Thus, the ROS load inside the cell increases and an oxidative process begins for lipids, proteins, and nucleic acids [47]. It has been accepted that iron and ROS may be increased, especially in tumor cells. The increased oxidative capacity of tumor cells may be effective in their growth. However, it is a contradictory opinion that increased ROS and iron content can increase ferroptosis. Toyokuni et al. reported the hypothesis that intracellular iron and thiol redox groups in tumor cells establish a balance for the cell to avoid ferroptosis. It is recognized that there are many more questions to be answered [25, 48]. Circulating non-heme iron can be transported bound and unbound to transferrin (Tf). Transferrin (Tf) is a glycoprotein with two high-affinity sites specific to ferric iron (Fe^{+3}). When circulating Tf is fully saturated, iron can be transported independently of Tf ferric iron is reduced to ferro (Fe^{+2}) iron by the presence of membrane-bound ferri reductases and taken into the cell by divalent metal transporter 1 (DMT1) [25, 49]. Most of the dietary heme iron is in the form of ferric iron. The absorption of inorganic iron from the lumen into the enterocyte in the duodenal villi is regulated in a very complex and molecularly controlled system. The first step in absorption is Fe^{+3} reduced to Fe^{+2} by ascorbate-dependent duodenal sitokrom *b* (DCYTB), a membrane-bound reductase. Ferrous iron is taken up into the enterocyte by DMT1 on the lumen-facing surface of the enterocyte. DMT1 is the most important molecule of nonheme iron intake. The synthesis of both DCYTB and DMT 1 is increased in iron deficiency [49–51]. Although intestinal absorption of heme iron (e.g., red meat) is effective for the human organism, it is by a mechanism that is not yet clearly understood. For heme absorption from the duodenum and upper jejunum, coordination of heme carrier protein 1 (HCP1) and heme responsive gene-1 is required [52, 53].

Under physiological conditions, the high-affinity Tf receptor 1 (TfR1) on the cell surface can bind two $\text{Fe}(\text{III})$. The $\text{Tf-Fe}^{+2}\text{-TfR1}$ complex is transported into cells via endocytosis to form endosomes. Endosomes release iron from the complex under acidic conditions. Free ferric iron is reduced to ferrous iron and then transported into the cytoplasm by DMT1. Endosomes containing the Apo-Tf-TfR1 complex return to the surface of the cells and ferrous iron becomes part of the labile iron pool (LIP) in preparation for the next

recycling [49, 50] Iron inside the cell can be stored in ferritin, transferred via ferroportin (FPN), or used in synthesis pathways [44]. Most of the intracellular iron is used for heme and iron-containing proteins, especially mitochondrial iron-sulfur-containing proteins (Fe-S) and iron-dependent enzymes. FPN is the only molecule known to transport iron out of the cell [54]. Iron transferred out of the cell via FPN is in ferrous form. In this transfer, ferrous iron is oxidized by extracellular ferroxidase and converted to ferric iron. The free ferric iron that becomes free is bound to Tf, forming Tf-Fe⁺² complexes, and iron is transported to other cells. The iron that is not transported out of the cell and not used in the cell is stored by binding to ferritin. Ferritin is a heterodimer consisting of 24 subunits as ferritin heavy chain 1 (FTH1) and ferritin light chain (FTL). FTH is the domain that binds iron molecules, and FTL can play a role in electron transport. FTH can bind 4500 iron atoms in the ferric form [55]. Iron release from ferritin is also controlled under physiological conditions [56]. In recent studies, nuclear receptor coactivator 4 (NCOA4)-mediated ferrophagy has been shown to induce iron release from ferritin. NCOA4 binds to ferritin and transports it to the lysosome, where ferric iron is decomposed and released [57]. The amount of iron in the cell increases. Therefore, it is postulated that NCOA4-mediated ferritinophagy can induce ferroptosis in the cell [58–61].

6. Regulation of systemic iron

Systemic iron level regulation is carried out through the FPN, which ensures the removal of iron from the cell. FPN is regulated both dependently and independently of hepcidin. In response to adequate systemic iron content, the liver secretes hepcidin into the systemic circulation. Hepcidin binds to FPN in the cell, causing a conformational change in the molecule. The modified FPN molecule is phosphorylated and ubiquitinated, then transported to the lysosome and inactivated by lysosomal enzymes. FPN can also be regulated independently of hepcidin. When there is not enough iron in the cell, FPN undergoes a similar conformational change and is again inactivated in the lysosome. In both cases, the removal of iron from the cell by the FPN is prevented [62].

7. Regulation of intracellular iron

Cell internal iron homeostasis is regulated by iron regulatory protein 1, 2 (IRP1, IRP2) and iron-responsive elements (IREs) molecules. IRPs are proteins that can bind 5',3' (UTR) of the mRNAs of IREs. These proteins are those involved in iron uptake (e.g., DMT1, TfR1), iron sequestration (e.g., subunits of ferritin: FTH1, FTL), and iron export (e.g., FPN). When intracellular iron is deficient, IRPs bind to 5' IREs of ferritin and FPN, and the translocation of these proteins is inhibited [63, 64]. Once iron demand is met, IRPs are degraded and these bonds are removed [63, 64]. Both cellular and systemic iron regulation is related to meeting iron needs. Systemic regulation is provided by the liver and hepcidin. The loss of binding activity of IRP1-IREs for cellular level iron regulation is related to the addition of 4Fe-4S. An E3 ligase complex linked to F-box and leucine-rich repeat protein 5 (FBXL5) drives ubiquitination and proteasomal degradation of IRP-2. FBXL5 requires sufficient iron and oxygen to remain stable. Other genes involved in iron metabolism are IREB2, FBXL5, TfR1, FTH1, and FTL [63–66].

It has been stated that the increase in iron level in the systemic circulation and intracellularly in vivo and in vivo conditions increases the susceptibility to ferroptosis. FPN decreases while Tf increases in ferroptosis-sensitive cells [58, 67]. Lysosomal high

Ferroptosis	
Morphological features	Shrink mitochondria within ceased mitochondrial membrane densities, outer mitochondrial membrane rupture, reduction or vanished of mitochondrial crista, normal nucleus
Biochemical features	Iron accumulation and lipid peroxidation
Death stimulus	Inhibition of cysteineimport (e.g.: erastin, SAS, glutamate Glutathione depletion GPX4 inactivation (e.g. BSO) A.A. depletion in presence of serum and glucose
Regulatory pathway	Xc/GPX4 pathway, P53/SLC7 A11 pathway, ATG5, ATG7-NCOA4 pathway, MVA sulfur transfer pathway, P53-SAT1-ALOX 15 pathway, HSPB1-TRF1, FSP1-COQ10-NAD(P) H pathway, P62-Keap-1NRF2 pathway
Hallmarks	Increased lipid peroxidation iron dependence
Keynes	GPX4, TFR1, SLC7A11, NRF2, NCOA4, P53, HSPB1, ACSL4, FSP1

A.A., amino acid; ACSL 4, acyl-Co Asynthetase long-chain family member 4; ALOX-15, arachidonate lipoxxygenase 15; AP-1, activator protein-1; ATG5, autophagy-related 5; ATG7, autophagy-related 7; COQ10, coenzyme Q10; DRAM3, damage regulated autophagy modulator 3; FSP1, ferroptosis suppressor protein 1; GPX4, glutathione peroxydase 4; HSPB1, heat shock protein beta-1; Keap1, Keleh-like ECH-associated protein 1; MAPK, mitogen-activated protein kinase; MLKL, mixed lineage kinase domain-like protein; m-TOR, mammalian target of rapamycin; MVA, mevalonate; LC3, microtubule-associated protein 1 light chain 3; NCOA4, nuclear coactivator 4; NRF2, nuclear factor erythroid 2-related factor 2; PKC, protein kinase C; RIP, receptor-interacting serine/threonine kinase; ROS, reactive oxygen species; SAT1, spermidine/spermine N1-acetyltransferase 1; SLC7A11, solute carrier family 7 member 11; system, Xc-cysteine/glutamate transporter; TFEB, transcription factor EB; TFR1, transferrin receptor 1; TNF-R1, tumor necrosis factor R1.

Table 1.
Characteristics of ferroptosis.

concentrations of iron can also prepare cells for ferroptosis [67]. Increased susceptibility to ferroptosis has been observed experimentally in mice with a high amount of iron in their diet and with increased extracellular matrix iron levels [68]. The heat shock protein family B member 1 (HSPB1) inhibits iron uptake via TfR1, reduces the level of iron into the cell, and inhibits ferroptosis by increasing the reduced form of GSH. HSPB1 also inhibits endocytosis and Trf1 reuptake by stabilizing the cortical actin cytoskeleton [69–71]. Both oxygenase 1 (HO-1) and phosphorylase kinase catalytic subunit gamma 2 (PHKG2) mediate ferroptosis when the intracellular iron level is increased [9, 72]. Almost all of the studies on this subject emphasize increased intracellular iron levels and ROS load to initiate ferroptosis. Until now, 3 basic processes that increase ROS in the cell due to iron have been reported: 1. by the Fenton reaction, which is inorganically not enzyme-catalyzed 2. via lipid autooxidation catalyzed by iron-containing enzymes 3. ROS formed by AA oxidation via iron-containing LOXs. However, how iron initiates and maintains ferroptosis and the process leading to cell death has not been fully elucidated (**Table 1**) [44].

8. P62 and NRF2 in ferroptosis

Nuclear factor erythroid 2-associated factor 2 (NRF2) is one of the proteins that create the most important antioxidant response in the cell against oxidative imbalance. Under normal conditions, it is preserved by Kelch-like ECH (erythroid cell-derived protein with CNC homology)-associated protein 1-mediated proteasomal degradation. NRF2 negatively regulates ferroptosis via the p62-keap1-NRF2 pathway. NRF2 and p62 competitively bind to Keap1 [73, 74]. Nrf2 plays a vital role in intracellular antioxidant balancing and activation of GPX4, in the re-synthesis of NADPH, 6PGD (phosphogluconate dehydrogenase, malic enzyme, and glucose 6-phosphate dehydrogenase)

and glutathione synthesis, cysteine supply via system Xc (glutathione peroxidase 4, glutathione reductase) play a key role for many genes. Ferroptosis inducers facilitate the interaction between p62 and Keap1. This interaction inhibits Keap1. Inhibition of Keap1 prevents binding between Keap1 and NRF2. The interaction of Keap1 and NRF2 triggers the degradation of NRF2 [75, 76]. It leads to NRF2-mediated ferroptosis by down-regulating genes involved in iron and ROS metabolisms. The most important of these are such as quinone oxidoreductase 1 (NQO1 and HO1) [75, 76].

NRF2 inhibits ferroptosis by increasing the expression of target genes involved in iron and ROS metabolism, such as NQO1 (NADPH Quinone Dehydrogenase 1) and HO1 (Heme oxygenase 1). NRF2-Keap1 pathway supports system Xc so NRF2 inhibits ferroptosis. It also negatively regulates ferroptosis by lowering intracellular reactive iron by gene regulation of Nrf2 ferritin (FTL/FTH) light chain and heavy chains, ferroportin (SLC40A1) subunit, and SLC7A11 component of Xc system. Nrf2 is also activated by oxidized lipids, which are also involved in the initiation of ligand-mediated transcription factor PPAR γ (peroxisome proliferator-activated receptor-gamma). Furthermore, a high NRF2 expression is associated with a worse overall survival rate in patients with glioma, and activation of the NRF2-Keap1 pathway supports system Xc so NRF2 inhibits ferroptosis [77]. The existence of studies reporting that NRF2 induces ferroptosis shows that there are still unanswered questions on this subject.

9. Tumor suppressor protein P53 and ferroptosis

P53 is a tumor suppressor that has been extensively studied. It has a tumor-suppressive effect by stopping metabolic cycles, mediating aging and apoptosis. It is involved in the cellular response to DNA damage, hypoxia, starvation, and oncogene activation. Activation of p53 ensures cell cycle slowdown at the low level of cellular stress, repair DNA damage, prevent ROS accumulation, and cell survival. However, severe cellular stress and damage induce a response of P53 to produce apoptosis and cell death [78]. On the one hand, p53 suppresses ferroptosis either through direct inhibition of DPP4 (dipeptidyl peptidase 4) activity [79] or through induction of CDKN1A/p21 (cyclin-dependent kinase inhibitor 1A) expression. On the other hand, p53 can increase ferroptosis by inhibiting the expression of SLC7A11 (solute transporter family 7 member 11) or by increasing the expression of SAT1 (spermidine/spermine N1-acetyltransferase 1) and GLS2 (glutaminase 2) [78–80].

It is accepted that the direction and intensity of the response of p53 are proportional to the level of stress to which the cell is exposed [80]. It is depleted by a mutation in many types of cancer and its anti-tumor effect is limited [81, 82]. Unlike nuclear p53, which acts as an autophagy-promoting transcription factor [82, 83], cytosolic p53 can block autophagy in response to nutrient starvation or mTOR inhibition [80, 83, 84]. These context-dependent roles of p53 in survival and death are regulated in a fine-tuned manner by its ubiquitination, phosphorylation, acetylation, and other modifications. Unlike nuclear p53, which functions as a transcription factor promoting autophagy, cytosolic p53 (BCL-2 family (BAX [BCL2 associated X, apoptosis regulator] and BBC3/PUMA [BCL2 binding component 3]) can suppress autophagy in response to cellular starvation and mTOR inhibition [81–84]. p53 cellular response is regulated by ubiquitination, phosphorylation, acetylation, and other modifications [81].

Classic ferroptosis model and cell culture studies have revealed that P53 is associated with ferroptosis [79, 82, 84]. The researchers have found that p53 promotes ferroptosis due to transrepression of SLC7A11 expression in fibroblasts and some cancer cells

(human breast cancer MCF7) and human osteosarcoma (U2OS) [79, 85, 86]. P53 plays a role in ferroptosis cascades, which can cause cell survival or death. It can function prodeath or prosurvival at the transcriptional or post-translational level. Depending on the type or severity of stress that the cell is exposed to, it may contribute to apoptosis or autophagy [87, 88].

Inhibition of SLC7A11 expression, increased expression of SAT1 (spermidine/Spermine N1-acetyltransferase 1), increased expression of GLS (Glutaminase) are required for p53-regulated ferroptosis [78]. SAT1 is a regulator of polyamine metabolism. Oxidative stress, inflammatory stimuli, and heat shock have been found to stimulate SAT1 activity. SAT1 is a transcriptional target of p53. An increase in SAT1 does not change SLC7A11 and GPX4 activity but increases ALOX15 (arachidonate 15-lipoxygenase) activity [84]. Thus, the required antioxidant response remains insufficient despite increased lipid peroxidation.

Acetylation of K98 is crucial for p53-mediated ferroptosis. In particular, p53 3KR, an acetylation-defective mutant in which 3 lysine residues (at positions 117, 161, and 162) have been replaced by arginine residues, is highly effective in repressing SLC711A [85–87]. In contrast, p53 4KR98 (an acetylation-defective mutant in which an additional lysine is replaced at position 98) cannot reduce SLC711A expression [80].

Perhaps p53 3KR gains a ferroptosis-inducing capacity while p53 4KR loses it. In human cancers, wild-type p53 is degraded by high levels of the oncogenic E3 ubiquitin-protein ligase MDM2. Thus, inhibition of MDM2-dependent proteasomal degradation of p53 offers an attractive therapeutic strategy for cancer therapy [88]. Since it will not be inactivated in MDM2–/– cells, the p53 level increases. p53 has been shown to contribute to the cell death cascade, which can be termed ferroptosis, which can be reversible by ferrostatin 1 in MDM2–/– mouse embryos. However, another study showed that ferrostatin-1 alone could not prevent cell death caused by MDM2 deficiency [89–91].

The anti-ferroptosis activation of ferrostatin-1 and liproxstatin-1 (another widely-used ferroptosis inhibitor) are mediated through their reactivity as radical-trapping antioxidants rather than their potency as inhibitors of lipoxygenases [90, 91]. The acetylation levels of p53 are localized by six different histone acetyltransferase: 1. REBBP/CBP (CREB binding protein), 2. EP300/p300 (E1A binding protein P300), 3. KAT2B/PCAF (lysine acetyltransferase 2B), 4. KAT5/Tip60 (lysine acetyltransferase), 5. KAT8/MOF (lysine acetyltransferase 8), and 6. KAT6A/MOZ (lysine acetyltransferase 6A). The ability of these acetyltransferases to regulate ferroptosis remains unclear [88, 92, 93].

GSL2 (glutaminase 2) is a mitochondrial enzyme, the first step of glutamine catabolism, and an important regulator of ferroptosis [94]. It is known as a transcriptional target of p53. It is responsible for oxygen consumption and ATP production in cancer cells. It is also known to offer antioxidant support through the production of GSH [95]. While all this is expected for negative regulation of ferroptosis, it has been shown that glutaminase degradation inhibits ferroptosis in fibroblast cells [96]. More research is needed for the relationship between glutaminase, p53, and ferroptosis.

DPP4 (dipeptidyl peptidase-4) is the most important regulator of survival in the ferroptosis-related function of P53. Cells with p53 knockout or pharmacologically inhibited become more sensitive to type I inducer of ferroptosis (erastin and SAS). However, there is no difference in response to typeII ferroptosis inducer (RSL3 and FIN56).

However, DPP4 inhibitors (linagliptin, vildagliptin, and alogliptin) together with other protease inhibitors (doxycycline, ritonavir, atazanavir, VX-222, semagacestat) completely block erastin-induced cell death in p53-deficient cells [79].

Another mediator of p53, CDKN1A/p21 (cyclin-dependent kinase inhibitor 1A), inhibits apoptosis. In cystine deficiency in cancer cells, p53-mediated CDKN1A

expression delays ferroptosis. Again, inhibition of MDM2 by nutlin-3 increases expression of p53, which blocks the ferroptosis induced by loss of Xc function [82]. More comprehensive and detailed studies on p53 and ferroptosis are needed.

10. Beclin-1 and ferroptosis

Beclin-1 (Vps30/Atg6 in yeast) is a well-known regulator of autophagy primarily involved in the formation of the PtdIns3K complex, which is involved in activating autophagy. Beclin-1 is a critical regulator of ferroptosis that is independent of the formation of the PtdIns3K complex. The beclin-1 expression only affects ferroptosis induced by the system Xc-inhibitor. Knockdown of Beclin-1 by RNA interference (RNAi) blocks ferroptosis, whereas knockdown of Beclin-1 by gene transfection promotes ferroptosis in cancer cells in response to system Xc-inhibitors (for example, erastin, sulfasalazine, and sorafenib). In contrast, it does not affect erastin-, sorafenib-, or sulfasalazine-induced ferroptosis. Beclin-1 mandatory for ferroptosis induced by system Xc – inhibitor [97, 98].

It needs ATG5 (related to autophagy 5) and NCOA4 (nuclear receptor coactivator 4). ATG5 is part of an E3-like ligase that is critical for the lipidation of members of GABARAP (GABA type-A receptor-associated protein families) and MAP1LC3 (microtubule-associated protein 1 light chain 3) members. However, NCOA4 is a transporter receptor that mediates FT/ferritin degradation via selective ferritinophagy. Inhibits elastin-induced conversion of MAP1LC3B-I to MAP1LC3B-II by inhibition of Atg5. Furthermore, suppression of NCOA4 blocks the degradation of FT/ferritin, resulting in suppression of ferroptosis. In contrast, knockdown of Beclin-1 does not affect the synthesis of lipidated MAP1LC3B and MAP1LC3B-positive points in ferroptosis. As a positive control in starvation-induced cells, knockdown of Beclin-1 stops the conversion of MAP1LC3B-I to MAP1LC3B-II. Significantly, the formation of a BECN1-PtdIns3K complex was observed in cancer cells only in response to starvation, but not to ferroptotic stimulus. These findings point to the regulatory roles of Beclin-1 in ferroptosis compared to induced autophagy [96–98].

11. AMPK ferroptosis

AMP-activated protein kinase (AMPK), a critical indicator of the cell's energy deficit, is activated through AMP binding, kinase phosphorylation, and other mechanisms. AMP-activated protein kinase (AMPK), a critical indicator of the cell's energy deficit, is activated through AMP binding, kinase phosphorylation, and other mechanisms. AMPK maintains the viability of the cell under energy stress. If this energy balance cannot be achieved, it leads the cell to apoptosis. AMPK exhibits various regulatory roles in lipid metabolism by mediating the phosphorylation of acetyl-CoA carboxylase as well as polyunsaturated fatty acid biosynthesis. AMPK has also been implicated in ferroptosis. The inhibitory effect of AMPK activation on ferroptosis does not include modulation of cystine uptake, iron metabolism autophagy, or mTORC1 signaling. Energy stress-mediated AMPK activation inhibits ferroptosis via mitochondria. The Loss of function of liver kinase B1 (LKB1) sensitizes mouse embryonic fibroblasts (MEFs) and human non-small cell lung carcinoma cell lines to ferroptosis. This LKB1-AMPK-ACC1 (ACC1—Acetyl-CoA carboxylase 1)-FAS (cell surface death receptor) axis has a vital role in regulating ferroptotic cell death [99].

A recent study also reported a supportive role of AMPK in the regulation of Beclin-1-mediated ferroptosis. Specifically, AMPK mediates the phosphorylation of Beclin-1 at Ser90/93/96. This is a prerequisite for the formation of the Beclin1-SLC7A11 complex in ferroptosis and subsequent lipid peroxidation. Inhibition of AMPK by siRNA or compound C reduces erastin-induced Beclin-1 phosphorylation at S93/96, thus inhibiting the formation of Beclin-1-SLC7A11 complex formation and subsequent ferroptosis. Thus, it is clear that Beclin-1 contributes to the core molecular machinery and signaling pathways involved in ferroptosis [100, 101]. The mechanisms of AMPK-mediated regulatory ferroptosis need further investigation.

12. Ataxia-telangiectasia-mutated kinase in ferroptosis

Ataxia-telangiectasia mutated kinase (ATM) is a crucial kinase for DNA damage responses. P53 is one of its sub-targets, which plays a decisive role in the regulation of ferroptosis, which activates its role in ferroptosis [102]. Genetic or pharmacological inhibition of ATM reduces intracellular labile iron by increasing FPN, FTH1, and FTL. Ataxia-telangiectasia mutated kinase (ATM) is a crucial kinase for DNA damage responses. P53 is one of its sub-targets, which plays a decisive role in the regulation of ferroptosis, which activates its role in ferroptosis [103]. Genetic or pharmacological inhibition of ATM reduces intracellular labile iron by increasing FPN and FTH and FTL. It relies on the transcriptional activity and nuclear translocation of metal regulatory transcription factor 1 (MTF1) upon TM inhibition. Under conditions of ATM inhibition, nuclear translocation of MTF1 is increased, resulting in changes in ferritin (FTH1) and ferroportin (FPN) expression, and the amount of intracellular unstable iron is reduced to prevent ferroptosis [104].

13. Iron and ferroptosis

The iron homeostasis in both manners is regulated by iron. In systemic iron regulation, the level of iron is sensed by the liver and the liver secretes the hormone hepcidin according to iron abundance. At the cellular iron level, the loss of IRP1-IREs binding activity depends on the insertion of 4Fe–4S cluster. As for the IRP2, a newly discovered FBXL5-dependent E3 ligase complex catalyzes the ubiquitination and proteasomal degradation of IRP2, while keeping the stability of FBXL5 requires iron and oxygen. IREB2, the main regulator of iron metabolism upon inhibition, reduces sensitivity to ferroptosis. Since iron metabolism is also affected by autophagy, it also regulates ferroptosis in many ways [58]. Ferritinophagy is the autophagy of selective ferritin, in which ferritin is recognized by the specific transport receptor NCOA4, which directs it to autophagosomes for lysosomal degradation. This lysosomal degradation of ferritin releases iron and thus increases ferroptosis susceptibility [45].

Apart from ferritin, HSPB1 and CISD1 are other proteins that affect ferroptosis susceptibility. In addition, heme oxygenase 1 (HO-1) and phosphorylase kinase catalytic subunit gamma 2 (PHKG2) mediate ferroptosis by regulating the abundance of iron [9, 72]. ROS accumulation initiated by labile iron in the cell occurs in three known ways, respectively. 1. non-enzymatic Fenton reaction; 2. ROS accumulated by lipid autooxidation catalyzed by iron-containing enzymes; 3. ROS accumulated by oxidation of arachidonic acid (AA) by lipid peroxidases (LOX). Although it is known that iron is an essential element in ferroptosis, it is not fully understood how iron regulates ferroptosis.

14. Biomarkers of ferroptosis

Prostaglandin-endoperoxide synthase 2 (PTGS2) increases with the formation of lipid peroxides and decreases in nicotinamide adenine dinucleotide phosphate (NADPH) [9, 32]. Moreover, the increase in PTGS2 cannot be suppressed by PTGS inhibitors. Malondialdehyde (MDA), the end product of lipid peroxidation, also increases. GPX4 protects cells against ferroptosis by catalyzing GSH and toxic PE-AA-OOH to oxidize GSH (GSSG) and non-toxic PE-AA-OH. GSSG is then converted to GSH by GSH reductase (GR) in the presence of NADPH. Therefore, NADPH, a coenzyme of GR, plays a vital role in maintaining the abundance of intracellular GSH. Furthermore, the basal NADPH abundance of a given cell is negatively correlated with ferroptosis susceptibility. NADPH necroptosis can establish a link between ferroptosis and GSH (**Figure 1**) [9, 32].

Figure 1.
ROS (reactive oxygen space) and ferroptosis.

15. Ferroptosis and other forms of cell death

Unlike ferroptosis, apoptosis, necrosis, and autophagy decreased mitochondrial volume, increased mitochondrial membrane density, reduction in mitochondrial cristae, and rupture of the outer membrane are observed. Also, ferroptosis cannot be stopped by inhibitors of apoptosis, necrosis, and autophagy [8, 9, 27]. Compared to ferroptosis, it may show common features with other regulated cell deaths. Although ferroptosis does show mitochondrial differences, it cannot be entirely attributed to it. The amount of mitochondrial ROS does not change in ferroptosis exposed to erastin. Moreover, ferroptosis also occurs in cells lacking a mitochondrial electron transport chain [8, 14, 105].

16. Ferroptosis and oxytosis

Oxidative glutamate toxicity leads to glutathione depletion by inhibiting cystine uptake via exogenous glutamate, the Xc system (cystine/glutamate antiporter).

Activation of lipoxygenase opens up cGMP-gated channels that allow reactive oxygen species production and extracellular calcium influx. In oxytosis paradigms in neuronal cells, mitochondrial disorders are mediated through mitochondrial transactivation of pro-apoptotic protein (BID). Upon translocation to mitochondria, BID mediates loss of mitochondrial integrity and function and deleterious translocation of mitochondrial AIF to the nucleus. Induced by stress and independently of mitochondrial death. In neuronal cells, ROS-induced transactivation of BID into mitochondria links both oxytosis and ferroptosis pathways and leads to irreversible morphological and functional damage. MMP (Matrix metallo proteinases) loss, reduction of ATP levels, and mitochondrial ROS generation are associated with the apoptosis-inducing factor (AIF). The BID inhibitor BI-6c9 and the ferroptosis inhibitors ferrostatin-1 and liproxstatin-1 can block these deadly pathways upstream of mitochondrial disorders. The analogy of ferroptosis and oxytosis seems to be the most promising for treatment options, especially in diseases related to iron accumulation such as neurodegenerative diseases, stroke, and reperfusion injury [106–108].

17. Ferroptosis and necroptosis

They used ACSL4 as a ferroptosis susceptibility marker and a Mixed Lineage Kinase domain-like (MLKL) marker for necroptosis. Interestingly, ACSL4 deficiency led to an increase in MLKL, and loss of MLKL increased the cells' sensitivity to ferroptosis. When one cell death pathway is inhibited, it evolves into the other pathway. It has been demonstrated that ferroptosis and necroptosis are different forms of cell death. They used ACSL4 as a ferroptosis susceptibility marker and a mixed lineage kinase domain-like (MLKL) marker for necroptosis. Interestingly, ACSL4 deficiency led to an increase in MLKL, and loss of MLKL increased the cells' sensitivity to ferroptosis. When one cell death pathway is inhibited, it evolves into the other pathway [15, 16, 109, 110].

18. Ferroptosis and autophagy

Ferritinophagy is the autophagic process of ferritin mediated by NCOA4. NCOA4 binds to FTH1 in autophagosomes during low intracellular iron and then autophagosomes are sent to lysosomes for ferritin degradation. Similarly, autophagy is the death of the cell by breaking down organelles and proteins in the cell with phagosomes and lysosomes [57]. Cell death can also be blocked by the inhibition of ferritinophagy in aged cells [60]. Ferritinophagy and unstable iron increase in fibroblast culture and cancer cells accelerated cell death [59]. Increased ferritinophagy in liver fibrosis and erastin-induced ferroptosis follow the same process [61]. Ferritinophagia has been reported at the onset of ferroptosis [100]. However, it differs from ferritinophagy in that specific autophagy inhibitors fail to rescue ferroptosis [11].

19. Conclusion

Ferroptosis differs from other forms of regulated cell death. It requires an unstable form of intracellular iron. It is associated with the increased reactive oxidative load. The susceptibility to ferroptosis differs from cell to cell. It presents new research areas for the treatment of cancer, circulatory diseases, and degenerative neurological disorders.

Abbreviations

AA	acyls-arachidonoyl
ACCC1	acetyl-CoA carboxylase 1
ACD	accidental cell death
ACSL4	acyl-CoA synthetase long-chain family member 4
AIF	apoptosis-inducing factor
ALOX-15	arachidonate lipoxygenase 15
AMPK	AMP-activated protein kinase
APAF1	apoptotic peptidase 1
ATG5	autophagy-related 5
ATG7	autophagy-related 7
ATM	ataxia-telangiectasia mutated kinase
BAX	BCL-2 associated protein
BBC3/PUMA	BCL2 binding component 3
BCL-2	B-cell lymphoma 2 gene
β-ME	β-mercaptoethanol
BSO	butionine sulfoximine
CDKN1A/p21	cyclin-dependent kinase inhibitor 1A
CED9	cell death abnormality gene 9
COQ10	Coenzyme Q10
DCYTB	ascorbate-dependent duodenal sitokrom <i>b</i> .
DPP4	dipeptidyl peptidase-4
DRAM3	damage regulated autophagy modulator 3
FBXL5	F-box and leucine-rich repeat protein 5
FIN56	ferroptosis inducing 56
FINO2	ferroptosis inducer endoperoxide containing 1,2-dioxolane
FPN	ferroportine
FSP1	ferroptosis suppressor protein 1
FTH	ferritin heavy chain
FTL	ferritin light chain
FPP	farnesyl pyrophosphate.
GABARAP	GABA type-A receptor-associated protein families
GPX4	glutathion peroxydase 4
GSH	glutathione
HO1	heme oxygenase
HSPB1	H shock protein beta-1
IREs	responsive elements
IRPs	iron regulatory proteins
KAT2B/PCAF	lysine acetyltransferase 2B/P300/CBP-associated factor
LC3	microtubule-associated protein 1 light chain3
LIP	labile iron pool
LKB1	liver kinase B1
LOXs	lipoxygenases
MAPK	mitogen-aktivated protein kinase
MAP1LC3	microtubule-associated protein 1 light chain 3
MEF	mouse embrionic fibroblast
MMP	matrix metalloproteinases
MLKL	mixed lineage kinase domain-like protein
MTF1	metal regulatory transcription factor 1

mTOR	mammalian target of rapamycin
MVA	mevalonate
NRF2	nuclear factor erythroid 2-associated factor 2
NADPH	nicotinamide adenine dinucleotide phosphate
NCCD	nomenclature committee on cell death
NCOA4	nuclear coactivator4
NQO1	NADPH quinone dehydrogenase1
NRF2	nuclear factor erythroid 2-related factor 2
PCD	programmed cell death
PE-OOH	phosphatidyl ethanol amine-hydroxyl
PHKG2	phosphorylase kinase catalytic subunit gamma 2
PKC	protein kinase C
PL	phospholipid
PTGS2	prostaglandin-endoperoxide synthase 2
PUFAs	polyunsaturated fatty acids
RAS-RAF-MEK	receptor tyrosine kinases
RCD	regulated cell death
REBBP/CBP	CREB binding protein
RIP	receptor-interacting serine/threonine kinase
ROS	reactive oxygen species
RSL3	RAS-selective lethal 3
RTAs	radical-trapping antioxidants
SAT1	spermidine/spermine N1-acetyltransferase 1
SLC7A11	solute carrier family 7 member 11
SQS	squalene synthase
VDAC	voltage-dependent anion channels
Tf	transferrin
TfR1	Tf receptor 1
TFEB	transcription factor EB
TfR1	transferrin receptor 1
TNF-R1	tumor necrosis factor R1
U-2 OS	cell line human osteosarcoma
Xc system	cystine/glutamate antiporter system

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