Review

American Society for Neurochemistry

ASN Neuro
Volume 13: 1–19

DOI: 10.1177/17590914211037505
journals.sagepub.com/home/asn
SAGE

Emerging Role of Ferroptosis in the Pathogenesis of Ischemic Stroke: A New Therapeutic Target?

Zhong-Qi Bu[1], Hai-Yang Yu[1], Jue Wang[1], Xin He[1], Yue-Ran Cui[1], Jia-Chun Feng[2] and Juan Feng[1]

Abstract

Ischemic stroke is one of the main causes of high morbidity, mortality, and disability worldwide; however, the treatment methods are limited and do not always achieve satisfactory results. The pathogenesis of ischemic stroke is complex, defined by multiple mechanisms; among them, programmed death of neuronal cells plays a significant role. Ferroptosis is a novel type of regulated cell death characterized by iron redistribution or accumulation and increased lipid peroxidation in the membrane. Ferroptosis is implicated in many pathological conditions, such as cancer, neurodegenerative diseases, and ischemia-reperfusion injury. In this review, we summarize current research findings on ferroptosis, including possible molecular mechanisms and therapeutic applications of ferroptosis regulators, with a focus on the involvement of ferroptosis in the pathogenesis and treatment of ischemic stroke. Understanding the role of ferroptosis in ischemic stroke will throw some light on the development of methods for diagnosis, treatment, and prevention of this devastating disease.

Keywords

ferroptosis, glutathione, iron, ischemic stroke, lipid peroxidation, treatment

Received March 19, 2021; Revised July 18, 2021; Accepted for publication July 18, 2021

Introduction

Ischemic stroke is one of the most serious diseases with high morbidity, disability, and mortality, which presents a serious public health problem throughout the world (Jickling et al., 2014). Severe cognitive and motor impairments caused by ischemic stroke can be a significant burden for families and the society in general (Naghavi et al., 2015). However, the only effective therapeutic approach in the acute phase of ischemic stroke is application of thrombolytic agents, which has a strict therapeutic time window of 4.5 hr (Cheng & Kim, 2015). The emerging intravascular thrombectomy has extended the time window to 24 hr (Saver et al., 2015), but many patients still cannot be observed; as a result, the optimum time for revascularization is missed and the consequences can be devastating (Kleindorfer et al., 2004). Furthermore, if recanalization occurs after the optimal period, it can even cause more serious adverse effects like hemorrhagic transformation. Therefore, it is necessary to improve our understanding of the pathogenic mechanisms underlying ischemic stroke in order to develop more effective treatment methods.

The pathogenesis of ischemic stroke is very complex and has not been fully understood. Possible mechanisms include excessive production of reactive oxygen species (ROS), increase of intracellular and mitochondrial calcium levels, glutamate excitotoxicity, spreading depolarization, activation of proinflammatory leukocytes in the central nervous system, decrease in cellular ATP, and induced neuronal cell death (Chamorro et al., 2016; Lambertsen et al., 2019; Moskowitz et al., 2010). In particular, many researchers focus on the role of different types of programmed cell death (PCD). Among them, apoptosis, which is the classical type, has been long considered as one of the causes of ischemic cell death (Shan et al., 2020; Zhang, Jin, et al., 2020). The

[1]Department of Neurology, Shengjing Hospital of China Medical University, Shenyang, China
[2]Department of Neurology and Neuroscience Center, The First Hospital of Jilin University, Changchun, China

Corresponding Author:
Juan Feng, Department of Neurology, Shengjing Hospital of China Medical University, Shenyang 110004, China.
Email: juanfeng@cmu.edu.cn

typical morphological features of apoptosis include chromatin condensation and nuclear fragmentation, formation of apoptotic bodies, cell shrinkage, and plasma membrane blebbing without membrane breakdown (Mughal et al., 2012). Furthermore, a proportion of neuronal cells die through regulated necrosis termed necroptosis, which is induced after cerebral ischemia (Degterev et al., 2005; Li, Mu, et al., 2020). Autophagy is another PCD type observed after cerebral ischemia (Wang et al., 2017; Yu, Yu, et al., 2019). During autophagy, macromolecules or organelles are enclosed into double-membrane vesicles called autophagosomes, which then fuse with lysosomes where the content is degraded. If autophagy exceeds a certain threshold, it could be detrimental and lead to cell death (Galluzzi et al., 2017; Hariharan et al., 2011). However, these mechanisms do not fully cover the molecular pathways underlying neuronal injury after ischemic stroke and it is suggested that other types of PCD may also be involved.

In 1997, Schubert and Maher reported that 12-lipoxygenase (12-LOX) plays a critical role in nerve cell death caused by glutathione depletion (Li et al., 1997). Later on molecular level, several studies revealed a central role of 12-LOX activation after glutathione depletion in mediating such oxidative stress-induced cell death (Canals et al., 2003; Khanna et al., 2003). Lo et al., further described that such oxidative glutamate toxicity could be decoupled from caspase activation (van Leyen et al., 2005). Moreover, the LOX-dependent cell death in neural cells might be dependent on the oxidation of intracellular membranes instead of free arachidonic acid (AA) (van Leyen et al., 2008). Dixon et al., firstly proposed the definition of ferroptosis in 2012 as is a new PCD type characterized by iron-dependent accumulation of lipid peroxides (Dixon et al., 2012). It is different from apoptosis, necrosis, and autophagy in morphological, biochemical, and genetic characteristics. Recent studies have shown that ferroptosis is implicated in the pathophysiology of many diseases, including cancer (Cheng et al., 2020), neurodegenerative conditions (Do Van et al., 2016), acute kidney injury (Adedoyin et al., 2018), and ischemic stroke (Li, Cao, et al., 2020; Tuo et al., 2017). In this review, we describe the discovery of ferroptosis, present the latest research findings on the underlying mechanisms and therapeutic applications of ferroptosis manipulation, and summarize the role of ferroptosis in the pathogenesis of ischemic stroke. Possible treatment methods of ischemic stroke that target ferroptosis are also discussed.

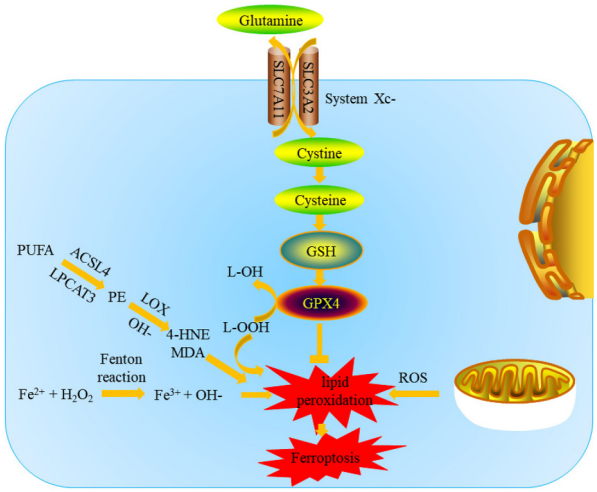

Figure 1. Regulatory mechanisms of ferroptosis. GPX4 is the pivotal regulator of ferroptosis; the GSH/GPX4 axis is inhibited by system Xc−. PUFAs are metabolized into 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) in the presence of ·OH, causing lipid peroxidation and ferroptosis. Mitochondria regulate ferroptosis through reactive oxygen species (ROS) production.

Discovery and Possible Mechanisms of Ferroptosis

Interestingly, activators and inhibitors of ferroptosis had been identified before the concept of ferroptosis was introduced. Thus, in 2003 it was reported that erastin could induce a type of cell death other than apoptosis in engineered human foreskin fibroblasts (Dolma et al., 2003). In 2008, the same group used high-throughput screening to show that Ras-selective lethal small molecules (RSL)3 and RSL5 caused iron-dependent oxidative cell death, which could be prevented by an antioxidant (vitamin E) and iron chelator desferrioxamine with high-throughput screening (Yang & Stockwell, 2008). In 2012, Dixon et al. termed the novel PCD type "ferroptosis" and since then, it has been extensively studied. In particular, the morphological characteristics of ferroptosis distinguishing it from the other PCD types have been described; thus, ferroptosis results in cell swelling, whereas apoptosis causes cell shrinkage and vacuolization. On the subcellular level, ferroptosis targets mitochondria, which show decreased volume, reduced number of cristae, and increased membrane density followed by outer membrane rupture (Angeli et al., 2017; Xie et al., 2016; Yu et al., 2017). The exact mechanism of ferroptosis is still under investigation, but the current data indicate that it includes abnormal metabolism of iron, amino acids, and lipids and deregulated expression of critical enzymes (Figure 1).

Abnormal Iron Metabolism

The excessive accumulation of iron is the pivotal characteristic of ferroptosis. Both ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron chelators (ciclopirox and deferoxamine, respectively), but not chelators of other metal ions, are effective inhibitors of ferroptosis (Stockwell et al., 2017). Fe^{2+} generated by intestinal absorption or erythrocyte degradation in the bloodstream can be oxidized to Fe^{3+}, which binds to transferrin (Tf) recognized by its cell membrane receptor TfR1, resulting in the

formation of the Tf-TfR1 complex (Frazer & Anderson, 2014). After Tf-TfR1 endocytosis, Fe^{3+} is released from Tf and reduced to Fe^{2+} by six-transmembrane epithelial antigen of the prostate 3 (STEAP3), a metal ion reductase in the acidic endosome. Fe^{2+} is then transported to the cytoplasm via divalent metal transporter 1 (DMT1) or zinc-iron transporters ZIP8 and ZIP 14 (Bogdan et al., 2016). Excessive iron is stored in the iron-storage protein complex, ferritin, composed of ferritin light and heavy chains (FTL and FTH1, respectively) (Xie et al., 2016); the latter has iron oxidase activity and can catalyze the oxidation of Fe^{2+} to Fe^{3+}, providing stable incorporation of iron into the ferritin shell, thus maintaining the equilibrium of the labile iron pool (Harrison & Arosio, 1996). A membrane protein ferroportin 1 is the only known iron efflux mediator, supporting iron export from the cell (Masaldan et al., 2019). This iron metabolism pathway exerts strict control over iron homeostasis, which, once deregulated, can lead to excessive iron accumulation and ferroptosis (Figure 2).

Interestingly, recent studies have shown a crosstalk between ferroptosis and another PCD type, autophagy, which can trigger ferroptosis by downregulating cellular ferritin levels. During autophagy, nuclear receptor coactivator 4 (NCOA4), a selective cargo receptor, binds to ferritin and transmits it to lysosomes for degradation, which can lead to the elevation of labile iron levels (Gao et al., 2016; Mancias et al., 2014; Santana-Codina & Mancias, 2018); consistently, it was shown that NCOA4 inhibition suppressed ferroptosis and its overexpression induced it (Hou et al., 2016; Figure 2). This newly observed process called "ferritinophagy" represents a bridge between autophagy and ferroptosis.

In fact, it is still unclear how iron promotes ferroptosis. Excessive Fe^{2+} can react with hydrogen peroxide (H_2O_2), producing a powerful oxidant, hydroxyl radical (·OH), in the Fenton reaction (Valko et al., 2005), which can induce ferroptosis through damage of lipids and other molecules (Imam et al., 2017). Furthermore, iron is a critical component of the catalytic subunit of lipid-oxidizing LOXs, which mediate lipid peroxidation (Shintoku et al., 2017). It was shown that LOX inhibitors (e.g., nordihydroguaiaretic acid and zileuton) can act as direct radical-trapping antioxidants (Shah et al., 2017, 2018) and suppress ferroptosis.

Overall, these findings indicate that the intracellular accumulation of labile iron is critical for ferroptosis initiation.

System Xc−

The amino-acid antiporter system Xc− regulates the exchange between extracellular cystine and intracellular glutamate across the plasma membrane in a 1:1 ratio (Galluzzi et al., 2017). The system consists of the light chain (SLC7A11), which is a 12-pass transmembrane transporter protein, and the glycosylated heavy chain (SLC3A2), which is a single-pass transmembrane regulatory protein (Sato et al., 1999). Inside the cell, cystine is reduced to cysteine, which is then used in the biosynthesis of proteins and nonribosomal tripeptide glutathione (GSH), an important antioxidant. Among the three amino acids of GSH, cysteine is the least abundant and is, therefore, a rate-limiting factor for the *de novo* GSH synthesis. In some cells, cysteine can be synthesized from methionine via the transsulfuration pathway (Stabler et al., 1993), but in many others, cystine import is necessary to maintain cysteine and GSH levels. GSH can act as a basic cofactor of glutathione peroxidase 4 (GPX4) that catalyzes the reduction of lipid peroxides. Thus, the inhibition of system Xc− results in the decrease of GSH and GPX4 activity, lipid peroxidation, and, ultimately, ferroptosis (Figure 1), which explains the activity of erastin to promote ferroptosis through inhibition of system Xc−. Mutant tumor suppressor protein p53 can inhibit system Xc− by downregulating SLC7A11, which results in the reduced activity of GPX4 and ferroptosis (Jiang, Hickman, et al., 2015; Jiang, Kon, et al., 2015). System Xc− function can also be inhibited by the excess of extracellular glutamate (Dixon, 2017), which is common for ischemia-reperfusion (IR) injury.

Thus, system Xc− can be considered a negative regulator of ferroptosis.

GPX4

GPX4, a selenium-dependent glutathione peroxidase expressed in mammalian cells, plays a key role in the regulation of ferroptosis by decreasing the amount of lipid peroxides (Yang et al., 2014). It is a unique enzyme that can reduce oxidized lipids (L-OOH) such as polyunsaturated fatty acids (PUFAs)-containing cholesterol and phospholipids (PLs) to harmless lipid alcohols (L-OH) by converting GSH into oxidized glutathione (GSSG) (Brigelius-Flohé & Maiorino, 2013). GPX4 is essential for preventing cell death and tissue damage in many organs such as the brain, skin, and endothelium (Wortmann et al., 2013). Pharmaceutical inhibition of GPX4 activity as well as GPX4 degradation or gene ablation can result in ferroptosis. A classical ferroptosis inducer RSL3 acts by irreversibly binding to seleno-cysteine in the GPX4 catalytic site and inhibiting GPX4 enzymatic activity; compounds DPI10 and DPI7 also directly inhibit GPX4 and induce ferroptosis through a similar mechanism (Yang et al., 2014). Genetic knockout of GPX4 in tubular cells leads to massive cell death showing pathological features of ferroptosis (Friedmann Angeli et al., 2014). However, the GPX4-mediated mechanism of ferroptosis still needs clarification.

Lipid Peroxidation

Lipid peroxidation is considered a critical process in the execution of ferroptosis (Magtanong et al., 2016). PUFA-PLs are most sensitive to peroxidation because they contain highly reactive hydrogen atoms in methylene bridges. PUFAs, particularly AA and adrenic acid (AdA), are enzymatically

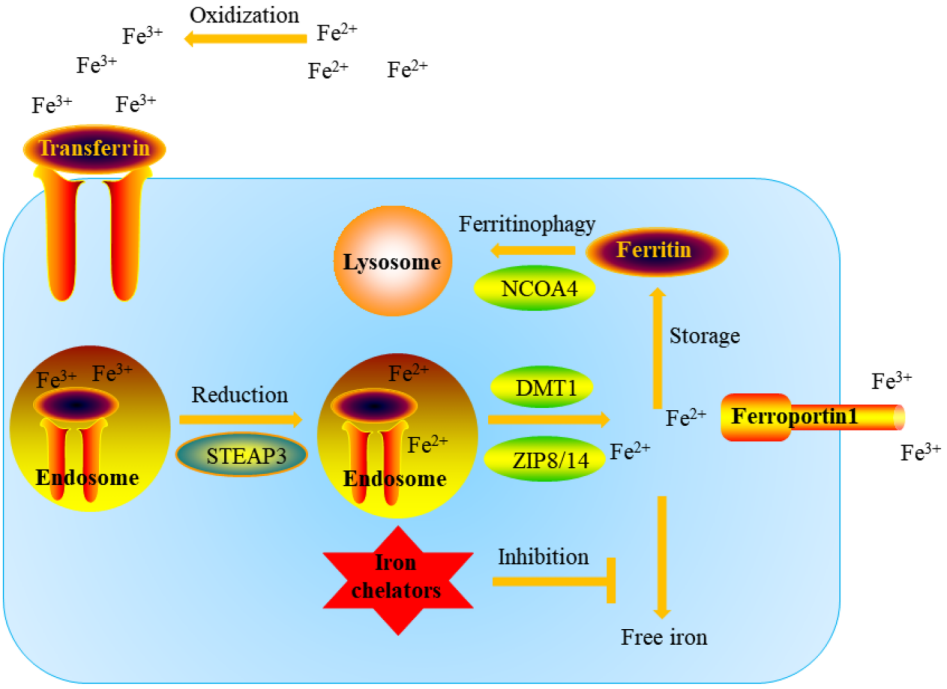

Figure 2. Iron metabolism and ferritinophagy. Blood Fe^{2+} can be oxidized to Fe^{3+}, which is transported into cells by Tf-TfR1 and reduced to Fe^{2+} in acidic endosomes. Intracellular Fe^{2+} exists in the form of free iron or stored in the ferritin complex, which can be degraded through ferritinophagy; ferroportin 1 transports Fe^{2+} outside the cell.

esterified to PLs, primarily phosphatidylethanolamine (PE) in a two-step process (Hao et al., 2018). First, acyl-CoA synthetase long-chain family member 4 (ACSL4) catalyzes the binding of AA/AdA to CoA required for their incorporation into PLs. It should be noted that ACSL4 has been identified as an enzyme essential for ferroptosis as it forms the lipid pool targeted by peroxidation (Doll et al., 2017). Next, lysophosphatidylcholine acyltransferase 3 (LPCAT3) inserts AA/AdA-CoA into membrane PE to form AA/AdA-PE, which subsequently activates ferroptotic signals (Hashidate-Yoshida et al., 2015). These lipids can undergo peroxidation catalyzed by LOX or induced by ·OH produced in the Fenton reaction; the resulting lipid peroxides can attack proximal PUFAs, thus triggering a chain reaction and leading to ferroptosis (Yang et al., 2016). The final products of lipid peroxidation, 4-hydroxynonenal and malondialdehyde, are frequently used as general markers of oxidative stress. Thus, lipid peroxidation is closely associated with ferroptosis and can be used as a tool to regulate the process.

Mitochondria

Mitochondria are organelles undergoing significant morphological changes in ferroptotic cells. The function of mitochondria in ferroptosis seems to be an interesting question. Mitochondria can synthesize ATP through oxidative phosphorylation producing ROS as a byproduct, which is essential for ferroptosis. Pharmacological induction of ferroptosis through inhibition of system Xc− was shown to induce mitochondrial ROS production, ATP depletion, membrane potential decrease, and fragmentation (Gao et al., 2019). In erastin-treated neurons, a Bcl-2 family member BH3-interacting domain death agonist translocates to mitochondria, leading to mitochondrial damage as the final step in ferroptosis execution (Neitemeier et al., 2017). Furthermore, 12/15 LOXs activated in ferroptosis directly attack the mitochondrial membrane leading to lipid peroxidation (Krainz et al., 2016). The role of mitochondria in ferroptosis is further supported by the presence of CDGSH iron sulphur domain 1 (CISD1), an iron-containing protein, on the mitochondrial outer membrane. CISD1 negatively regulates ferroptotic cell death and its genetic inhibition leads to iron accumulation inside mitochondria, which increases lipid peroxidation and contributes to ferroptosis (Yuan et al., 2016).

However, in the initial description of ferroptosis it was indicated that mitochondrial DNA-knockout cells remained sensitive to ferroptosis induction (Galluzzi et al., 2017). Therefore, the question whether mitochondria is involved in the ferroptosis pathway in all cell types remains open and further studies are needed to address it.

Other Pathways

Recent studies have reported a special role of ferroptosis suppressor protein 1 (FSP1) in the process, suggesting a novel mechanism of ferroptosis regulation in parallel to GPX4 (Bersuker et al., 2019; Doll et al., 2019). Thus, FSP1 protects

human cancer cells from ferroptosis caused by GPX4 deletion, whereas FSP1 knockout increases cell sensitivity to ferroptosis inducers, which is reversed by FSP1 overexpression. FSP1 suppresses ferroptosis by regulating ubiquinone (also named coenzyme Q10 [CoQ10]): the reduced form, ubiquinol, neutralizes lipid peroxyl radicals and decreases lipid peroxidation, whereas FSP1 uses NAD(P)H to promote CoQ10 regeneration. It was also shown that inhibition of MDM2 or MDMX, negative regulators of p53, resulted in FSP1 overexpression and elevation of CoQ10 levels, suggesting that MDM2/MDMX inhibition might be used to prevent degenerative diseases involving ferroptosis (Venkatesh et al., 2020). In summary, the discovery of FSP1 and its function defines a new mechanism in the development of ferroptosis; therefore, FSP1 might be a promising therapeutic target in many diseases.

The occurrence of cell ferroptosis can also be regulated by the transsulfuration pathway. When astrocytes are under oxidative stress, sulfur-containing methionine can be converted into cysteine through this pathway, and GSH can be synthesized subsequently to exert antioxidant effects (McBean, 2012). Cystathionine β-synthase (CBS), which catalyzes the first step of the transsulfuration pathway, plays an important role in ferroptosis. Thus, CBS inhibition leads to ferroptosis in hepatocellular carcinoma (Wang et al., 2018), whereas its overexpression promotes resistance to ferroptosis in ovarian cancer cells; furthermore, the antioxidant transcription factor NRF2, which controls CBS expression, is closely linked to the regulation of ferroptosis in cancer cells (Liu et al., 2020). A recent study shows that inhibition of protein deglycase DJ-1 enhances the sensitivity of cancer cells to ferroptosis inducers by blocking the formation of the S-adenosyl homocysteine hydrolase tetramer and inhibiting its activity in the transsulfuration pathway (Cao et al., 2020). Thus, the transsulfuration pathway plays an important role in the ferroptosis regulatory network.

Interestingly, tumor suppressor p53 may regulate ferroptosis in a bidirectional manner. On the one hand, p53 can inhibit SLC7A11 expression and reduce cystine uptake by system Xc −, which downregulates GPX4 and cell antioxidant activity, leading to ROS accumulation and ferroptosis (Jiang, Kon, et al., 2015). On the other hand, p53 overexpression has been shown to inhibit ferroptosis in colorectal cancer cells (Xie et al., 2017).

In addition, the TAZ-ANGPTL4-NOX2 (Yang et al., 2020), p62-Keap1-NRF2 (Sun et al., 2016), and glutaminolysis (Gao et al., 2015) pathways may also play a regulatory role in ferroptosis.

Table 1. Classical Inducers and Inhibitors of Ferroptosis.

Molecule	Mode of action	Target	Effect
Erastin	Inducer	System Xc −	Inhibition
Sorafenib	Inducer	System Xc −	Inhibition
Sulfasalazine	Inducer	System Xc −	Inhibition
RSL3	Inducer	GPX4	Inhibition
FIN56	Inducer	GPX4, CoQ10	GPX4 degradation, depletion of CoQ10
Ferrostatin-1	Inhibitor	Lipid radicals	Lipid peroxidation inhibition
Liproxstatin-1	Inhibitor	Lipid radicals	Lipid peroxidation inhibition
Deferoxamine	Inhibitor	Iron	Free iron reduction
α-Tocopherol	Inhibitor	Iron	Free iron reduction

Targeting Ferroptosis: A Promising Perspective for Many Diseases

The inducers and inhibitors of ferroptosis identified to date are summarized in Table 1. As ferroptosis is involved in the pathogenesis of many diseases (Chen et al., 2020; Li, Liu, et al., 2020; Tadokoro et al., 2020), its regulation may be an effective treatment approach. Therefore, in recent years significant attention has been paid to the search for ferroptosis inducers and inhibitors, which can be effective treatment tools (Table 1). Ferroptosis-associated diseases can be divided into two categories depending on the pathogenic mechanism, which involves either inhibition or induction of ferroptosis. Cancer belongs to the first category because the proliferation of cancer cells is negatively regulated by ferroptosis. Therefore, the search of ferroptosis inducers in cancer cells is currently a focus of research. The three approaches to induce ferroptosis and kill cancer cells are small molecules, nanomaterials, and gene therapy. Thus, it was shown that a small molecule sorafenib, considered a multikinase inhibitor, promoted ferroptosis in hepatocellular carcinoma cells (Louandre et al., 2013), whereas a benzopyran derivative 2-imino-6-methoxy-2H-chromene-3-carbothioamide induced ferroptosis through decreasing SLC7A11 activity in colorectal cancer cells *in vitro* and *in vivo* (Zhang, Liu, et al., 2020). Other small molecules such as sulfasalazine, artemisinin, and artesunate, which also showed anticancer activity through induction of ferroptosis, may have potential therapeutic application (Isani et al., 2019; Wang et al., 2019; Yu, Yang, et al., 2019). In addition, a number of nanomaterials can promote ferroptosis and might be used for cancer therapy. Among them, iron-based nanoparticles have received the closest attention (Ma et al., 2017). Specialized nanoparticles can accurately identify cancer cells and, through target iron delivery, enhance the therapeutic effect. Finally, genetic technologies can modulate the expression of genes associated with ferroptosis and reduce cancer cell viability. Thus, depletion of a glycoprotein ceruloplasmin, which was shown to reduce intracellular iron levels and suppress

ferroptosis in hepatocellular carcinoma cells, accelerates erastin-induced ferroptosis (Shang et al., 2020). The regulation of ferroptosis in cancer needs to be further investigated in order to develop effective therapies.

In addition to cancer, manipulation of ferroptosis mechanisms may be used to treat nonneoplastic diseases such as neurodegeneration, IR injury, or acute kidney disease. Thus, inhibition of ferroptosis by trihydroxy chalcones was found to be effective in Alzheimer's disease (Cong et al., 2019) and that by liproxstatin-1 was shown to reduce myocardial damage after IR by downregulating VDAC1 and upregulating GPX4 (Feng et al., 2019). Curcumin, a powerful antioxidant, can inhibit ferroptosis and alleviate rhabdomyolysis-associated renal injury (Guerrero-Hue et al., 2019). Below, we focus on inhibition of ferroptosis in neuronal cells, which should be beneficial for post-stroke recovery.

Mechanisms of Ferroptosis in the Pathogenesis of Ischemic Stroke

The main pathological mechanism of ischemic stroke is local cerebral ischemia and hypoxia caused by cerebral blood flow occlusion. In ischemic stroke, the affected brain tissue can be divided into ischemic core and penumbra areas based on blood supply (Kasasbeh et al., 2019). The functional damage of neurons in the penumbra is reversible (Xu et al., 2019). However, prolonged periods of ischemia increase neuronal cell death and worsen clinical symptoms; furthermore, many secondary factors such as excitotoxicity induced by dying cerebral tissues and inflammation near the ischemic region may come into play (Lambertsen et al., 2019; Pineda-Ramírez et al., 2020). Therefore, protection of neurons in the ischemic penumbra is critical for the treatment of ischemic stroke. Along with the other types of cell death, ferroptosis plays an important role in the pathogenesis of brain ischemia (Hanson et al., 2009; Selim, 2010). A detailed account of the chemical biology of ferroptosis in the central nervous system has been well summarized by Ratan (2020). In the next sections, we describe ferroptosis occurring in the course of ischemic stroke in detail (Table 2).

Iron Accumulation and Redistribution

Before the concept of ferroptosis was put forward, the accumulation of iron had been found in the ischemic regions of the brain, including the hippocampus and basal ganglia (Dietrich & Bradley, 1988; Kondo et al., 1995; Park et al., 2011). It is well known that the prognosis of ischemic stroke is worse in the elderly, who have iron deposits in the brain (Valdés Hernández et al., 2019), suggesting that iron accumulation may aggravate ferroptosis of neurons in ischemic brain injury. Furthermore, clinical studies revealed the association between serum ferritin levels and poor prognosis for patients with acute cerebral infarction (Dávalos et al., 1994; Millan et al., 2007; Millán et al., 2008), as well as between serum Tf levels and lower risk of stroke (Gill et al., 2018). Another clinical study showed that iron regulatory hormone hepcidin was significantly higher in patients with ischemic stroke (Słomka et al., 2015). Iron from blood may enter brain tissue through the broken blood–brain barrier, leading to excessive iron accumulation in neuronal cells and their death by ferroptosis. However, the specific mechanism of iron transport to the injured brain tissue in the course of ischemic stroke still needs clarification.

Early in 1995, Hunt and his colleagues reported that iron chelators could abrogate ischemia metabolic changes (Hurn et al., 1995). Zaman et al. further revealed that iron chelators were neuroprotective against glutathione depletion-induced toxicity by inducing transcription factors like HIF-1 and ATF-1/CREB and upregulating glycolytic enzymes like $p21^{waf1/cip1}$ and erythropoietin (Zaman et al., 1999). They could inhibit the activity of HIF prolyl 4-hydroxylases, a class of iron-dependent enzymes, while a number of structurally diverse prolyl 4-hydroxylase inhibitors have been demonstrated to confer neuroprotection against oxidative stress both in vivo and in vitro (Karuppagounder et al., 2016; Siddiq et al., 2005; Smirnova et al., 2010).

Intracellular iron accumulation may be a direct cause of ferroptosis. Fari et al. (Ryan et al., 2018) used a mouse model of permanent middle cerebral artery occlusion (MCAO), and found that the loss of ceruloplasmin, a ferroxidase responsible for iron efflux from the cell, may compromise iron homeostasis, increase oxidative injury and lesion size, and impair the recovery of neural function, suggesting that the deregulation of iron intracellular balance may lead to iron deposition-induced ferroptosis. Iron export was also found to be related to the expression of the microtubule-associated protein tau. When 12-month-old tau-knockout mice were subjected to MCAO, they had more severe symptoms than wild-type mice, but the injury could be relieved by iron-targeting interventions, indicating that tau may induce iron export and inhibit ferroptosis (Tuo et al., 2017). In addition, Rosaria et al. (Ingrassia et al., 2012) observed increased expression of NF-κB-regulated 1B isoform of the divalent metal transporter-1 (1B/DMT1) in animal and cellular models of ischemic stroke, which was associated with increased iron uptake into neuronal cells. Recently, Wang et al. reported that mitochondrial ferritin attenuates neuronal ferroptosis by decreasing the intracellular labile iron pool during cerebral IR injury (Wang et al., 2021).

A recent study indicates that noncoding (nc)RNAs could regulate iron transport and induce ferroptosis in cerebral IR injury (Lu et al., 2020). It was shown that lncRNA PVT1 promoted and miR-214 inhibited ferroptosis in *in vivo* and *in vitro* models and that the levels of these ncRNAs were increased and decreased, respectively, in plasma of patients with acute ischemic stroke. Mechanistically, miR-214 could bind to 3′-untranslated regions of PVT1, p53, and TfR1 genes, suggesting that PVT1 induced ferroptosis through

Table 2. Research on Ferroptosis in Ischemic Stroke.

Ferroptosis mechanism	Ferroptosis target	Results	References
Iron metabolism	Iron accumulation	Found in ischemic regions and brain tissue of elderly people	Valdés Hernández et al. (2019)
	Serum ferritin	Higher levels indicate poorer prognosis after acute cerebral infarction	Dávalos et al. (1994); Millan et al. (2007); Millán et al. (2008)
	Serum transferrin	Higher levels indicate lower stroke risk	Gill et al. (2018)
	Hepcidin	Higher levels in patients with ischemic stroke	Słomka et al. (2015)
	Ceruloplasmin	Loss leads to intracellular iron deposition-induced ferroptosis	Ryan et al. (2018)
	Tau	May induce iron export and inhibit ferroptosis	Tuo et al. (2017)
	1B/DMT1	Increases iron uptake in neuronal cells	Ingrassia et al. (2012)
	Mitochondrial ferritin	Attenuates neuronal ferroptosis by decreasing the intracellular labile iron pool during cerebral IR injury	Wang et al. (2021)
	Non-coding RNA	LncRNA PVT1 is upregulated and miR-214 downregulated in plasma of patients with acute ischemic stroke	Lu et al. (2020)
Glutamate	System Xc−	Glutamate blocks system Xc− and promotes ferroptosis	Novgorodov et al. (2018)
	ATF4	ATF4 upregulates Chac1, Trib3, or CHOP to promote ferroptosis	Crawford et al. (2015), etc.
Oxidative stress	Mitochondrial genes	Upregulation of mitochondrial ROS production; overexpression of mitochondrial haplogroup F1 in patients	Tsai et al. (2020)
	MiR-532-3p	Inhibits NOX2 expression; downregulated in ischemic stroke	Mao et al. (2020)
	ERK	Activate epigenetic modulators like transglutaminases, which add monoamines or polyamines to histones or transcription factors to promote transcription	Farrelly et al. (2019)
	Antioxidant system	N-acetylcysteine,1,2,4-triazole derivative compound 11 can improve antioxidant defense	Liao et al. (2020)
Lipid peroxidation	Peroxidation induction	Compound A3, sulfasalazine, and *Momordica charantia* polysaccharide inhibit lipid peroxidation	Mohsin Alvi et al. (2020)
	Phospholipase A2	Promotes hydrolysis of phospholipids, arachidonic acid release, and ROS generation	Adibhatla et al. (2003)
	TLR4	Lipid peroxidation marker malondialdehyde is decreased in the brain of TLR4-deficient mice	Caso et al. (2007)
	15-A(2t)-cyclopentenone isoprostane	Elevated expression in stroke-affected human cortical tissue	Zeiger et al. (2009)
	MiR-17-92	Protects endothelial cells from ferroptosis	Xiao et al. (2019)
Epigenetic regulation	Sp1	Sp1 inhibitor, MTM, could target several pathways involved in oxidative stress-mediated neuronal cell death	Sleiman et al. (2011)
	HDACs	HDAC inhibition could acylate p53 and decrease its transcriptional activity, and thus suppressing the expression of pro-apoptotic PUMA	Brochier et al. (2013)
	Transglutaminases	Inhibitors of transglutaminases could confer protection against ferroptotic stimulus by acting downstream of nuclear ERK activation	Basso et al. (2012)

miR-214-regulated expression of TfR1 and p53. The detailed mechanism of ncRNA-mediated iron accumulation and ferroptosis in neuronal cells during ischemic stroke needs further investigation.

How iron plays a role in neuronal ferroptosis is an interesting issue. Karuppagounder et al. reported that the hypoxia-inducible factor prolyl hydroxylase domain (HIF-PHD) family is iron dependent. An inhibitor of the HIF-PHD enzymes, adaptaquin, could reduce behavioral deficits after ICH (intracerebral hemorrhage) in some animal models. And the protective effect of adaptaquin was related to the reduced activity of activating transcription factor 4 (ATF4) (Karuppagounder et al., 2016; Lange et al., 2008). It is suggested that iron may participate in loading of iron-dependent enzymes and induce neuronal ferroptosis. However, no evidence of Fenton reaction and production of hydroxyl radicals or iron dependence of lipid peroxidation in stroke has been reported yet.

Glutamate Induced Ferroptosis

Glutamate plays an important role in the maintenance of nerve function as an excitatory neurotransmitter in the physiological state (Kaelberer et al., 2018). However, in ischemic stroke, increased release and impaired cellular reuptake of glutamate results in its accumulation in the brain, which causes damage and death of neuronal cells. Neuronal death caused by accumulating glutamate was conventionality considered to result in excessive activation of synaptic and extrasynaptic ionotropic glutamate receptors, which is called excitotoxicity (Choi et al., 1987; Lynch & Dawson, 1994; Sattler & Tymianski, 2000). Another study suggested that glutamate toxicity is caused by inhibition of cystine import, which is ionotropic glutamate receptor independent (Murphy et al., 1989). The antiporter which can import cystine and export glutamate was identified as system Xc− by Bannai (Bannai, 1986; Bannai et al., 1977; Bannai & Kitamura, 1980; Sagara et al., 1993). Concentrations of glutamate required to inhibit system Xc− are 1,000 fold higher than those required to activate glutamate receptors. Later, when the concept of ferroptosis was proposed, glutamate-induced cell death in organotypic hippocampal slice cultures was proved to be inhibited by iron chelation (Dixon et al., 2012), indicating that glutamate may cause cell ferroptosis.

The role of glutamate and system Xc− in ferroptosis has been investigated in several studies. Glutamate blocked system Xc− function and promoted ferroptosis in primary oligodendrocytes through activation of acid sphingomyelinase, which resulted in the opening of the mitochondrial permeability transition pore and induction of ferroptosis (Novgorodov et al., 2018). Although inhibition of system Xc− is known to induce ferroptosis, unexpectedly, system Xc− was upregulated in astrocytes and microglia in a rat model of stroke, whereas its inhibition decreased inflammation and alleviated IR injury (Domercq et al., 2016). Furthermore, studies by Sandra Hewett et al. showed that deletion of system Xc− in the brain would alleviate the neuronal injury (Evonuk et al., 2015; Jackman et al., 2012; Sosnoski et al., 2020). Jackman et al. suggested that increased system Xc− expression in astrocytes could potentiate hypoglycaemic neuronal death. They administrated drugs known to inhibit system Xc− and found that neuronal death induced by glucose deprivation was notably ameliorated. Evonuk et al. found that pharmacological and genetic suppression of system Xc− could alleviate chronic and relapsing-remitting experimental autoimmune encephalomyelitis. Coculture studies showed that myelin-specific CD4(+) Th1 cells could enhance the release of glutamate by microglia through the system Xc−, leading to the death of mature myelin-producing oligodendrocytes. Thus, the functions of system Xc− in different neural cell types in ischemic stroke remain to be further elucidated.

The regulatory mechanism of glutamate-induced ferroptosis in neurons may be related to an important transcription factor, ATF4. One study showed that 119 genes were abnormally regulated when the cortical neurons were exposed to excessive glutamate, but only three genes were altered when glutamate was added to neurons with germline deletion of ATF4. In addition, the infarct size of the ATF4(−/−) mice was significantly smaller than that of the wild-type mice, and behavioral recovery was improved in ATF4(−/−) mice with the same blood flow in the rodent model of ischemic stroke (Lange et al., 2008). This research suggested that ATF4 may be a particularly significant regulatory factor of ferroptosis. ATF4 can regulate the expression of a series of proteins related to ferroptosis. Chac1 (glutathione-specific γ-glutamyl cyclotransferase) is regulated by ATF4 in neurons when ferroptotic stimuli occurs, and it can catalyze the cleavage of glutathione to promote ferroptosis(Crawford et al., 2015). Trib3 (Tribbles homolog 3) is another protein regulated by ATF4. Trib3 has been proved to mediate neuronal death by reducing the mitophagy regulator Parkin (Aimé et al., 2020). ATF4 can upregulate CHOP (C/EBP homologous protein), which can aggravate ischemic stroke (Li, Zhang, et al., 2020). Other studies showed that REDD1 (regulated in development and DNA damage response-1) or CARS (cysteinyl-tRNA synthetase) can be induced by ATF4 and facilitate ferroptosis (Hayano et al., 2016; Malagelada et al., 2008). However, the exact mechanism of ATF4 in ischemic stroke remains to be revealed. The downstream targets of ATF4 require further study.

Oxidative Stress

Oxidative cell damage has been implicated in the pathogenesis of many diseases. Oxidative stress is caused by the accumulation of pro-oxidant and the loss of antioxidant species in the cell, which results in the damage of DNA, lipids, and proteins, thus creating pathological conditions (Mukherjee et al., 2015). It is suggested that oxidative stress may be one of the mechanisms inducing ferroptosis in cerebral stroke. During IR, neuronal cells in the penumbra may be subjected to oxidative stress caused by pro-oxidants ROS and reactive nitrogen species (RNS) (Orellana-Urzúa et al., 2021). ROS comprise free radicals such as ·OH and superoxide (O_2^-) as well as some nonradical molecules such as H_2O_2, whereas commonly observed RNS are nitric oxide (NO·) and peroxynitrite ($ONOO^-$) (Prasad et al., 2019). The production of ROS in mitochondria can depend on mitochondrial gene expression. Using RNA sequencing, it was shown that the mitochondrial haplogroup F1 (Mthapg F1)-encoding gene was overexpressed in patients with ischemic stroke and that Mthapg F1 upregulation induced ROS production in mitochondria and decreased cell viability (Tsai et al., 2020). NADPH oxidase 2 (NOX2) is considered an important enzyme catalyzing ROS formation in the brain. It was shown that miR-532-3p, which could directly inhibit NOX2 expression, was significantly decreased in cell and animal models of ischemic stroke, which exacerbated oxidative

damage of neuronal cells (Mao et al., 2020). However, the mechanism inducing ROS and RNS accumulation during ischemic stroke is still not clear and needs further research.

Mitogen-activated protein kinase (MAPK) pathway has been demonstrated to mediate ROS-induced cell death (Guyton et al., 1996). Extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinase or stress-activated protein kinase (JNK/SAPK), and the p38 MAP kinase (p38 MAPK) are main members of MAPK family (Davis, 1993). DeFranco et al. showed that Ras-MEK-ERK pathway was crucial to glutamate-induced ferroptosis (Xiao et al., 1999), and delayed and persistent activation of ERKs is associated with glutamate-induced oxidative toxicity (Stanciu & DeFranco, 2002). Nuclear translocation of ERK1/2 could phosphorylate proto-oncogenic c-Myc (myelocytomatosis oncogene) on serine 62, leading to its stabilization and increased transcriptional activity (Chatterjee et al., 2001), while the later could bind to its response element in the promoters of Tfr1 (transferrin receptor 1), ferritin (heavy and light chains), IRP2 (iron regulatory protein 2), and Nramp1 (natural resistance associated macrophage protein 1) genes, thus coordinately regulating iron metabolism (Dang, 2012; Wu et al., 1999). In addition, ERK could activate epigenetic modulators like transglutaminases, which add monoamines or polyamines to histones or transcription factors to promote transcription (Farrelly et al., 2019). But it remains to be revealed whether transglutaminases can directly modify the transcription factors which are involved in pro-death responses to neuronal ferroptosis in ischemic conditions.

The loss of antioxidants is another key factor leading to oxidative stress. Antioxidants prevent cell damage by converting ROS/RNS to harmless molecules. Thus, O_2^- can be converted to H_2O_2 by superoxide dismutase (SOD)1 in the cytoplasm and SOD2 in mitochondria; in turn, H_2O_2 can be reduced to water by peroxidases (Cheung et al., 2000). However, after ischemic stroke the level of endogenous antioxidants decreases, leading to oxidative stress. When patients with acute ischemic stroke were treated with a neuroprotective drug N-acetylcysteine, serum levels of SOD and glutathione peroxidase significantly increased, whereas the National Institute of Health Stroke Scale scores decreased (Sabetghadam et al., 2020). Other drugs were also shown to regulate antioxidant levels and exert neuroprotective effects in animal models of cerebral ischemia. Thus, a 1,2,4-triazole derivative compound 11 could induce antioxidant defense mechanisms by promoting the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and SOD (Liao et al., 2020). These data suggest that effective antioxidants may improve the prognosis of ischemic stroke.

Lipid Peroxidation

Lipid peroxidation, considered a direct inducing factor of ferroptosis (Homma et al., 2019), is promoted by oxidative stress. In ischemic stroke, lipid peroxidation can be inhibited by certain drugs. Thus, a synthetic polyphenol compound A3 can reverse the increase in lipid peroxidation induced by ischemic stroke and exert a neuroprotective effect in a rat model of permanent MCAO (Mohsin Alvi et al., 2020). Sulfasalazine can inhibit lipid peroxidation cascades in the acute stage of IR injury and improve brain conditions in a rat model (Cetin et al., 2017). A polysaccharide of *Momordica charantia* has been shown to provide neuroprotection against cerebral IR injury by scavenging O_2^-, NO·, and $ONOO^-$ and inhibiting lipid peroxidation (Gong et al., 2015). These results suggest that many drugs with antioxidant activity can reduce lipid peroxidation and through this prevent brain IR injury. However, the number of such drugs in clinical practice is still limited, indicating the need for further research.

The brain tissue is rich in PUFAs, which makes it more susceptible to lipid peroxidation in ischemic conditions. However, the mechanism of lipid peroxidation induced by ischemic stroke remains unclear. It was shown that during brain ischemia, phospholipase A2 catalyzed PL hydrolysis and AA release, which in turn promoted ROS formation and lipid peroxidation; these detrimental effects could be alleviated by citicoline (Adibhatla et al., 2003). Caso et al. (2007) found that TLR4 deficiency in mice with permanent MCAO caused a decrease in lipid peroxidation marker malondialdehyde, suggesting TLR4 involvement in the induction of lipid peroxidation after stroke. Another study showed that the levels of 15-A(2t)-cyclopentenone isoprostane (15-A(2t)-IsoP) formed after free radical-mediated peroxidation of AA were elevated in stroke-affected human cortical tissue, where 15-A(2t)-IsoP mediated the opening of the permeability transition pore and release of mitochondrial cytochrome C, which resulted in neuronal cell death (Zeiger et al., 2009).

It is established that the death of endothelial cells causes vascular injury, atherosclerosis, and ischemia. It was shown that ferroptosis was involved in the pathogenesis of atherosclerosis, which could be alleviated by the inhibition of ferroptosis through decrease of lipid peroxidation (Bai et al., 2020). Another study suggested that the overexpression of miR-17-92 could protect endothelial cells from ferroptosis by directly binding to zinc lipoprotein A20-encoding mRNA and inhibiting A20 biosynthesis, which decreased ACSL4 expression and, consequently, lipid peroxidation (Xiao et al., 2019). However, the relationship between lipid peroxidation and ferroptosis in ischemic stroke is still unclear and this question should be addressed in future research.

Epigenetic Regulation

Epigenetics are crucial for cells to adapt to various signals, conditions, and stressors, which include DNA methylation, histone modifications, chromatin remodeling, and noncoding RNAs (Katada et al., 2012). Mounting evidence has

suggested epigenetic mechanisms to be novel, fundamental factors in regulating the expression of stroke-related genes and closely related to ferroptosis. In ischemic cell models, oxidative stress could activate Sp1 by enhancing its acetylation and histone deacetylase (HDAC) inhibitors could prevent oxidative neuronal death, later known as ferroptosis, by augmenting this Sp1-dependent adaptive response (Ryu et al., 2003). Moreover, Sp1 inhibitor, MTM, could target several pathways involved in oxidative stress-mediated neuronal cell death, including ERK, c-Src, HIF1α, and $p21^{waf1/cip1}$ (Sleiman et al., 2011). Langley et al. further revealed that HDAC inhibition could acylate p53 and decrease its transcriptional activity, and thus suppressing the expression of pro-apoptotic PUMA (Brochier et al., 2013), and the HDAC inhibitors can lead to neuroprotection independent of HDAC inhibition (Sleiman et al., 2014). Also, transglutaminases, a group of enzymes that also modulate transcription, were suggested to show an elevated transamidating activity in different models of stroke (McConoughey et al., 2010; Paschen et al., 1990). Inhibitors of transglutaminases, regardless of their structures, could confer protection against ferroptotic stimulus by acting downstream of nuclear ERK activation, a well-established mediator of ferroptosis in neurons (Basso et al., 2012). Collectively, these findings suggest an important role of these epigenetic regulators in mediating ferroptotic death. Molecular or pharmacological agents that target these epigenetic proteins are promising in treating ischemic stroke.

Inhibition of Ferroptosis: A New Hope for Cure of Ischemic Stroke

Ischemic stroke is a serious disease, which can result in permanent disability and even death, representing a serious burden to the society. However, the only therapeutic drug currently approved by the Food and Drug Administration for acute ischemic stroke is recombinant tissue plasminogen activator, a thrombolytic agent (Snow, 2016). Another standard treatment method is thrombectomy, which should induce vascular recanalization. However, the narrow therapeutic time window (<4.5 hr) limits the effect of these methods. Thus, only 5%–10% patients can be successfully treated by vascular recanalization; furthermore, this procedure increases the risk of cerebral IR injury, which can aggravate the patient's condition (Henninger & Fisher, 2016). Therefore, current research efforts are focused on the development of new treatment methods for ischemic stroke. Some studies reported a therapeutic potential of neuroprotective agents in experimental models; however, these drugs have not been clinically proven to have a definite effect. Stem cell therapy, which has a wide application perspective, has also shown effects in the acute, subacute, and chronic phases of ischemic stroke (Kwak et al., 2018). With respect to novel treatment approaches, ferroptosis, a new type of PCD which has been confirmed to participate in the pathogenesis of ischemic stroke, is a promising therapeutic target and its inhibition during ischemia may have a clinical potential.

Among ferroptosis inhibitors, only one drug, edaravone, has been used to treat patients with acute ischemic stroke. Edaravone was verified to act as a free radical scavenger and to attenuate ferroptotic cell death in ischemic brain tissues suffering from cystine deprivation (Homma et al., 2019). Other drugs were found effective in animal and cell models of stroke. A ferroptosis inhibitor deferoxamine, also acting as iron chelator, was shown to improve the post-stroke cognitive impairment in diabetic rats with MCAO (Abdul et al., 2020). Deferoxamine decreased vasoregression and polarization of microglia by improving aquaporin-4 polarity and permeability of the blood–brain barrier, and reduced lipid ROS and other ferroptotic markers in brain microvascular endothelial cells *in vitro*; as a result, endothelial ferroptosis was downregulated and the functional conditions of the animals improved (Abdul et al., 2020). Another drug which can inhibit ferroptosis in ischemic stroke is selenium (Se), an essential element for GPX4 activity. Selenium promoted GPX4 expression through coordinated activation of neuroprotective transcription factors TFAP2c and Sp1, whereas a brain-penetrating selenopeptide induced homeostatic transcriptional activation of GPX4, which in turn inhibited ferroptosis after ischemic stroke (Alim et al., 2019).

Some traditional Chinese herbal medicines have been also shown to inhibit ferroptosis in ischemic stroke. Thus, a monoterpenoid phenol carvacrol was reported to protect hippocampal neurons against cerebral IR injury in gerbils by inhibiting ferroptosis through upregulation of GPX4 expression; in the process, lipid peroxidation in the brain tissue was decreased and learning and memory abilities of gerbils improved (Guan et al., 2019). Naotaifang extract improved neural functions in rats after ischemia by inhibiting neuronal ferroptosis through downregulation of TFR1/DMT1 and upregulation of SCL7A11/GPX4 pathways (Lan et al., 2020). In addition, Galangin, a flavonoid isolated from *Alpinia officinarum*, attenuated cerebral IR injury by inhibiting ferroptosis via activating the SLC7A11 and GPX4 in gerbils (Guan et al., 2021). Although these traditional Chinese drugs have not been clinically verified to improve patients' conditions after ischemic stroke, their safety and lack of toxicity may facilitate their testing in clinical trials and application to patients.

Conclusions and Prospects

The role of ferroptosis in ischemic stroke is currently under investigation, and there are still many questions to be answered. First, the exact mechanism of ferroptosis remains unclear. Although many studies suggest that ferroptosis can be assessed based on iron levels, ROS production, GPX4 expression, and cell viability, there are still no specific markers of ferroptosis, in contrast to other types of PCD, which have definite signatures such as caspase activation in

apoptosis (Nagata, 2018) or autophagic lysosome formation in autophagy (Cao et al., 2019). It is important to identify specific ferroptosis markers in order to perform comprehensive investigation of the process. Although iron metabolism and lipid peroxidation during pathological conditions may induce ferroptosis, the underlying mechanisms need further investigation. For example, the expression of iron metabolism regulators such as TfR1, DMT1, and ferroportin 1 can affect the occurrence and severity of ferroptosis. However, the questions remain whether these proteins can regulate other signaling pathways and whether in addition to iron, other metal ions can trigger ferroptosis through different molecular mechanisms. These issues should be resolved.

Another issue is that the mechanism of ferroptosis has mostly been investigated in cancer field. Whether the ferroptosis processes in tumors and ischemic brain are identical remains unclear. In tumors, ferroptosis has often been studied using ferroptotic stimulators such as erastin or RSL3 (Kathman et al., 2020; Zhang, Deng, et al., 2020). However, more *in vitro* and *in vivo* models should be developed to analyze ferroptosis after ischemic stroke. In addition, different cell types in brain the tissue, including neurons, microglia, astrocytes, oligodendrocytes, and so on, are stimulated by ischemia simultaneously. But the role of ferroptosis in these different cell types in brain tissue remains to be illuminated. Ischemia can induce inflammation in the affected brain tissue. Many studies demonstrate that ferroptosis can be accompanied with inflammation (Li et al., 2018; von Mässenhausen et al., 2018); however, the mechanistic link between ferroptosis and inflammation during ischemic stroke is not yet known. Investigation of the role of ferroptosis in ischemic stroke and the underlying molecular pathways is still in its early stages and the current data are mostly obtained using cellular and animal models, indicating the need of conducting clinical studies in patients with ischemic stroke.

The development of drugs targeting ferroptosis in ischemic stroke is an important aspect of research. The identified mechanisms of ferroptosis following IR injury offer molecular targets to interfere with in order to improve neuroprotection in the brain. However, during ischemic stroke, neuronal cells may experience different types of PCD, including apoptosis, autophagy, necrosis, and/or ferroptosis. Which PCD type should be mostly focused on? Can a combined therapy targeting distinct PCD types achieve better results? Since the pathological process in ischemic stroke includes acute, subacute, and chronic phases, when should the drugs be administered? These questions should be addressed by further basic and clinical research on ferroptosis in ischemic stroke.

Summary Statement

Based on the collection of the recent progress in the mechanism of ferroptosis, this review summarized the possible function of ferroptosis in the pathophysiological process of ischemic stroke to seek more approaches to the diagnosis and treatment of this disease.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

Our study was supported financially by the Key Research and Development Program of Liaoning Province (2020JH2/10300047) and National Natural Science Foundation of China (81771271).

ORCID iD


Juan Feng https://orcid.org/0000-0002-1815-7036

References

Abdul, Y., Li, W., Ward, R., Abdelsaid, M., Hafez, S., Dong, G., Jamil, S., Wolf, V., Johnson, M. H., Fagan, S. C., & Ergul, A. (2020). Deferoxamine treatment prevents post-stroke vasoregression and neurovascular unit remodeling leading to improved functional outcomes in type 2 male diabetic rats: Role of endothelial ferroptosis. *Translational Stroke Research*, *12*(4), 615–630. https://doi.org/10.1007/s12975-020-00844-7

Adedoyin, O., Boddu, R., Traylor, A., Lever, J. M., Bolisetty, S., George, J. F., & Agarwal, A. (2018). Heme oxygenase-1 mitigates ferroptosis in renal proximal tubule cells. *American Journal of Physiology Renal Physiology*, *314*(5), F702–F714. https://doi.org/10.1152/ajprenal.00044.2017

Adibhatla, R. M., Hatcher, J. F., & Dempsey, R. J. (2003). Phospholipase A2, hydroxyl radicals, and lipid peroxidation in transient cerebral ischemia. *Antioxidants & Redox Signaling*, *5*(5), 647–654. https://doi.org/10.1089/152308603770310329

Aimé, P., Karuppagounder, S. S., Rao, A., Chen, Y., Burke, R. E., Ratan, R. R., & Greene, L. A. (2020). The drug adaptaquin blocks ATF4/CHOP-dependent pro-death Trib3 induction and protects in cellular and mouse models of Parkinson's disease. *Neurobiology of Disease*, *136*, 104725. https://doi.org/10.1016/j.nbd.2019.104725

Alim, I., Caulfield, J. T., Chen, Y., Swarup, V., Geschwind, D. H., Ivanova, E., Seravalli, J., Ai, Y., Sansing, L. H., Ste Marie, E. J., Hondal, R. J., Mukherjee, S., Cave, J. W., Sagdullaev, B. T., Karuppagounder, S. S., & Ratan, R. R. (2019). Selenium drives a transcriptional adaptive program to block ferroptosis and treat stroke. *Cell*, *177*(5), 1262–1279.e25. https://doi.org/10.1016/j.cell.2019.03.032

Angeli, J. P. F., Shah, R., Pratt, D. A., & Conrad, M. (2017). Ferroptosis inhibition: Mechanisms and opportunities. *Trends in Pharmacological Sciences*, *38*(5), 489–498. https://doi.org/10.1016/j.tips.2017.02.005

Bai, T., Li, M., Liu, Y., Qiao, Z., & Wang, Z. (2020). Inhibition of ferroptosis alleviates atherosclerosis through attenuating lipid peroxidation and endothelial dysfunction in mouse aortic endothelial cell. *Free Radical Biology & Medicine*, *160*, 92–102. https://doi.org/10.1016/j.freeradbiomed.2020.07.026

Bannai, S. (1986). Exchange of cystine and glutamate across plasma membrane of human fibroblasts. *The Journal of Biological Chemistry*, *261*(5), 2256–2263. https://doi.org/10.1016/S0021-9258(17)35926-4

Bannai, S., & Kitamura, E. (1980). Transport interaction of L-cystine and L-glutamate in human diploid fibroblasts in culture. *The Journal of Biological Chemistry*, *255*(6), 2372–2376. https://doi.org/10.1016/S0021-9258(19)85901-X

Bannai, S., Tsukeda, H., & Okumura, H. (1977). Effect of antioxidants on cultured human diploid fibroblasts exposed to cystine-free medium. *Biochemical and Biophysical Research Communications*, *74*(4), 1582–1588. https://doi.org/10.1016/0006-291X(77)90623-4

Basso, M., Berlin, J., Xia, L., Sleiman, S. F., Ko, B., Haskew-Layton, R., Kim, E., Antonyak, M. A., Cerione, R. A., Iismaa, S. E., Willis, D., Cho, S., & Ratan, R. R. (2012). Transglutaminase inhibition protects against oxidative stress-induced neuronal death downstream of pathological ERK activation. *Journal of Neuroscience*, *32*(19), 6561–6569. https://doi.org/10.1523/JNEUROSCI.3353-11.2012

Bersuker, K., Hendricks, J. M., Li, Z., Magtanong, L., Ford, B., Tang, P. H., Roberts, M. A., Tong, B., Maimone, T. J., Zoncu, R., Bassik, M. C., Nomura, D. K., Dixon, S. J., & Olzmann, J. A. (2019). The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature*, *575*(7784), 688–692. https://doi.org/10.1038/s41586-019-1705-2

Bogdan, A. R., Miyazawa, M., Hashimoto, K., & Tsuji, Y. (2016). Regulators of iron homeostasis: New players in metabolism, cell death, and disease. *Trends in Biochemical Sciences*, *41*(3), 274–286. https://doi.org/10.1016/j.tibs.2015.11.012

Brigelius-Flohé, R., & Maiorino, M. (2013). Glutathione peroxidases. *Biochimica et Biophysica Acta*, *1830*(5), 3289–3303. https://doi.org/10.1016/j.bbagen.2012.11.020

Brochier, C., Dennis, G., Rivieccio, M. A., McLaughlin, K., Coppola, G., Ratan, R. R., & Langley, B. (2013). Specific acetylation of p53 by HDAC inhibition prevents DNA damage-induced apoptosis in neurons. *Journal of Neuroscience*, *33*(20), 8621–8632. https://doi.org/10.1523/JNEUROSCI.5214-12.2013

Canals, S., Casarejos, M. J., de Bernardo, S., Rodríguez-Martín, E., & Mena, M. A. (2003). Nitric oxide triggers the toxicity due to glutathione depletion in midbrain cultures through 12-lipoxygenase. *Journal of Biological Chemistry*, *278*(24), 21542–21549. https://doi.org/10.1074/jbc.M213174200

Cao, J., Chen, X., Jiang, L., Lu, B., Yuan, M., Zhu, D., Zhu, H., He, Q., Yang, B., & Ying, M. (2020). DJ-1 suppresses ferroptosis through preserving the activity of S-adenosyl homocysteine hydrolase. *Nature Communications*, *11*(1), 1251. https://doi.org/10.1038/s41467-020-15109-y

Cao, Y., Luo, Y., Zou, J., Ouyang, J., Cai, Z., Zeng, X., Ling, H., & Zeng, T. (2019). Autophagy and its role in gastric cancer. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *489*, 10–20. https://doi.org/10.1016/j.cca.2018.11.028

Caso, J. R., Pradillo, J. M., Hurtado, O., Lorenzo, P., Moro, M. A., & Lizasoain, I. (2007). Toll-like receptor 4 is involved in brain damage and inflammation after experimental stroke. *Circulation*, *115*(12), 1599–1608. https://doi.org/10.1161/CIRCULATIONAHA.106.603431

Cetin, C., Erdogan, A. M., Dincel, G. C., Bakar, B., & Kisa, U. (2017). Effects of sulphasalazine in cerebral ischemia reperfusion injury in rat. *Archives of Medical Research*, *48*(3), 247–256. https://doi.org/10.1016/j.arcmed.2017.06.004

Chamorro, Á., Dirnagl, U., Urra, X., & Planas, A. M. (2016). Neuroprotection in acute stroke: Targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *The Lancet Neurology*, *15*(8), 869–881. https://doi.org/10.1016/S1474-4422(16)00114-9

Chatterjee, S., Zaman, K., Ryu, H., Conforto, A., & Ratan, R. R. (2001). Sequence-selective DNA binding drugs mithramycin A and chromomycin A3 are potent inhibitors of neuronal apoptosis induced by oxidative stress and DNA damage in cortical neurons. *Annals of Neurology*, *49*(3), 345–354. https://doi.org/10.1002/ana.71

Chen, L. D., Wu, R. H., Huang, Y. Z., Chen, M. X., Zeng, A. M., Zhuo, G. F., Xu, F. S., Liao, R., & Lin, Q. C. (2020). The role of ferroptosis in chronic intermittent hypoxia-induced liver injury in rats. *Sleep & Breathing = Schlaf & Atmung*, *24*(4), 1767–1773. https://doi.org/10.1007/s11325-020-02091-4

Cheng, J., Fan, Y. Q., Liu, B. H., Zhou, H., Wang, J. M., & Chen, Q. X. (2020). ACSL4 Suppresses glioma cells proliferation via activating ferroptosis. *Oncology Reports*, *43*(1), 147–158. https://doi.org/10.3892/or.2019.7419

Cheng, N. T., & Kim, A. S. (2015). Intravenous thrombolysis for acute ischemic stroke within 3 hours versus between 3 and 4.5 hours of symptom onset. *The Neurohospitalist*, *5*(3), 101–109. https://doi.org/10.1177/1941874415583116

Cheung, P. Y., Wang, W., & Schulz, R. (2000). Glutathione protects against myocardial ischemia-reperfusion injury by detoxifying peroxynitrite. *Journal of Molecular and Cellular Cardiology*, *32*(9), 1669–1678. https://doi.org/10.1006/jmcc.2000.1203

Choi, D. W., Maulucci-Gedde, M., & Kriegstein, A. R. (1987). Glutamate neurotoxicity in cortical cell culture. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *7*(2), 357–368. https://doi.org/10.1523/JNEUROSCI.07-02-00357.1987

Cong, L., Dong, X., Wang, Y., Deng, Y., Li, B., & Dai, R. (2019). On the role of synthesized hydroxylated chalcones as dual functional amyloid-β aggregation and ferroptosis inhibitors for potential treatment of Alzheimer's disease. *European Journal of Medicinal Chemistry*, *166*, 11–21. https://doi.org/10.1016/j.ejmech.2019.01.039

Crawford, R. R., Prescott, E. T., Sylvester, C. F., Higdon, A. N., Shan, J., Kilberg, M. S., & Mungrue, I. N. (2015). Human CHAC1 protein degrades glutathione, and mRNA induction is regulated by the transcription factors ATF4 and ATF3 and a bipartite ATF/CRE regulatory element. *The Journal of Biological Chemistry*, *290*(25), 15878–15891. https://doi.org/10.1074/jbc.M114.635144

Dang, C. V. (2012). MYC On the path to cancer. *Cell*, *149*(1), 22–35. https://doi.org/10.1016/j.cell.2012.03.003

Dávalos, A., Fernandez-Real, J. M., Ricart, W., Soler, S., Molins, A., Planas, E., & Genís, D. (1994). Iron-related damage in acute ischemic stroke. *Stroke*, *25*(8), 1543–1546. https://doi.org/10.1161/01.STR.25.8.1543

Davis, R. J. (1993). The mitogen-activated protein kinase signal transduction pathway. *Journal of Biological Chemistry*, *268*(20), 14553–14556. https://doi.org/10.1016/S0021-9258(18)82362-6

Degterev, A., Huang, Z., Boyce, M., Li, Y., Jagtap, P., Mizushima, N., Cuny, G. D., Mitchison, T. J., Moskowitz, M. A., & Yuan, J. (2005). Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nature Chemical Biology*, *1*(2), 112–119. https://doi.org/10.1038/nchembio711

Dietrich, R. B., & Bradley, W. G. Jr. (1988). Iron accumulation in the basal ganglia following severe ischemic-anoxic insults in children.

Radiology, *168*(1), 203–206. https://doi.org/10.1148/radiology.168.1.3380958

Dixon, S. J. (2017). Ferroptosis: Bug or feature? *Immunological Reviews*, *277*(1), 150–157. https://doi.org/10.1111/imr.12533

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., Patel, D. N., Bauer, A. J., Cantley, A. M., Yang, W. S., Morrison, B. 3rd, & Stockwell, B. R. (2012). Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell*, *149*(5), 1060–1072. https://doi.org/10.1016/j.cell.2012.03.042

Doll, S., Freitas, F. P., Shah, R., Aldrovandi, M., da Silva, M. C., Ingold, I., Grocin, A. G., da Silva, T. N. X., Panzilius, E., Scheel, C. H., Mourão, A., Buday, K., Sato, M., Wanninger, J., Vignane, T., Mohana, V., Rehberg, M., Flatley, A., & Schepers, A., … Conrad, M. (2019). FSP1 Is a glutathione-independent ferroptosis suppressor. *Nature*, *575*(7784), 693–698. https://doi.org/10.1038/s41586-019-1707-0

Doll, S., Proneth, B., Tyurina, Y. Y., Panzilius, E., Kobayashi, S., Ingold, I., Irmler, M., Beckers, J., Aichler, M., Walch, A., Prokisch, H., Trümbach, D., Mao, G., Qu, F., Bayir, H., Füllekrug, J., Scheel, C. H., Wurst, W., & Schick, J. A., … Conrad, M. (2017). ACSL4 Dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nature Chemical Biology*, *13*(1), 91–98. https://doi.org/10.1038/nchembio.2239

Dolma, S., Lessnick, S. L., Hahn, W. C., & Stockwell, B. R. (2003). Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. *Cancer Cell*, *3*(3), 285–296. https://doi.org/10.1016/S1535-6108(03)00050-3

Domercq, M., Szczupak, B., Gejo, J., Gómez-Vallejo, V., Padro, D., Gona, K. B., Dollé, F., Higuchi, M., Matute, C., Llop, J., & Martín, A. (2016). PET Imaging with [(18)F]FSPG evidences the role of system xc(-) on brain inflammation following cerebral ischemia in rats. *Theranostics*, *6*(11), 1753–1767. https://doi.org/10.7150/thno.15616

Do Van, B., Gouel, F., Jonneaux, A., Timmerman, K., Gelé, P., Pétrault, M., Bastide, M., Laloux, C., Moreau, C., Bordet, R., Devos, D., & Devedjian, J. C. (2016). Ferroptosis, a newly characterized form of cell death in Parkinson's disease that is regulated by PKC. *Neurobiology of Disease*, *94*, 169–178. https://doi.org/10.1016/j.nbd.2016.05.011

Evonuk, K. S., Baker, B. J., Doyle, R. E., Moseley, C. E., Sestero, C. M., Johnston, B. P., De Sarno, P., Tang, A., Gembitsky, I., Hewett, S. J., Weaver, C. T., Raman, C., & DeSilva, T. M. (2015). Inhibition of system Xc(-) transporter attenuates autoimmune inflammatory demyelination. *Journal of Immunology (Baltimore, MD: 1950)*, *195*(2), 450–463. https://doi.org/10.4049/jimmunol.1401108

Farrelly, L. A., Thompson, R. E., Zhao, S., Lepack, A. E., Lyu, Y., Bhanu, N. V., Zhang, B., Loh, Y.-H. E., Ramakrishnan, A., Vadodaria, K. C., Heard, K. J., Erikson, G., Nakadai, T., Bastle, R. M., Lukasak, B. J., Zebroski, H., Alenina, N., Bader, M., & Berton, O., … Maze, I. (2019). Histone serotonylation is a permissive modification that enhances TFIID binding to H3K4me3. *Nature*, *567*(7749), 535–539. https://doi.org/10.1038/s41586-019-1024-7

Feng, Y., Madungwe, N. B., Imam Aliagan, A. D., Tombo, N., & Bopassa, J. C. (2019). Liproxstatin-1 protects the mouse myocardium against ischemia/reperfusion injury by decreasing VDAC1 levels and restoring GPX4 levels. *Biochemical and Biophysical Research Communications*, *520*(3), 606–611. https://doi.org/10.1016/j.bbrc.2019.10.006

Frazer, D. M., & Anderson, G. J. (2014). The regulation of iron transport. *BioFactors (Oxford, England)*, *40*(2), 206–214. https://doi.org/10.1002/biof.1148

Friedmann Angeli, J. P., Schneider, M., Proneth, B., Tyurina, Y. Y., Tyurin, V. A., Hammond, V. J., Herbach, N., Aichler, M., Walch, A., Eggenhofer, E., Basavarajappa, D., Rådmark, O., Kobayashi, S., Seibt, T., Beck, H., Neff, F., Esposito, I., Wanke, R., & Förster, H., … Conrad, M. (2014). Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nature Cell Biology*, *16*(12), 1180–1191. https://doi.org/10.1038/ncb3064

Galluzzi, L., Baehrecke, E. H., Ballabio, A., Boya, P., Pedro, J. M. B.-S., Cecconi, F., Choi, A. M., Chu, C. T., Codogno, P., Colombo, M. I., Cuervo, A. M., Debnath, J., Deretic, V., Dikic, I., Eskelinen, E.-L., Fimia, G. M., Fulda, S., Gewirtz, D. A., & Green, D. R., … Kroemer, G. (2017). Molecular definitions of autophagy and related processes. *The EMBO Journal*, *36*(13), 1811–1836. https://doi.org/10.15252/embj.201796697

Gao, M., Monian, P., Pan, Q., Zhang, W., Xiang, J., & Jiang, X. (2016). Ferroptosis is an autophagic cell death process. *Cell Research*, *26*(9), 1021–1032. https://doi.org/10.1038/cr.2016.95

Gao, M., Monian, P., Quadri, N., Ramasamy, R., & Jiang, X. (2015). Glutaminolysis and transferrin regulate ferroptosis. *Molecular Cell*, *59*(2), 298–308. https://doi.org/10.1016/j.molcel.2015.06.011

Gao, M., Yi, J., Zhu, J., Minikes, A. M., Monian, P., Thompson, C. B., & Jiang, X. (2019). Role of mitochondria in ferroptosis. *Molecular Cell*, *73*(2), 354–363.e353. https://doi.org/10.1016/j.molcel.2018.10.042

Gill, D., Monori, G., Tzoulaki, I., & Dehghan, A. (2018). Iron status and risk of stroke. *Stroke*, *49*(12), 2815–2821. https://doi.org/10.1161/STROKEAHA.118.022701

Gong, J., Sun, F., Li, Y., Zhou, X., Duan, Z., Duan, F., Zhao, L., Chen, H., Qi, S., & Shen, J. (2015). Momordica charantia polysaccharides could protect against cerebral ischemia/reperfusion injury through inhibiting oxidative stress mediated c-Jun N-terminal kinase 3 signaling pathway. *Neuropharmacology*, *91*, 123–134. https://doi.org/10.1016/j.neuropharm.2014.11.020

Guan, X., Li, X., Yang, X., Yan, J., Shi, P., Ba, L., Cao, Y., & Wang, P. (2019). The neuroprotective effects of carvacrol on ischemia/reperfusion-induced hippocampal neuronal impairment by ferroptosis mitigation. *Life Sciences*, *235*, 116795. https://doi.org/10.1016/j.lfs.2019.116795

Guan, X., Li, Z., Zhu, S., Cheng, M., Ju, Y., Ren, L., Yang, G., & Min, D. (2021). Galangin attenuated cerebral ischemia-reperfusion injury by inhibition of ferroptosis through activating the SLC7A11/GPX4 axis in gerbils. *Life Sciences*, *264*, 118660. https://doi.org/10.1016/j.lfs.2020.118660

Guerrero-Hue, M., García-Caballero, C., Palomino-Antolín, A., Rubio-Navarro, A., Vázquez-Carballo, C., Herencia, C., Martín-Sanchez, D., Farré-Alins, V., Egea, J., Cannata, P., Praga, M., Ortiz, A., Egido, J., Sanz, A. B., & Moreno, J. A. (2019). Curcumin reduces renal damage associated with rhabdomyolysis by decreasing ferroptosis-mediated cell death. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *33*(8), 8961–8975. https://doi.org/10.1096/fj.201900077R

Guyton, K. Z., Liu, Y., Gorospe, M., Xu, Q., & Holbrook, N. J. (1996). Activation of mitogen-activated protein kinase by H2O2. Role in cell survival following oxidant injury. *Journal of Biological Chemistry*, *271*(8), 4138–4142. https://doi.org/10.1074/jbc.271.8.4138

Hanson, L. R., Roeytenberg, A., Martinez, P. M., Coppes, V. G., Sweet, D. C., Rao, R. J., Marti, D. L., Hoekman, J. D., Matthews, R. B., Frey, W. H. 2nd, & Panter, S. S. (2009). Intranasal deferoxamine provides increased brain exposure and significant protection in rat ischemic stroke. *The Journal of Pharmacology and Experimental Therapeutics*, *330*(3), 679–686. https://doi.org/10.1124/jpet.108.149807

Hao, S., Liang, B., Huang, Q., Dong, S., Wu, Z., He, W., & Shi, M. (2018). Metabolic networks in ferroptosis. *Oncology Letters*, *15*(4), 5405–5411. https://doi.org/10.3892/ol.2018.8066

Hariharan, N., Zhai, P., & Sadoshima, J. (2011). Oxidative stress stimulates autophagic flux during ischemia/reperfusion. *Antioxidants & Redox Signaling*, *14*(11), 2179–2190. https://doi.org/10.1089/ars.2010.3488

Harrison, P. M., & Arosio, P. (1996). The ferritins: Molecular properties, iron storage function and cellular regulation. *Biochimica et Biophysica Acta*, *1275*(3), 161–203. https://doi.org/10.1016/0005-2728(96)00022-9

Hashidate-Yoshida, T., Harayama, T., Hishikawa, D., Morimoto, R., Hamano, F., Tokuoka, S. M., Eto, M., Tamura-Nakano, M., Yanobu-Takanashi, R., Mukumoto, Y., Kiyonari, H., Okamura, T., Kita, Y., Shindou, H., & Shimizu, T. (2015). Fatty acid remodeling by LPCAT3 enriches arachidonate in phospholipid membranes and regulates triglyceride transport. *eLife*, *4*, e06328. https://doi.org/10.7554/eLife.06328

Hayano, M., Yang, W. S., Corn, C. K., Pagano, N. C., & Stockwell, B. R. (2016). Loss of cysteinyl-tRNA synthetase (CARS) induces the transsulfuration pathway and inhibits ferroptosis induced by cystine deprivation. *Cell Death and Differentiation*, *23*(2), 270–278. https://doi.org/10.1038/cdd.2015.93

Henninger, N., & Fisher, M. (2016). Extending the time window for endovascular and pharmacological reperfusion. *Translational Stroke Research*, *7*(4), 284–293. https://doi.org/10.1007/s12975-015-0444-4

Homma, T., Kobayashi, S., Sato, H., & Fujii, J. (2019). Edaravone, a free radical scavenger, protects against ferroptotic cell death in vitro. *Experimental Cell Research*, *384*(1), 111592. https://doi.org/10.1016/j.yexcr.2019.111592

Hou, W., Xie, Y., Song, X., Sun, X., Lotze, M. T., Zeh, H. J. 3rd, Kang, R., & Tang, D. (2016). Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy*, *12*(8), 1425–1428. https://doi.org/10.1080/15548627.2016.1187366

Hurn, P. D., Koehler, R. C., Blizzard, K. K., & Traystman, R. J. (1995). Deferoxamine reduces early metabolic failure associated with severe cerebral ischemic acidosis in dogs. *Stroke*, *26*(4), 688–694; discussion 694–685. https://doi.org/10.1161/01.STR.26.4.688

Imam, M. U., Zhang, S., Ma, J., Wang, H., & Wang, F. (2017). Antioxidants mediate both iron homeostasis and oxidative stress. *Nutrients*, *9*(7), 671. https://doi.org/10.3390/nu9070671

Ingrassia, R., Lanzillotta, A., Sarnico, I., Benarese, M., Blasi, F., Borgese, L., Bilo, F., Depero, L., Chiarugi, A., Spano, P. F., & Pizzi, M. (2012). 1B/(-)IRE DMT1 expression during brain ischemia contributes to cell death mediated by NF-κB/RelA acetylation at Lys310. *PLoS One*, *7*(5), e38019. https://doi.org/10.1371/journal.pone.0038019

Isani, G., Bertocchi, M., Andreani, G., Farruggia, G., Cappadone, C., Salaroli, R., Forni, M., & Bernardini, C. (2019). Cytotoxic effects of artemisia annua L. and pure artemisinin on the D-17 canine osteosarcoma cell line. *Oxidative Medicine and Cellular Longevity*, *2019*, 1615758. https://doi.org/10.1155/2019/1615758

Jackman, N. A., Melchior, S. E., Hewett, J. A., & Hewett, S. J. (2012). Non-cell autonomous influence of the astrocyte system Xc− on hypoglycaemic neuronal cell death. *ASN Neuro*, *4*(1), e00074. https://doi.org/10.1042/AN20110030

Jiang, L., Hickman, J. H., Wang, S. J., & Gu, W. (2015). Dynamic roles of p53-mediated metabolic activities in ROS-induced stress responses. *Cell Cycle (Georgetown, TX)*, *14*(15), 2881–2885. https://doi.org/10.1080/15384101.2015.1068479

Jiang, L., Kon, N., Li, T., Wang, S. J., Su, T., Hibshoosh, H., Baer, R., & Gu, W. (2015). Ferroptosis as a p53-mediated activity during tumour suppression. *Nature*, *520*(7545), 57–62. https://doi.org/10.1038/nature14344

Jickling, G. C., Liu, D., Stamova, B., Ander, B. P., Zhan, X., Lu, A., & Sharp, F. R. (2014). Hemorrhagic transformation after ischemic stroke in animals and humans. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *34*(2), 185–199. https://doi.org/10.1038/jcbfm.2013.203

Kaelberer, M. M., Buchanan, K. L., Klein, M. E., Barth, B. B., Montoya, M. M., Shen, X., & Bohórquez, D. V. (2018). A gut-brain neural circuit for nutrient sensory transduction. *Science (New York, NY)*, *361*(6408), eaat5236. https://doi.org/10.1126/science.aat5236

Karuppagounder, S. S., Alim, I., Khim, S. J., Bourassa, M. W., Sleiman, S. F., John, R., Thinnes, C. C., Yeh, T.-L., Demetriades, M., Neitemeier, S., Cruz, D., Gazaryan, I., Killilea, D. W., Morgenstern, L., Xi, G., Keep, R. F., Schallert, T., Tappero, R. V., & Zhong, J., … Ratan, R. R. (2016). Therapeutic targeting of oxygen-sensing prolyl hydroxylases abrogates ATF4-dependent neuronal death and improves outcomes after brain hemorrhage in several rodent models. *Science Translational Medicine*, *8*(328), 328ra329. https://doi.org/10.1126/scitranslmed.aac6008

Kasasbeh, A. S., Christensen, S., Parsons, M. W., Campbell, B., Albers, G. W., & Lansberg, M. G. (2019). Artificial neural network computer tomography perfusion prediction of ischemic core. *Stroke*, *50*(6), 1578–1581. https://doi.org/10.1161/STROKEAHA.118.022649

Katada, S., Imhof, A., & Sassone-Corsi, P. (2012). Connecting threads: Epigenetics and metabolism. *Cell*, *148*(1-2), 24–28. https://doi.org/10.1016/j.cell.2012.01.001

Kathman, S. G., Boshart, J., Jing, H., & Cravatt, B. F. (2020). Blockade of the lysophosphatidylserine lipase ABHD12 potentiates ferroptosis in cancer cells. *ACS Chemical Biology*, *15*(4), 871–877. https://doi.org/10.1021/acschembio.0c00086

Khanna, S., Roy, S., Ryu, H., Bahadduri, P., Swaan, P. W., Ratan, R. R., & Sen, C. K. (2003). Molecular basis of vitamin E action: Tocotrienol modulates 12-lipoxygenase, a key mediator of glutamate-induced neurodegeneration. *Journal of Biological Chemistry*, *278*(44), 43508–43515. https://doi.org/10.1074/jbc.M307075200

Kleindorfer, D., Kissela, B., Schneider, A., Woo, D., Khoury, J., Miller, R., Alwell, K., Gebel, J., Szaflarski, J., Pancioli, A., Jauch, E., Moomaw, C., Shukla, R., & Broderick, J. P. (2004). Eligibility for recombinant tissue plasminogen activator in acute ischemic stroke: A population-based study. *Stroke*, *35*(2), e27–e29. https://doi.org/10.1161/01.STR.0000109767.11426.17

Kondo, Y., Ogawa, N., Asanuma, M., Ota, Z., & Mori, A. (1995). Regional differences in late-onset iron deposition, ferritin,

transferrin, astrocyte proliferation, and microglial activation after transient forebrain ischemia in rat brain. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *15*(2), 216–226. https://doi.org/10.1038/jcbfm.1995.27

Krainz, T., Gaschler, M. M., Lim, C., Sacher, J. R., Stockwell, B. R., & Wipf, P. (2016). A mitochondrial-targeted nitroxide is a potent inhibitor of ferroptosis. *ACS Central Science*, *2*(9), 653–659. https://doi.org/10.1021/acscentsci.6b00199

Kwak, K. A., Kwon, H. B., Lee, J. W., & Park, Y. S. (2018). Current perspectives regarding stem cell-based therapy for ischemic stroke. *Current Pharmaceutical Design*, *24*(28), 3332–3340. https://doi.org/10.2174/1381612824666180604111806

Lambertsen, K. L., Finsen, B., & Clausen, B. H. (2019). Post-stroke inflammation-target or tool for therapy? *Acta Neuropathologica*, *137*(5), 693–714. https://doi.org/10.1007/s00401-018-1930-z

Lan, B., Ge, J. W., Cheng, S. W., Zheng, X. L., Liao, J., He, C., Rao, Z. Q., & Wang, G. Z. (2020). Extract of naotaifang, a compound Chinese herbal medicine, protects neuron ferroptosis induced by acute cerebral ischemia in rats. *Journal of Integrative Medicine*, *18*(4), 344–350. https://doi.org/10.1016/j.joim.2020.01.008

Lange, P. S., Chavez, J. C., Pinto, J. T., Coppola, G., Sun, C. W., Townes, T. M., Geschwind, D. H., & Ratan, R. R. (2008). ATF4 Is an oxidative stress-inducible, prodeath transcription factor in neurons in vitro and in vivo. *The Journal of Experimental Medicine*, *205*(5), 1227–1242. https://doi.org/10.1084/jem.20071460

Li, Y., Cao, Y., Xiao, J., Shang, J., Tan, Q., Ping, F., Huang, W., Wu, F., Zhang, H., & Zhang, X. (2020). Inhibitor of apoptosis-stimulating protein of p53 inhibits ferroptosis and alleviates intestinal ischemia/reperfusion-induced acute lung injury. *Cell Death and Differentiation*, *27*(9), 2635–2650. https://doi.org/10.1038/s41418-020-0528-x

Li, C., Deng, X., Xie, X., Liu, Y., Friedmann Angeli, J. P., & Lai, L. (2018). Activation of glutathione peroxidase 4 as a novel anti-inflammatory strategy. *Frontiers in Pharmacology*, *9*, 1120. https://doi.org/10.3389/fphar.2018.01120

Li, Y., Liu, Y., Wu, P., Tian, Y., Liu, B., Wang, J., Bihl, J., & Shi, H. (2020). Inhibition of ferroptosis alleviates early brain injury after subarachnoid hemorrhage in vitro and in vivo via reduction of lipid peroxidation. *Cellular and Molecular Neurobiology*, *41*, 263–278. https://doi.org/10.1007/s10571-020-00850-1

Li, Y., Maher, P., & Schubert, D. (1997). A role for 12-lipoxygenase in nerve cell death caused by glutathione depletion. *Neuron*, *19*(2), 453–463. https://doi.org/10.1016/S0896-6273(00)80953-8

Li, C., Mu, N., Gu, C., Liu, M., Yang, Z., Yin, Y., Chen, M., Wang, Y., Han, Y., Yu, L., & Ma, H. (2020). Metformin mediates cardioprotection against aging-induced ischemic necroptosis. *Aging Cell*, *19*(2), e13096. https://doi.org/10.1111/acel.13096

Li, Y., Zhang, Y., Fu, H., Huang, H., Lu, Q., Qin, H., Wu, Y., Huang, H., Mao, G., Wei, Z., & Liao, P. (2020). Hes1 knockdown exacerbates ischemic stroke following tMCAO by increasing ER stress-dependent apoptosis via the PERK/eIF2α/ATF4/CHOP signaling pathway. *Neuroscience Bulletin*, *36*(2), 134–142. https://doi.org/10.1007/s12264-019-00411-7

Liao, L., Jiang, C., Chen, J., Shi, J., Li, X., Wang, Y., Wen, J., Zhou, S., Liang, J., Lao, Y., & Zhang, J. (2020). Synthesis and biological evaluation of 1,2,4-triazole derivatives as potential neuroprotectant against ischemic brain injury. *European Journal of Medicinal Chemistry*, *190*, 112114. https://doi.org/10.1016/j.ejmech.2020.112114

Liu, N., Lin, X., & Huang, C. (2020). Activation of the reverse transsulfuration pathway through NRF2/CBS confers erastin-induced ferroptosis resistance. *British Journal of Cancer*, *122*(2), 279–292. https://doi.org/10.1038/s41416-019-0660-x

Louandre, C., Ezzoukhry, Z., Godin, C., Barbare, J. C., Mazière, J. C., Chauffert, B., & Galmiche, A. (2013). Iron-dependent cell death of hepatocellular carcinoma cells exposed to sorafenib. *International Journal of Cancer*, *133*(7), 1732–1742. https://doi.org/10.1002/ijc.28159

Lu, J., Xu, F., & Lu, H. (2020). LncRNA PVT1 regulates ferroptosis through miR-214-mediated TFR1 and p53. *Life Sciences*, *260*, 118305. https://doi.org/10.1016/j.lfs.2020.118305

Lynch, D. R., & Dawson, T. M. (1994). Secondary mechanisms in neuronal trauma. *Current Opinion in Neurology*, *7*(6), 510–516. https://doi.org/10.1097/00019052-199412000-00007

Ma, P., Xiao, H., Yu, C., Liu, J., Cheng, Z., Song, H., Zhang, X., Li, C., Wang, J., Gu, Z., & Lin, J. (2017). Enhanced cisplatin chemotherapy by iron oxide nanocarrier-mediated generation of highly toxic reactive oxygen Species. *Nano Letters*, *17*(2), 928–937. https://doi.org/10.1021/acs.nanolett.6b04269

Magtanong, L., Ko, P. J., & Dixon, S. J. (2016). Emerging roles for lipids in non-apoptotic cell death. *Cell Death and Differentiation*, *23*(7), 1099–1109. https://doi.org/10.1038/cdd.2016.25

Malagelada, C., Jin, Z. H., & Greene, L. A. (2008). RTP801 is induced in Parkinson's disease and mediates neuron death by inhibiting Akt phosphorylation/activation. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *28*(53), 14363–14371. https://doi.org/10.1523/JNEUROSCI.3928-08.2008

Mancias, J. D., Wang, X., Gygi, S. P., Harper, J. W., & Kimmelman, A. C. (2014). Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature*, *509*(7498), 105–109. https://doi.org/10.1038/nature13148

Mao, L., Zuo, M. L., Wang, A. P., Tian, Y., Dong, L. C., Li, T. M., Kuang, D. B., Song, G. L., & Yang, Z. B. (2020). Low expression of miR–532–3p contributes to cerebral ischemia/reperfusion oxidative stress injury by directly targeting NOX2. *Molecular Medicine Reports*, *22*(3), 2415–2423. https://doi.org/10.3892/mmr.2020.11325

Masaldan, S., Bush, A. I., Devos, D., Rolland, A. S., & Moreau, C. (2019). Striking while the iron is hot: Iron metabolism and ferroptosis in neurodegeneration. *Free Radical Biology & Medicine*, *133*, 221–233. https://doi.org/10.1016/j.freeradbiomed.2018.09.033

McBean, G. J. (2012). The transsulfuration pathway: A source of cysteine for glutathione in astrocytes. *Amino Acids*, *42*(1), 199–205. https://doi.org/10.1007/s00726-011-0864-8

McConoughey, S. J., Basso, M., Niatsetskaya, Z. V., Sleiman, S. F., Smirnova, N. A., Langley, B. C., Mahishi, L., Cooper, A. J. L., Antonyak, M. A., Cerione, R. A., Li, B., Starkov, A., Chaturvedi, R. K., Flint Beal, M., Coppola, G., Geschwind, D. H., Ryu, H., Xia, L., & Iismaa, S. E., … Ratan, R. R. (2010). Inhibition of transglutaminase 2 mitigates transcriptional dysregulation in models of Huntington disease. *Embo Molecular Medicine*, *2*(9), 349–370. https://doi.org/10.1002/emmm.201000084

Millán, M., Sobrino, T., Arenillas, J. F., Rodríguez-Yáñez, M., García, M., Nombela, F., Castellanos, M., de la Ossa, N. P., Cuadras, P., Serena, J., Castillo, J., & Dávalos, A. (2008). Biological signatures of brain damage associated with high

serum ferritin levels in patients with acute ischemic stroke and thrombolytic treatment. *Disease Markers*, *25*(3), 181–188. https://doi.org/10.1155/2008/380356

Millan, M., Sobrino, T., Castellanos, M., Nombela, F., Arenillas, J. F., Riva, E., Cristobo, I., García, M. M., Vivancos, J., Serena, J., Moro, M. A., Castillo, J., & Dávalos, A. (2007). Increased body iron stores are associated with poor outcome after thrombolytic treatment in acute stroke. *Stroke*, *38*(1), 90–95. https://doi.org/10.1161/01.STR.0000251798.25803.e0

Mohsin Alvi, A., Tariq Al Kury, L., Umar Ijaz, M., Ali Shah, F., Khan M, T., Sheikh A, S., Nadeem, H., Khan, A. U., Zeb, A., & Li, S. (2020). Post-treatment of synthetic polyphenolic 1,3,4 oxadiazole compound A3, attenuated ischemic stroke-induced neuroinflammation and neurodegeneration. *Biomolecules*, *10*(6), 816. https://doi.org/10.3390/biom10060816

Moskowitz, M. A., Lo, E. H., & Iadecola, C. (2010). The science of stroke: Mechanisms in search of treatments. *Neuron*, *67*(2), 181–198. https://doi.org/10.1016/j.neuron.2010.07.002

Mughal, W., Dhingra, R., & Kirshenbaum, L. A. (2012). Striking a balance: Autophagy, apoptosis, and necrosis in a normal and failing heart. *Current Hypertension Reports*, *14*(6), 540–547. https://doi.org/10.1007/s11906-012-0304-5

Mukherjee, K., Chio, T. I., Sackett, D. L., & Bane, S. L. (2015). Detection of oxidative stress-induced carbonylation in live mammalian cells. *Free Radical Biology & Medicine*, *84*, 11–21. https://doi.org/10.1016/j.freeradbiomed.2015.03.011

Murphy, T. H., Miyamoto, M., Sastre, A., Schnaar, R. L., & Coyle, J. T. (1989). Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. *Neuron*, *2*(6), 1547–1558. https://doi.org/10.1016/0896-6273(89)90043-3

Nagata, S. (2018). Apoptosis and clearance of apoptotic cells. *Annual Review of Immunology*, *36*, 489–517. https://doi.org/10.1146/annurev-immunol-042617-053010

Naghavi, M., Wang, H., Lozano, R., Davis, A., Liang, X., Zhou, M., Vollset, S. E., Ozgoren, A. A., Abd-Allah, F., Aziz, M. I. A., Abera, S. F., Aboyans, V., Abraham, B., Abraham, J. P., Abuabara, K. E., Abubakar, I., Abu-Raddad, L. J., Abu-Rmeileh, N. M. E., Achoki, T., & , … Murray, C. J. L. (2015). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: A systematic analysis for the Global Burden of Disease Study 2013.. *Lancet (London, England)*, *385*(9963), 117–171. https://doi.org/10.1016/S0140-6736(14)61682-2.

Neitemeier, S., Jelinek, A., Laino, V., Hoffmann, L., Eisenbach, I., Eying, R., Ganjam, G. K., Dolga, A. M., Oppermann, S., & Culmsee, C. (2017). BID links ferroptosis to mitochondrial cell death pathways. *Redox Biology*, *12*, 558–570. https://doi.org/10.1016/j.redox.2017.03.007

Novgorodov, S. A., Voltin, J. R., Gooz, M. A., Li, L., Lemasters, J. J., & Gudz, T. I. (2018). Acid sphingomyelinase promotes mitochondrial dysfunction due to glutamate-induced regulated necrosis. *Journal of Lipid Research*, *59*(2), 312–329. https://doi.org/10.1194/jlr.M080374

Orellana-Urzúa, S., Claps, G., & Rodrigo, R. (2021). Improvement of a novel proposal for antioxidant treatment against brain damage occurring in ischemic stroke patients. *CNS & Neurological Disorders Drug Targets*, *20*, 3–21. https://doi.org/10.2174/1871527319666200910153431

Park, U. J., Lee, Y. A., Won, S. M., Lee, J. H., Kang, S. H., Springer, J. E., Lee, Y. B., & Gwag, B. J. (2011). Blood-derived iron mediates free radical production and neuronal death in the hippocampal CA1 area following transient forebrain ischemia in rat. *Acta Neuropathologica*, *121*(4), 459–473. https://doi.org/10.1007/s00401-010-0785-8

Paschen, W., Röhn, G., & Schmidt-Kastner, R. (1990). Transglutaminase activity in reversible cerebral ischemia in the rat. *Neuroscience Letters*, *110*(1-2), 232–236. https://doi.org/10.1016/0304-3940(90)90817-S

Pineda-Ramírez, N., Calzada, F., Alquisiras-Burgos, I., Medina-Campos, O. N., Pedraza-Chaverri, J., Ortiz-Plata, A., Pinzón Estrada, E., Torres, I., & Aguilera, P. (2020). Antioxidant properties and protective effects of some species of the annonaceae, lamiaceae, and geraniaceae families against neuronal damage induced by excitotoxicity and cerebral ischemia. *Antioxidants (Basel, Switzerland)*, *9*(3), 253. https://doi.org/10.3390/antiox9030253

Prasad, A., Sedlářová, M., Balukova, A., Rác, M., & Pospíšil, P. (2019). Reactive oxygen species as a response to wounding: In vivo imaging in Arabidopsis thaliana. *Frontiers in Plant Science*, *10*, 1660. https://doi.org/10.3389/fpls.2019.01660

Ratan, R. R. (2020). The chemical biology of ferroptosis in the central nervous system. *Cell Chemical Biology*, *27*(5), 479–498. https://doi.org/10.1016/j.chembiol.2020.03.007

Ryan, F., Zarruk, J. G., Lößlein, L., & David, S. (2018). Ceruloplasmin plays a neuroprotective role in cerebral ischemia. *Frontiers in Neuroscience*, *12*, 988. https://doi.org/10.3389/fnins.2018.00988

Ryu, H., Lee, J., Olofsson, B. A., Mwidau, A., Dedeoglu, A., Escudero, M., Flemington, E., Azizkhan-Clifford, J., Ferrante, R. J., & Ratan, R. R. (2003). Histone deacetylase inhibitors prevent oxidative neuronal death independent of expanded polyglutamine repeats via an Sp1-dependent pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(7), 4281–4286. https://doi.org/10.1073/pnas.0737363100

Sabetghadam, M., Mazdeh, M., Abolfathi, P., Mohammadi, Y., & Mehrpooya, M. (2020). Evidence for a beneficial effect of oral N-acetylcysteine on functional outcomes and inflammatory biomarkers in patients with acute ischemic stroke. *Neuropsychiatric Disease and Treatment*, *16*, 1265–1278. https://doi.org/10.2147/NDT.S241497

Sagara, J., Miura, K., & Bannai, S. (1993). Cystine uptake and glutathione level in fetal brain cells in primary culture and in suspension. *Journal of Neurochemistry*, *61*(5), 1667–1671. https://doi.org/10.1111/j.1471-4159.1993.tb09801.x

Santana-Codina, N., & Mancias, J. D. (2018). The role of NCOA4-mediated ferritinophagy in health and disease. *Pharmaceuticals (Basel, Switzerland)*, *11*(4), 114. https://doi.org/10.3390/ph11040114

Sato, H., Tamba, M., Ishii, T., & Bannai, S. (1999). Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. *The Journal of Biological Chemistry*, *274*(17), 11455–11458. https://doi.org/10.1074/jbc.274.17.11455

Sattler, R., & Tymianski, M. (2000). Molecular mechanisms of calcium-dependent excitotoxicity. *Journal of Molecular Medicine (Berlin, Germany)*, *78*(1), 3–13. https://doi.org/10.1007/s001090000077

Saver, J. L., Goyal, M., Bonafe, A., Diener, H.-C., Levy, E. I., Pereira, V. M., Albers, G. W., Cognard, C., Cohen, D. J.,

Hacke, W., Jansen, O., Jovin, T. G., Mattle, H. P., Nogueira, R. G., Siddiqui, A. H., Yavagal, D. R., Baxter, B. W., Devlin, T. G., & Lopes, D. K., … Jahan, R. (2015). Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. *The New England Journal of Medicine*, *372*(24), 2285–2295. https://doi.org/10.1056/NEJMoa1415061

Selim, M. (2010). Treatment with the iron chelator, deferoxamine mesylate, alters serum markers of oxidative stress in stroke patients. *Translational Stroke Research*, *1*(1), 35–39. https://doi.org/10.1007/s12975-009-0001-0

Shah, R., Margison, K., & Pratt, D. A. (2017). The potency of diarylamine radical-trapping antioxidants as inhibitors of ferroptosis underscores the role of autoxidation in the mechanism of cell death. *ACS Chemical Biology*, *12*(10), 2538–2545. https://doi.org/10.1021/acschembio.7b00730

Shah, R., Shchepinov, M. S., & Pratt, D. A. (2018). Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. *ACS Central Science*, *4*(3), 387–396. https://doi.org/10.1021/acscentsci.7b00589

Shan, H. M., Zang, M., Zhang, Q., Shi, R. B., Shi, X. J., Mamtilahun, M., Liu, C., Luo, L. L., Tian, X., Zhang, Z., Yang, G. Y., Tang, Y., Pu, J., & Wang, Y. (2020). Farnesoid X receptor knockout protects brain against ischemic injury through reducing neuronal apoptosis in mice. *Journal of Neuroinflammation*, *17*(1), 164. https://doi.org/10.1186/s12974-020-01838-w

Shang, Y., Luo, M., Yao, F., Wang, S., Yuan, Z., & Yang, Y. (2020). Ceruloplasmin suppresses ferroptosis by regulating iron homeostasis in hepatocellular carcinoma cells. *Cellular Signalling*, *72*, 109633. https://doi.org/10.1016/j.cellsig.2020.109633

Shintoku, R., Takigawa, Y., Yamada, K., Kubota, C., Yoshimoto, Y., Takeuchi, T., Koshiishi, I., & Torii, S. (2017). Lipoxygenase-mediated generation of lipid peroxides enhances ferroptosis induced by erastin and RSL3. *Cancer Science*, *108*(11), 2187–2194. https://doi.org/10.1111/cas.13380

Siddiq, A., Ayoub, I. A., Chavez, J. C., Aminova, L., Shah, S., LaManna, J. C., Patton, S. M., Connor, J. R., Cherny, R. A., Volitakis, I., Bush, A. I., Langsetmo, I., Seeley, T., Gunzler, V., & Ratan, R. R. (2005). Hypoxia-inducible factor prolyl 4-hydroxylase inhibition. A target for neuroprotection in the central nervous system. *Journal of Biological Chemistry*, *280*(50), 41732–41743. https://doi.org/10.1074/jbc.M504963200

Sleiman, S. F., Langley, B. C., Basso, M., Berlin, J., Xia, L., Payappilly, J. B., Kharel, M. K., Guo, H., Marsh, J. L., Thompson, L. M., Mahishi, L., Ahuja, P., MacLellan, W. R., Geschwind, D. H., Coppola, G., Rohr, J., & Ratan, R. R. (2011). Mithramycin is a gene-selective Sp1 inhibitor that identifies a biological intersection between cancer and neurodegeneration. *Journal of Neuroscience*, *31*(18), 6858–6870. https://doi.org/10.1523/JNEUROSCI.0710-11.2011

Sleiman, S. F., Olson, D. E., Bourassa, M. W., Karuppagounder, S. S., Zhang, Y. L., Gale, J., Wagner, F. F., Basso, M., Coppola, G., Pinto, J. T., Holson, E. B., & Ratan, R. R. (2014). Hydroxamic acid-based histone deacetylase (HDAC) inhibitors can mediate neuroprotection independent of HDAC inhibition. *Journal of Neuroscience*, *34*(43), 14328–14337. https://doi.org/10.1523/JNEUROSCI.1010-14.2014

Słomka, A., Świtońska, M., & Żekanowska, E. (2015). Hepcidin levels are increased in patients with acute ischemic stroke: Preliminary report. *Journal of Stroke and Cerebrovascular Diseases: The Official Journal of National Stroke Association*, *24*(7), 1570–1576. https://doi.org/10.1016/j.jstrokecerebrovasdis.2015.03.031

Smirnova, N. A., Rakhman, I., Moroz, N., Basso, M., Payappilly, J., Kazakov, S., Hernandez-Guzman, F., Gaisina, I. N., Kozikowski, A. P., Ratan, R. R., & Gazaryan, I. G. (2010). Utilization of an in vivo reporter for high throughput identification of branched small molecule regulators of hypoxic adaptation. *Chemistry & Biology*, *17*(4), 380–391. https://doi.org/10.1016/j.chembiol.2010.03.008

Snow, S. J. (2016). Stroke and t-PA–triggering new paradigms of care. *The New England Journal of Medicine*, *374*(9), 809–811. https://doi.org/10.1056/NEJMp1514696

Sosnoski, H. M., Sears, S. M. S., He, Y., Frare, C., & Hewett, S. J. (2020). Sexually dimorphic and brain region-specific transporter adaptations in system x(c)(-) null mice. *Neurochemistry International*, *141*, 104888. https://doi.org/10.1016/j.neuint.2020.104888

Stabler, S. P., Lindenbaum, J., Savage, D. G., & Allen, R. H. (1993). Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. *Blood*, *81*(12), 3404–3413. https://doi.org/10.1182/blood.V81.12.3404.3404

Stanciu, M., & DeFranco, D. B. (2002). Prolonged nuclear retention of activated extracellular signal-regulated protein kinase promotes cell death generated by oxidative toxicity or proteasome inhibition in a neuronal cell line. *Journal of Biological Chemistry*, *277*(6), 4010–4017. https://doi.org/10.1074/jbc.M104479200

Stockwell, B. R., Friedmann Angeli, J. P., Bayir, H., Bush, A. I., Conrad, M., Dixon, S. J., Fulda, S., Gascón, S., Hatzios, S. K., Kagan, V. E., Noel, K., Jiang, X., Linkermann, A., Murphy, M. E., Overholtzer, M., Oyagi, A., Pagnussat, G. C., Park, J., & Ran, Q., … Zhang, D. D. (2017). Ferroptosis: A regulated cell death nexus linking metabolism, redox biology, and disease. *Cell*, *171*(2), 273–285. https://doi.org/10.1016/j.cell.2017.09.021

Sun, X., Ou, Z., Chen, R., Niu, X., Chen, D., Kang, R., & Tang, D. (2016). Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology (Baltimore, MD)*, *63*(1), 173–184. https://doi.org/10.1002/hep.28251

Tadokoro, T., Ikeda, M., Ide, T., Deguchi, H., Ikeda, S., Okabe, K., Ishikita, A., Matsushima, S., Koumura, T., Yamada, K. I., Imai, H., & Tsutsui, H. (2020). Mitochondria-dependent ferroptosis plays a pivotal role in doxorubicin cardiotoxicity. *JCI Insight*, *5*(9), e132747. https://doi.org/10.1172/jci.insight.132747

Tsai, M. H., Kuo, C. W., Lin, T. K., Ho, C. J., Wang, P. W., Chuang, J. H., & Liou, C. W. (2020). Ischemic stroke risk associated with mitochondrial haplogroup F in the Asian population. *Cells*, *9*(8), 1885. https://doi.org/10.3390/cells9081885

Tuo, Q. Z., Lei, P., Jackman, K. A., Li, X.-L., Xiong, H., Li, X.-L., Liuyang, Z.-Y., Roisman, L., Zhang, S.-T., Ayton, S., Wang, Q., Crouch, P. J., Ganio, K., Wang, X.-C., Pei, L., Adlard, P. A., Lu, Y.-M., Cappai, R., & Wang, J.-Z., … Bush, A. I. (2017). Tau-mediated iron export prevents ferroptotic damage after ischemic stroke. *Molecular Psychiatry*, *22*(11), 1520–1530. https://doi.org/10.1038/mp.2017.171

Valdés Hernández, M. D. C., Case, T., Chappell, F. M., Glatz, A., Makin, S., Doubal, F., & Wardlaw, J. M. (2019). Association between striatal brain iron deposition, microbleeds and cognition 1 year after a minor ischaemic stroke. *International Journal of Molecular Sciences*, *20*(6), 1293. https://doi.org/10.3390/ijms20061293

Valko, M., Morris, H., & Cronin, M. T. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, *12*(10), 1161–1208. https://doi.org/10.2174/0929867053764635

van Leyen, K., Arai, K., Jin, G., Kenyon, V., Gerstner, B., Rosenberg, P. A., Holman, T. R., & Lo, E. H. (2008). Novel lipoxygenase inhibitors as neuroprotective reagents. *Journal of Neuroscience Research*, *86*(4), 904–909. https://doi.org/10.1002/jnr.21543

van Leyen, K., Siddiq, A., Ratan, R. R., & Lo, E. H. (2005). Proteasome inhibition protects HT22 neuronal cells from oxidative glutamate toxicity. *Journal of Neurochemistry*, *92*(4), 824–830. https://doi.org/10.1111/j.1471-4159.2004.02915.x

Venkatesh, D., O'Brien, N. A., Zandkarimi, F., Tong, D. R., Stokes, M. E., Dunn, D. E., Kengmana, E. S., Aron, A. T., Klein, A. M., Csuka, J. M., Moon, S. H., Conrad, M., Chang, C. J., Lo, D. C., D'Alessandro, A., Prives, C., & Stockwell, B. R. (2020). MDM2 And MDMX promote ferroptosis by PPARα-mediated lipid remodeling. *Genes & Development*, *34*(7-8), 526–543. https://doi.org/10.1101/gad.334219.119

von Mässenhausen, A., Tonnus, W., & Linkermann, A. (2018). Cell death pathways drive necroinflammation during acute kidney injury. *Nephron*, *140*(2), 144–147. https://doi.org/10.1159/000490807

Wang, L., Cai, H., Hu, Y., Liu, F., Huang, S., Zhou, Y., Yu, J., Xu, J., & Wu, F. (2018). A pharmacological probe identifies cystathionine β-synthase as a new negative regulator for ferroptosis. *Cell Death & Disease*, *9*(10), 1005. https://doi.org/10.1038/s41419-018-1063-2

Wang, J., Cao, B., Han, D., Sun, M., & Feng, J. (2017). Long non-coding RNA H19 induces cerebral ischemia reperfusion injury via activation of autophagy. *Aging and Disease*, *8*(1), 71–84. https://doi.org/10.14336/AD.2016.0530

Wang, P., Cui, Y., Ren, Q., Yan, B., Zhao, Y., Yu, P., Gao, G., Shi, H., Chang, S., & Chang, Y. Z. (2021). Mitochondrial ferritin attenuates cerebral ischaemia/reperfusion injury by inhibiting ferroptosis. *Cell Death & Disease*, *12*(5), 447. https://doi.org/10.1038/s41419-021-03725-5

Wang, N., Zeng, G. Z., Yin, J. L., & Bian, Z. X. (2019). Artesunate activates the ATF4-CHOP-CHAC1 pathway and affects ferroptosis in Burkitt's lymphoma. *Biochemical and Biophysical Research Communications*, *519*(3), 533–539. https://doi.org/10.1016/j.bbrc.2019.09.023

Wortmann, M., Schneider, M., Pircher, J., Hellfritsch, J., Aichler, M., Vegi, N., Kölle, P., Kuhlencordt, P., Walch, A., Pohl, U., Bornkamm, G. W., Conrad, M., & Beck, H. (2013). Combined deficiency in glutathione peroxidase 4 and vitamin E causes multiorgan thrombus formation and early death in mice. *Circulation Research*, *113*(4), 408–417. https://doi.org/10.1161/CIRCRESAHA.113.279984

Wu, K. J., Polack, A., & Dalla-Favera, R. (1999). Coordinated regulation of iron-controlling genes, H-ferritin and IRP2, by c-MYC. *Science (New York, NY)*, *283*(5402), 676–679. https://doi.org/10.1126/science.283.5402.676

Xiao, N., Callaway, C. W., Lipinski, C. A., Hicks, S. D., & DeFranco, D. B. (1999). Geldanamycin provides posttreatment protection against glutamate-induced oxidative toxicity in a mouse hippocampal cell line. *Journal of Neurochemistry*, *72*(1), 95–101. https://doi.org/10.1046/j.1471-4159.1999.0720095.x

Xiao, F. J., Zhang, D., Wu, Y., Jia, Q. H., Zhang, L., Li, Y. X., Yang, Y. F., Wang, H., Wu, C. T., & Wang, L. S. (2019). miRNA-17-92 protects endothelial cells from erastin-induced ferroptosis through targeting the A20-ACSL4 axis. *Biochemical and Biophysical Research Communications*, *515*(3), 448–454. https://doi.org/10.1016/j.bbrc.2019.05.147

Xie, Y., Hou, W., Song, X., Yu, Y., Huang, J., Sun, X., Kang, R., & Tang, D. (2016). Ferroptosis: Process and function. *Cell Death and Differentiation*, *23*(3), 369–379. https://doi.org/10.1038/cdd.2015.158

Xie, Y., Zhu, S., Song, X., Sun, X., Fan, Y., Liu, J., Zhong, M., Yuan, H., Zhang, L., Billiar, T. R., Lotze, M. T., Zeh, H. J. 3rd, Kang, R., Kroemer, G., & Tang, D. (2017). The tumor suppressor p53 limits ferroptosis by blocking DPP4 activity. *Cell Reports*, *20*(7), 1692–1704. https://doi.org/10.1016/j.celrep.2017.07.055

Xu, B., Wang, T., Xiao, J., Dong, W., Wen, H. Z., Wang, X., Qin, Y., Cai, N., Zhou, Z., Xu, J., & Wang, H. (2019). FCPR03, a novel phosphodiesterase 4 inhibitor, alleviates cerebral ischemia/reperfusion injury through activation of the AKT/GSK3β/ β-catenin signaling pathway. *Biochemical Pharmacology*, *163*, 234–249. https://doi.org/10.1016/j.bcp.2019.02.023

Yang, W. H., Huang, Z., Wu, J., Ding, C. C., Murphy, S. K., & Chi, J. T. (2020). A TAZ-ANGPTL4-NOX2 axis regulates ferroptotic cell death and chemoresistance in epithelial ovarian cancer. *Molecular Cancer Research: MCR*, *18*(1), 79–90. https://doi.org/10.1158/1541-7786.MCR-19-0691

Yang, W. S., Kim, K. J., Gaschler, M. M., Patel, M., Shchepinov, M. S., & Stockwell, B. R. (2016). Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(34), E4966–E4975. https://doi.org/10.1073/pnas.1603244113

Yang, W. S., SriRamaratnam, R., Welsch, M. E., Shimada, K., Skouta, R., Viswanathan, V. S., Cheah, J. H., Clemons, P. A., Shamji, A. F., Clish, C. B., Brown, L. M., Girotti, A. W., Cornish, V. W., Schreiber, S. L., & Stockwell, B. R. (2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell*, *156*(1-2), 317–331. https://doi.org/10.1016/j.cell.2013.12.010

Yang, W. S., & Stockwell, B. R. (2008). Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. *Chemistry & Biology*, *15*(3), 234–245. https://doi.org/10.1016/j.chembiol.2008.02.010

Yu, H., Guo, P., Xie, X., Wang, Y., & Chen, G. (2017). Ferroptosis, a new form of cell death, and its relationships with tumourous diseases. *Journal of Cellular and Molecular Medicine*, *21*(4), 648–657. https://doi.org/10.1111/jcmm.13008

Yu, H., Yang, C., Jian, L., Guo, S., Chen, R., Li, K., Qu, F., Tao, K., Fu, Y., Luo, F., & Liu, S. (2019). Sulfasalazine–induced ferroptosis in breast cancer cells is reduced by the inhibitory effect of estrogen receptor on the transferrin receptor. *Oncology Reports*, *42*(2), 826–838. https://doi.org/10.3892/or.2019.7189

Yu, S., Yu, M., He, X., Wen, L., Bu, Z., & Feng, J. (2019). KCNQ1OT1 promotes autophagy by regulating miR-200a/FOXO3/ATG7 pathway in cerebral ischemic stroke. *Aging Cell*, *18*(3), e12940. https://doi.org/10.1111/acel.12940

Yuan, H., Li, X., Zhang, X., Kang, R., & Tang, D. (2016). CISD1 inhibits ferroptosis by protection against mitochondrial lipid peroxidation. *Biochemical and Biophysical Research Communications*, *478*(2), 838–844. https://doi.org/10.1016/j.bbrc.2016.08.034

Zaman, K., Ryu, H., Hall, D., O'Donovan, K., Lin, K. I., Miller, M. P., Marquis, J. C., Baraban, J. M., Semenza, G. L., & Ratan, R. R.

(1999). Protection from oxidative stress-induced apoptosis in cortical neuronal cultures by iron chelators is associated with enhanced DNA binding of hypoxia-inducible factor-1 and ATF-1/CREB and increased expression of glycolytic enzymes, p21(waf1/cip1), and erythropoietin. *Journal of Neuroscience*, *19*(22), 9821–9830. https://doi.org/10.1523/JNEUROSCI.19-22-09821.1999

Zeiger, S. L., Musiek, E. S., Zanoni, G., Vidari, G., Morrow, J. D., Milne, G. J., & McLaughlin, B. (2009). Neurotoxic lipid peroxidation species formed by ischemic stroke increase injury. *Free Radical Biology & Medicine*, *47*(10), 1422–1431. https://doi.org/10.1016/j.freeradbiomed.2009.08.011

Zhang, H., Deng, T., Liu, R., Ning, T., Yang, H., Liu, D., Zhang, Q., Lin, D., Ge, S., Bai, M., Wang, X., Zhang, L., Li, H., Yang, Y., Ji, Z., Wang, H., Ying, G., & Ba, Y. (2020). CAF secreted miR-522 suppresses ferroptosis and promotes acquired chemo-resistance in gastric cancer. *Molecular Cancer*, *19*(1), 43. https://doi.org/10.1186/s12943-020-01168-8

Zhang, D., Jin, W., Liu, H., Liang, T., Peng, Y., Zhang, J., & Zhang, Y. (2020). ENT1 Inhibition attenuates apoptosis by activation of cAMP/pCREB/Bcl2 pathway after MCAO in rats. *Experimental Neurology*, *331*, 113362. https://doi.org/10.1016/j.expneurol.2020.113362

Zhang, L., Liu, W., Liu, F., Wang, Q., Song, M., Yu, Q., Tang, K., Teng, T., Wu, D., Wang, X., Han, W., & Li, Y. (2020). IMCA induces ferroptosis mediated by SLC7A11 through the AMPK/mTOR pathway in colorectal cancer. *Oxidative Medicine and Cellular Longevity*, *2020*, 1675613. https://doi.org/10.1155/2020/1675613