ZOOLOGICAL RESEARCH

The pathological role of ferroptosis in ischemia/reperfusion-related injury

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ABSTRACT

Ischemia/reperfusion (I/R) is a pathological process that occurs in numerous organs throughout the human body, and it is frequently associated with severe cellular damage and death. Recently it has emerged that ferroptosis, a new form of regulated cell death that is caused by iron-dependent lipid peroxidation, plays a significantly detrimental role in many I/R models. In this review, we aim to revise the pathological process of I/R and then explore the molecular pathogenesis of ferroptosis. Furthermore, we aim to evaluate the role that ferroptosis plays in I/R, providing evidence to support the targeting of ferroptosis in the I/R pathway may present as a therapeutic intervention to alleviate ischemia/ reperfusion injury (IRI) associated cell damage and death.

Keywords: Ischemia/reperfusion; Ferroptosis; Reactive oxygen species; Lipid peroxidation; Iron

INTRODUCTION

Ischemia/reperfusion (I/R) is a pathological event that occurs in numerous disease states. As the name implies, I/R consists of two significant events, the results of which can cause detrimental cellular damage. Ischemia, the first significant event, refers to the restriction of blood supply to an organ, usually as a result of a blockage within the arterial blood supply by an embolus. Ischemic events are almost always associated with cellular metabolic imbalances and deleterious hypoxia. The second significant event is the reperfusion, or restoration of blood flow and reoxygenation to the affected ischemic area, which can further cause excessive tissue deterioration, initiating destructive inflammatory responses (Eltzschig & Eckle, 2011; Yellon & Hausenloy, 2007).

Ischemia/reperfusion injury (IRI) is a significant contributor to the pathology of numerous disease states, particularly post-cardiac trauma. Moreover, IRI can delay the recovery of organ transplantation and can impede patient recovery undergoing treatments. The role of IRI has been investigated in many organs. However, most research has focused on the heart (Yellon & Hausenloy, 2007), brain (Hanson et al., 2009), and kidney (Friedmann Angeli et al., 2014). The precise molecular mechanism and pathways associated with IRI is not well understood and is heavily debated. As such, the implementation of pharmaceutical strategies has been hampered. Cellular death, however, is a steadfast pathological indicator of IRI. Accordingly, it seems likely that efforts to prevent or arrest the cell death cascade associated with IRI might present as a new and currently unmet therapeutic strategy (Eltzschig & Eckle, 2011; Gudipaty et al., 2018;

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Linkermann et al., 2013; Sun et al., 2018). Indeed, the investigation and the requirement of a thorough understanding of I/R-related cell death have already been proposed, and likely will be a critical step to develop useful treatment paradigms for IRI (Yeo et al., 2013; Yu et al., 2017).

Ferroptosis is a recently recognized novel form of cell death and is a potential therapeutic target with an application for many disease states (Stockwell et al., 2017). In this review, we intend to discuss the ferroptosis cell death pathway, and how it may negatively impact outcomes of I/R-related cell death in different organs and diseases.

THE CURRENT UNDERSTANDING OF THE PATHOMECHANISM OF ISCHEMIA/REPERFUSION-RELATED CELL DEATH

The sudden reduction of available tissue oxygen and nutrients is the critical event initiating cellular injury in ischemic tissue. Under ischemic conditions, mitochondria switch from aerobic to anaerobic metabolism, subsequently leading to a reduction in ATP with associated tissue acidification (Gores et al., 1988; Raat et al., 2009). The inhibition of ATP generation initiates a rise in intracellular sodium and extracellular potassium levels, thus depolarizing the cell (Kimura et al., 1986), and leading to a compensatory transient calcium influx. Concomitantly, calcium-dependent proteolytic enzymes are activated, resulting in apoptosis and necrosis (Halestrap, 2006; Nayler, 1981). Furthermore, during this cellular cascade, other essential ATP-dependent cell functions, such as phosphorylation and enzymatic activity, are also suppressed (Granger et al., 2001), contributing to the cell stress and cell death cascades. Under normal, non-ischemic physiological conditions, several endogenous mechanisms are responsible for reactive oxygen species (ROS) scavenging. However, these mechanisms, and their capacity to effectively scavenge for ROS, is severely negated after I/R (Weisfeldt et al., 1988; Zweier et al., 1987). It has been demonstrated that reperfusion of ischemic tissue can lead to a "burst" of ROS, and this oxidative burst can mediate IRI (Becker & Ambrosio, 1987; Hess & Manson, 1984). The mitochondrial respiratory chain and NADPH oxidases of the NADPH oxidase (NOX) family are believed to be significant sources of ROS (Cadenas, 2018). Antioxidants, endogenous or exogenous, have been shown to protect from IRI in the liver, kidney, heart, and brain (Dare et al., 2015; Jiang et al., 2015; Ni et al., 2019; Zhou et al., 2018). In Figure 1, we describe the current knowledge of IRI.

I/R leads to the activation of cell death pathways, where necrosis, apoptosis, and autophagy-associated cell death are suggested to be the key contributors responsible for the pathology of I/R (Eltzschig & Eckle, 2011; Linkermann et al., 2013; Sun et al., 2018). Furthermore, IRI is punctuated by several cellular events such as the reperfusion associated

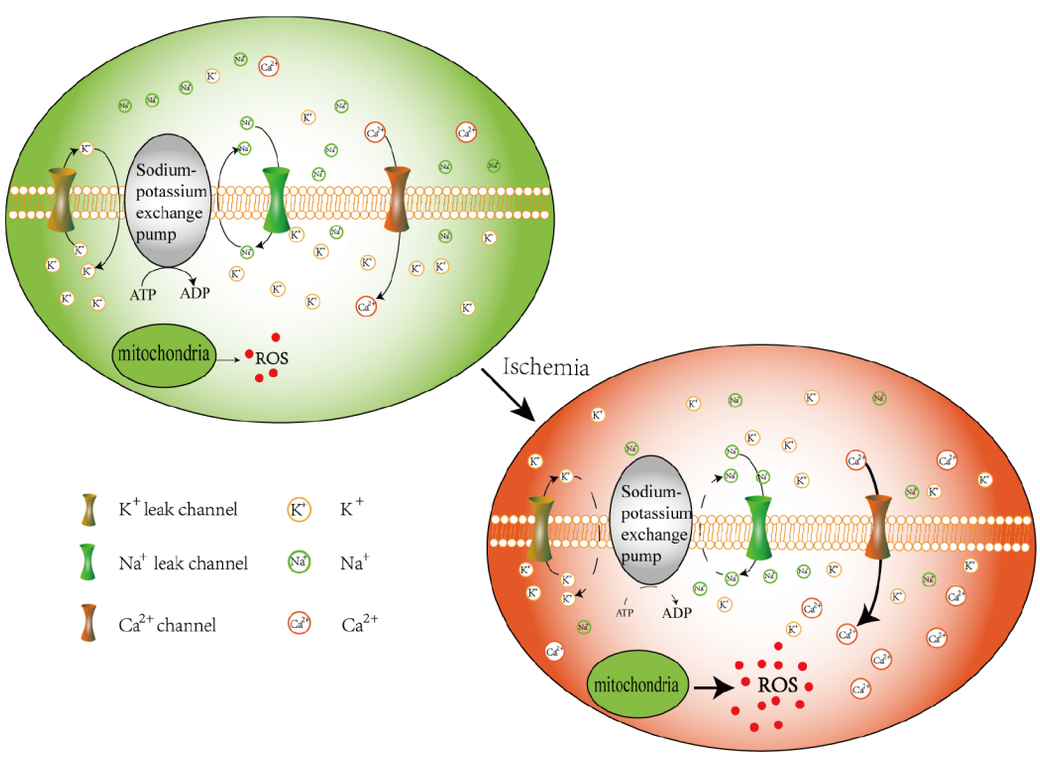

Figure 1 Changes in the cytoplasmic environment during ischemia

After ischemia, the amount of ATP in cells decreased with the lack of tissue energy supply. The resting potential maintained by active transport breaks down with the large outflow of calcium. Compensatory calcium influx activates downstream calcium-dependent signaling pathways. During this process, the mitochondria produce excess ROS.

oxidation burst accompanied by lipid peroxidation (Farmer & Mueller, 2013), and elevated intracellular iron levels (Scindia et al., 2019; Zhao et al., 2018).

These cellular events, which are consistent with the presentation of iron-dependent non-apoptotic ferroptosis, are preventable by iron chelation and antioxidants. Indeed, Iron chelation has been reported to be beneficial in some animal models of IRI (Galaris et al., 2006; Scindia et al., 2015). Furthermore, the observation that iron and ferroptosis mediate cellular damage to organs, and cell death has been frequently documented in recent literature (Friedmann Angeli et al., 2014; Gao et al., 2015; Linkermann et al., 2014; Skouta et al., 2014; Tuo et al., 2017).

REGULATION OF FERROPTOSIS

Ferroptosis is a non-apoptotic form of cell death that is characterized by the accumulation of iron-dependent lipid hydroperoxides to lethal levels (Stockwell et al., 2017). Ferroptosis was clearly defined in 2012, when a small chemical screened from a large library, namely erastin, was shown to inhibit antioxidant glutathione synthesis, and subsequently initiate ferroptosis-related cell death, a cell death pathway that could not be rescued by inhibitors of other known cell death forms (Dixon et al., 2012). Therefore, ferroptosis was determined to be morphologically, biochemically, and genetically distinct from other forms of cell death, and may also be involved in various diseases (Stockwell et al., 2017; Wu et al., 2018). In Figure 2, we summarize the mechanism and key regulators of ferroptosis from the perspectives of oxidation and antioxidation. Hyperactivity of the oxidation mechanism and weakening of the antioxidant mechanism will both result in ferroptosis via the accumulation of toxic lipid peroxidation.

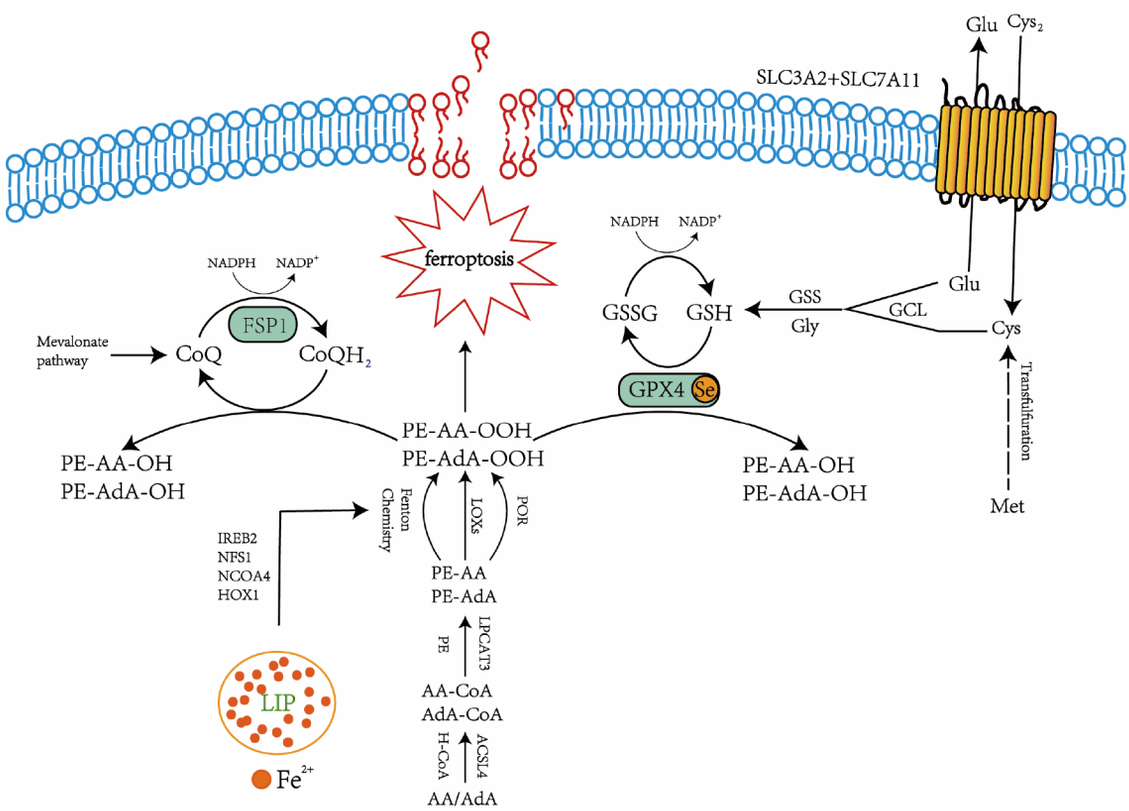

Figure 2 The indicated pathways control the sensitivity of ferroptosis

Lipid ROS accumulation is achieved through following major pathways: (1) iron promotes lipid oxidation by Fenton reaction; (2) the arachidonic acid (AA)-containing phosphatidylethanolamine (PE) (AA-PE)/adrenoyl (AdA)-PE is generated by acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) and oxidized by lipoxygenases (LOXs). (3) POR can also control lipid peroxidation in ferroptosis by distinct mechanisms. Glutathione (GSH)-dependent glutathione peroxidase 4 (GPX4) and ferroptosis suppressor protein 1 (FSP1)-dependent coenzyme Q10 (CoQ10), as two parallel pathways, control generation of lipid ROS. The accumulation of lipid ROS leads to ferroptosis. PE: Phosphatidylethanolamine; LIP: Labile iron pool; NADPH: Nicotinamide adenine dinucleotide phosphate; Gln: Glutamine; Met: Methionine; Glu: Glutamate; Cys: cysteine; Gly: Glycine; GSSG: Oxidized GSH; GCL: Glutamate-cysteine ligase; GSS: Glutathione synthetase; IREB2: Iron-responsive element binding protein 2; NCOA4: Nuclear receptor coactivator 4; NFS1: Cysteine desulfurase; HO-1: Heme oxygenase-1; POR: Cytochrome P450 oxidoreductase.

Antioxidant mechanism

Glutathione peroxidase 4 (GPx4) is a selenium-dependent enzyme, which primarily functions as an endogenous antioxidant (Cardoso et al., 2017). The catalytic center of GPx4 is a tetrad, comprising of hydrogen bonds between a redox-active cysteine or selenocysteine (Sec), coupled with the nitrogen atoms from the surrounding asparagine (Asn), glutamine (Gln) and tryptophan (Trp) residues. This structure is the key to the extraordinary catalytic efficiency of GPx4, whereby the exposure of surface positive charges account for rapid and selective oxidation of mainly cysteine residues (Tosatto et al., 2008). GPx4 has a unique ability as a cytosolic "antioxidant enzyme", whereby it can modulate substrates including H_2O_2, small hydroperoxides, hydroperoxides in complex lipids such as phospholipid, cholesterol, and cholesterol ester hydroperoxides, even when inserted into biomembranes or lipoproteins (Brigelius-Flohé & Maiorino, 2013; Thomas et al., 1990). The chemical properties of GPx4 enable it to act as the central regulator of anti-lipid peroxidation and ferroptosis resistance. Ferroptosis agonists act directly (RSL3) or indirectly (Erastin or Fin56) on GPx4 by attenuating its activity (Ke et al., 2016). Glutathione (GSH) and selenium are necessary for the maintenance of GPx4 function and activity (Ingold et al., 2018; Stockwell et al., 2017).

Erastin and RSL3 were found to trigger RAS mutation-dependent cytotoxicity in 2003 (Dolma et al., 2003), and several proteins involved in glutathione metabolism have been implicated in ferroptosis (Yagoda et al., 2007). It is now understood that glutamine-induced neuronal death shares the characteristics of ferroptosis (Choi, 1988; Murphy et al., 1989), which indirectly highlights the role of glutathione synthesis in the process. GSH is also an important endogenous antioxidant (Meister & Anderson, 1983) and is involved in the regeneration of GPx4 (Maiorino et al., 2018). GSH is synthesized from glutamate, cysteine, and glycine in two-steps, under the catalysis of cytosolic enzymes, glutamate-cysteine ligase (GCL), and glutathione synthetase (GSS) (Liang et al., 2019). Numerous studies indicate that cysteine is essential for cell survival. Human fibroblasts cultured in the cystine-free medium cannot survive without it, and the cystine deprived cell death is caused by glutathione depletion, which in turn can be arrested by the lipophilic antioxidant a-tocopherol (Bannai et al., 1977). Cystine is a disulfide formed between two cysteine molecules and is the predominant form of cysteine in the extracellular space (Conrad & Sato, 2012). The exchange of cystine and glutamate across the plasma membrane is facilitated by a cystine/glutamate antiporter system (Xct), which is a disulfide-linked heterodimer composed of two subunits. The first subunit is solute carrier family 3 member 2 (SLC3A2), and the second is the catalytic subunit solute carrier family 7 member 11 (SLC7A11) (Liang et al., 2019). Once cystine is imported into the cell, it is quickly reduced to cysteine. The exchange of cystine/cystathionine with glutamate is the most important event of ferroptosis and is the main target of erastin (Dixon et al., 2012). Interestingly, cysteine may be synthesized from methionine by the transsulfuration pathway in certain types of cells (McBean, 2012), and this pathway may be resistant to erastin.

When intracellular selenium is deficient, cysteine replaces selenocysteine in the GPx4 active center (Xu et al., 2010). This recombinant Cys mutant GPx4 expressed in *Escherichia coli* exhibits a significant reduction in catalytic efficiency and a 1 000-fold lower activity (Yu et al., 2014) when compared with the natural selenoenzyme. However, sodium selenite (Na_2SeO_3) supplementation can restore GPx4 activity in methamphetamine-treated SH-SY5Y cells (Barayuga et al., 2013). Selenium is required by GPx4 to prevent ferroptosis from utilizing Sec deprotonation to suppress irreversible hyper-oxidation (Brigelius-Flohé & Maiorino, 2013; Ingold et al., 2018). In the central nervous system, Sec is preferred over Cys with its thiol groups, since Sec can deprotonate rapidly under acidic conditions seen in the brain (Cardoso et al., 2017; Song et al., 2014).

It was previously believed that ferroptosis was regulated exclusively by GPx4 (Friedmann Angeli et al., 2014; Yang et al., 2014). However, inhibition of GPx4 fails to trigger ferroptosis regardless of acyl-CoA synthetase long-chain family member 4 (ACSL4) expression, a pro-ferroptosis gene (Doll et al., 2017). This suggests that alternative resistance mechanisms may exist. Recent evidence indicates that the ferroptosis suppressor protein 1 (FSP1)-coenzyme Q10 (CoQ10)-(nicotinamide adenine dinucleotide phosphate)NAD(P)H pathway co-operates with GPx4 and glutathione to suppress phospholipid peroxidation and ferroptosis, as a stand-alone parallel system (Bersuker et al., 2019; Doll et al., 2019). FSP1, previously called apoptosis-inducing factor mitochondrial 2 (AIFM2), was predicted to induce apoptosis by a caspase-1 independent pathway, due to its biochemical similarities to a previously known AIFM1 (Wu et al., 2002). Instead of inducing apoptosis, however, FSP1 is recruited to the plasma membrane by myristoylation, where it functions as an oxidoreductase that catalyzes the regeneration coenzyme Q10 (CoQ10) using NAD(P)H (Bersuker et al., 2019; Doll et al., 2019). Ubiquinol, the reduced form of CoQ10, is a lipophilic radical-trapping antioxidant (RTA) (Frei et al., 1990), that regulates ferroptosis by halting the propagation of lipid peroxides. Ubiquinol is generated by the mevalonate pathway, which is an essential anabolic pathway using acetyl-CoA (Mullen et al., 2016). Small molecules have been shown to initiate ferroptosis by blocking this pathway. For example, FIN56 binds to, and activates, the enzyme squalene synthase, resulting in the depletion of endogenous CoQ10 (Shimada et al., 2016).

Oxidation mechanisms

Lipid metabolism plays an indispensable role in physiological and pathological functions in the human body. For example, fatty acids are essential building blocks of cellular membranes and are the mediator of signaling and energy metabolism in cells (Olzmann & Carvalho, 2019). Long-chain fatty acids containing two or more double bonds are called polyunsaturated fatty acids (PUFAs), and they are involved in

plasma membrane generation and maintenance (Gill & Valivety, 1997a, 1997b). However, the cis double bonds of the methylene groups of PUFAs, are easily oxidized, and the more methylene groups present, the more susceptible the fatty acid is to autoxidation (Conrad & Pratt, 2019; Rouzer & Marnett, 2003). Ultimately, the accumulation of lipid hydroperoxides is a crucial factor that triggers ferroptosis (Dixon et al., 2012; Stockwell et al., 2017). Oxidized arachidonic acid (AA)-containing phosphatidylethanolamine (PE) (AA-PE) is a ferroptosis cell death signal. AA is a type of PUFA that can be elongated into adrenoyl (AdA) by elongase (Kagan et al., 2017). A recent study indicated that AA-OOH-PE, rather than other types of phospholipids (PL)-OOH, induced ferroptosis (Kagan et al., 2017). In this process, the acyl-CoA synthetase long-chain family 4 (ACSL4) catalyzed the formation of AA-CoA (Doll et al., 2017), which esterified into AA-PE by lysophosphatidylcholine acyltransferase 3 (LPCAT3) (Dixon et al., 2015). Indeed, AA-PE can be oxidized to AA-OOH-PE by lipoxygenases (LOXs) and reactive oxygen radicals (Forcina & Dixon, 2019; Yang et al., 2016). Additionally, cytochrome P450 oxidoreductase (POR) facilitates lipid peroxidation via the donation of electrons to downstream effectors in cells undergoing ferroptosis inducing stress (Zou et al., 2020). When the level of AA-OOH-PE exceeds the capacity of the reduction system (GPX4 et al.,), ferroptosis occurs (Doll et al., 2017; Lagarde et al., 2015; Liang et al., 2019).

Iron is a redox-active metal that can be involved in lipid peroxidation (Hassannia et al., 2019), of which there are two contributory pathways. Firstly, iron can be released from the labile iron pool (LIP), subsequently promoting ROS accumulation by the Fenton reaction (Kakhlon & Cabantchik, 2002; Kruszewski, 2003). Secondly, irons role as an essential reactive element in many enzymes such as lipoxygenases (LOXs), and NADPH oxidases, is directly involved in lipid peroxidation (Grivennikova & Vinogradov, 2006; Halliwell & Cross, 1994). These results strongly indicate that proteins associated with iron homeostasis can also regulate ferroptosis. Moreover, the silencing of the iron response element binding protein 2 (IREB2) by sh-RNA has been shown to reduce sensitivity to ferroptosis (Dixon et al., 2012). Cysteine desulfurase (NFS1), the iron-sulfur cluster biosynthetic enzyme in eukaryotes, has also been found to inhibit ferroptosis in lung cancer (Alvarez et al., 2017). And prominin2, a ferroptosis stress response protein, increases the resistance to ferroptosis via stimulating iron export (Brown et al., 2019). Autophagy of ferritin in lysosomes can also increase the content of the reduced form of iron (Terman & Kurz, 2013), thus facilitating ferroptosis. Meanwhile, ferritinophagy cargo receptor nuclear receptor coactivator 4 (NCOA4), can mediate the autophagic degradation of ferritin, and its subsequent inhibition is protective to cells from ferroptosis damage (Gao et al., 2016; Hou et al., 2016). Heme oxygenase-1 (HO-1) can catalyze heme degradation to release ferrous iron, and early research indicates that oxidative stress can induce HO-1 expression via the activation of the p62-Keap1-NRF2 pathway, to antagonize ferroptosis (Sun et al., 2016). However, overexpression of HO-1 may accelerate ferroptosis in cancer cells (Chang et al., 2018; Hassannia et al., 2018).

FERROPTOSIS AS THE MAJOR FORM OF CELL DEATH OCCURRED DURING ISCHEMIA-REPERFUSION

In a variety of human conditions, I/R events cause extensive tissue damage, heightened inflammatory responses, and is a major cause of morbidity and mortality. furthermore, there is evidence suggesting that the reduction of IRI may increase organ transplantation success rates (Raat et al., 2009). It is, therefore, important to determine and understand the type of cell death in I/R events, to identify appropriate therapeutic target opportunities. As noted above, recent investigations have provided strong evidence demonstrating how ferroptosis can participate in IRI, and how targeting ferroptosis might be beneficial for I/R conditions.

Brain I/R events

According to the data released by the World Health Organization (WHO)(WHO, 2016), a stroke occurs on average every 5 seconds affecting 15 million people annually, and it is the leading cause of brain injury and subsequent permanent disability. The majority of strokes are caused by the occlusion of a cerebral artery (ischemic stroke) (Iadecola & Anrather, 2011). To date, no significant effective therapeutic interventions have been developed to counter the deleterious effects of cerebral I/R (Krishnamurthi et al., 2013; Yeo et al., 2013).

The physiological functions of the brain post-stroke result in increased vulnerability to oxidative stress. The brain requires a constant production of high levels of ATP to maintain metabolic activity and neuronal homeostasis (Bélanger et al., 2011), and ATP production is impeded in I/R. Additionally, the brain also accumulates more deleterious byproducts of mitochondrial metabolism under ischemic conditions when compared to other organs (Cardoso et al., 2017). Further, neuronal membranes are rich in PUFAs, which are easily oxidized (Conrad & Pratt, 2019). Accordingly, antioxidant production in the brain is tightly regulated and balanced. Under I/R conditions, iron accumulation in affected brain areas has been observed in both patients, and experimental animal models of cerebral ischemia (Ding et al., 2011; Park et al., 2011) (Dietrich & Bradley, 1988; Fang et al., 2013), and has been proposed as the key mediator of neuronal damage and death (Castellanos et al., 2002; Kondo et al., 1995, 1997). Consistently, iron chelation therapy has been shown to attenuate the cellular damage observed in the brains of experimental IRI rodent animal models (Hanson et al., 2009; Patt et al., 1990).

Tuo et al. (2017) have recently shown that ischemic stroke can cause pro-ferroptotic iron accumulation via the acute suppression of tau, an Alzheimer's disease protein that can facilitate iron export (Lei et al., 2012, 2017), leading to worsening of IRI-related cellular damage and death (Bi et al.,

2017). However, IRI-related neuronal damage can be rescued by ferroptosis inhibitors such as liprostatin-1 and ferrostatin-1, strongly suggesting a direct involvement of ferroptosis in brain IRI (Tuo et al., 2017). Furthermore, pharmacological supplementation of selenium has been shown to promote the expression of GPx4, which blocks ferroptosis and protects neurons from IRI (Alim et al., 2019). Additionally, the ion channel activating monoterpenoid phenol carvacrol can protect hippocampal neurons against I/R in gerbils by inhibiting GPX4-dependent ferroptosis (Guan et al., 2019). PEBP1 is a scaffold protein inhibitor of protein kinase cascades, and it combines with 15-LOX to promote ferroptosis by the generation of lipid death signals (Wenzel et al., 2017). When the PEBP1/15-LOX is upregulated, GPX4 levels and its enzymatic activity is decreased in the cortex following experimentally induced brain injury, further supporting the notion that ferroptosis is involved in IRI pathogenesis of the brain post-injury (Wenzel et al., 2017). These findings strongly support the hypothesis that ferroptosis is intricately involved in and likely modulates the extent of brain pathology after IRI.

Heart I/R events

Cardiovascular disease is considered the leading cause of morbidity and mortality worldwide among all diseases (Stamenkovic et al., 2019). Myocardial reperfusion injury, also known as lethal reperfusion injury, results in the death of cardiac myocytes, which are viable immediately before reperfusion. The destruction of viable cardiac myocytes upon reperfusion ensures that the rate of death or cardiac failure is still high, even with optimally controlled myocardial reperfusion (Yellon & Hausenloy, 2007). Clinical studies indicate that residual myocardial iron is a risk factor for inadequate left ventricular remodeling after reperfusion (Bulluck et al., 2016). Notably, evidence indicates that the mitochondria-specific overexpression of GPx4 in mitochondria alleviates cardiac dysfunction following I/R (Dabkowski et al., 2008). Inhibition of glutaminolysis, a component of the GSH generation pathway, can also attenuate I/R-associated heart injury by blocking ferroptosis (Gao et al., 2015). The cardiac mechanistic target of rapamycin (mTOR) protects the heart against IRI (Aoyagi et al., 2012) and was found to exert protective effects against excess iron and ferroptosis (Baba et al., 2018). Ferrostatin-1 and iron chelation can also ameliorate heart failure induced by both acute and chronic I/R (Fang et al., 2019), consistent with the notion that targeting ferroptosis can serve as a potential strategy to prevent cardiomyopathy.

Kidney I/R events

Renal IRI, a common cause of acute renal failure, has been widely observed in a variety of clinical events, including renal transplantation, embolic or thrombotic events, and surgical interventions, thus playing a significant role on the morbidity and mortality of patients (Pefanis et al., 2019; Zhang et al., 2014). Similar to other I/R events summarized above, reperfusion in the kidney generates an increase in reactive oxygen species that can induce cell death (Castaneda et al., 2003; Perico et al., 2004). For some time now, iron has been suggested to play an important role in renal IRI, and iron chelators have been shown to inhibit renal tubular cell death (Linkermann 2016; Sogabe et al., 1996).

Due to its specific ability to reduce lipid peroxides, the role of GPx4 in renal IRI has recently been investigated. Knockout of GPx4 induces kidney failure in mice, which presents with molecular features of ferroptosis and can be inhibited by the application of ferroptosis inhibitors (Friedmann Angeli et al., 2014). Moreover, subsequent studies have also shown that ferroptosis inhibitors can attenuate renal IRI (Friedmann Angeli et al., 2014; Linkermann et al., 2014). The ubiquitous multi-functional protein, augmenter of liver regeneration (ALR), can alleviate IRI, and the silencing of ALR aggravates ferroptosis and is intricately linked to the glutathione–glutathione peroxidase system (Huang et al., 2019). However, the targeting of ferroptosis alone may not be an optimal therapeutic strategy, as two recent studies of renal IRI demonstrate the coexistence of both necrosis and ferroptosis pathways (Pefanis et al., 2019), indicating that necrosis and ferroptosis may play a synergistic role in renal IRI (Müller et al., 2017). By using CRISPR/Cas9 technology, Müller et al. (2017) found that the pseudokinase mixed lineage kinase domain-like protein (MLKL), a molecular switch to induce necroptotic cell death, drives basal resistance to ferroptosis via the depletion PUFAs, while ACSL4 drives basal resistance to necroptosis rendering the cell membrane less amenable to MLKL-driven membrane permeabilization in renal IRI. These observations suggest that combined therapy should be considered for the treatment of renal IRI. Interestingly, the activin receptor-like kinase (ALK4/5), also known as activin-transforming growth factor (TGF) β receptor, is involved in the stress-induced renal injury, and can suppress cadmium and erastin-induced renal tubular cell death (Fujiki et al., 2019), indicating that the renal IRI model may be useful for studying the relationship of ferroptosis and other forms of cell death.

I/R events of other organs

The role of ferroptosis has been investigated in other organs that can undergo IR events. For example, testicular IRI induces cell death of germ cells and Sertoli cells, and the death of Sertoli cells is explicitly associated with ferroptosis, as indicated by the fact that ferroptosis inhibitors only, and not inhibitors of apoptosis, necrosis or autophagy, protect Sertoli cell from oxygen-glucose deprivation/reoxygenation (Li et al., 2018). Also, ACSL4 was shown to play a critical role in intestinal IRI, which can be protected by ACSL4 inhibition and ferroptosis inhibitors (Li et al., 2019).

CONCLUDING REMARKS

Elevated iron and oxidative stress exist in many organs or tissues after I/R, and iron chelating agents have been tested for and indeed demonstrated efficacy in the improvement of outcomes in a variety of symptoms associated with I/R. (Davis et al., 1997; Patt et al., 1990; Prass et al., 2002). However, the

use of iron chelators in the clinical setting is hampered by the potential negative impact on blood physiology, and by an inability to target the specific organs requiring therapeutic intervention effectively.

Over the last ten years, clinical trials for iron chelators in IRI have shown limited success (Chan et al., 2012; Drossos et al., 1995; Lesnefsky et al., 1990). The limitations are likely due to a variety of reasons, including chelating efficiency in iron affected areas, the specific timing of use, heterogeneity among patients, and other adverse off-target side effects, suggesting that by itself iron may not be an optimal target.

A growing number of recent studies link ferroptosis with IRI, and this should not be surprising, given the fact IRI is essentially related to oxidative damage, which is one of the main causes of ferroptosis. These observations should also provide a window for intervention, whereby instead of targeting the initial cause of the disease, it may be possible to target the pathways of cell death initiated by IRI directly. Multiple studies in numerous organs demonstrate that inhibition of ferroptosis, either by chemical inhibitors or genetic ablation of key genes involved in ferroptosis, can protect cells during IRI, strongly suggesting that ferroptosis can serve as a target for drug development. It is therefore imperative that research efforts are undertaken to screen new drug-like compounds for preclinical and clinical tests of ferroptosis inhibitors to treat IRI in the future.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

H.Y. and P.L. conceived the review and prepared the draft. All authors contributed to the discussions. All authors read and approved the final version of the manuscript.

REFERENCES

Alim I, Caulfield JT, Chen YX, Swarup V, Geschwind DH, Ivanova E, Seravalli J, Ai YX, Sansing LH, Marie EJS, Hondal RJ, Mukherjee S, Cave JW, Sagdullaev BT, Karuppagounder SS, Ratan RR. 2019. Selenium drives a transcriptional adaptive program to block ferroptosis and treat stroke. *Cell*, **177**(5): 1262−1279.

Alvarez SW, Sviderskiy VO, Terzi EM, Papagiannakopoulos T, Moreira AL, Adams S, Sabatini DM, Birsoy K, Possemato R. 2017. Nfs1 undergoes positive selection in lung tumours and protects cells from ferroptosis. *Nature*, **551**(7682): 639−643.

Aoyagi T, Kusakari Y, Xiao CY, Inouye BT, Takahashi M, Scherrer-Crosbie M, Rosenzweig A, Hara K, Matsui T. 2012. Cardiac mTOR protects the heart against ischemia-reperfusion injury. *American Journal of Physiology-Heart and Circulatory Physiology*, **303**(1): H75−H85.

Baba Y, Higa JK, Shimada BK, Horiuchi KM, Suhara T, Kobayashi M, Woo JD, Aoyagi H, Marh KS, Kitaoka H, Matsui T. 2018. Protective effects of the mechanistic target of rapamycin against excess iron and ferroptosis in cardiomyocytes. *American Journal of Physiology-Heart and Circulatory Physiology*, **314**(3): H659−H668.

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.

Barayuga SM, Pang XS, Andres MA, Panee J, Bellinger FP. 2013. Methamphetamine decreases levels of glutathione peroxidases 1 and 4 in SH-SY5Y neuronal cells: protective effects of selenium. *Neurotoxicology*, **37**: 240−246.

Becker LC, Ambrosio G. 1987. Myocardial consequences of reperfusion. *Progress in Cardiovascular Diseases*, **30**(1): 23−44.

Bélanger M, Allaman I, Magistretti PJ. 2011. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metabolism*, **14**(6): 724−738.

Bersuker K, Hendricks JM, Li ZP, Magtanong L, Ford B, Tang PH, Roberts MA, Tong BQ, Maimone TJ, Zoncu R, Bassik MC, Nomura DK, Dixon SJ, Olzmann JA. 2019. The coq oxidoreductase fsp1 acts parallel to GPX4 to inhibit ferroptosis. *Nature*, **575**(7784): 688−692.

Bi M, Gladbach A, van Eersel J, Ittner A, Przybyla M, van Hummel A, Chua SW, van der Hoven J, Lee WS, Müller J, Parmar J, von Jonquieres G, Stefen H, Guccione E, Fath T, Housley GD, Klugmann M, Ke YD, Ittner LM. 2017. Tau exacerbates excitotoxic brain damage in an animal model of stroke. *Nature Communications*, **8**(1): 473.

Brigelius-Flohé R, Maiorino M. 2013. Glutathione peroxidases. *Biochimica et Biophysica Acta (BBA) - General Subjects*, **1830**(5): 3289−3303.

Brown CW, Amante JJ, Chhoy P, Elaimy AL, Liu HB, Zhu LJ, Baer CE, Dixon SJ, Mercurio AM. 2019. Prominin2 drives ferroptosis resistance by stimulating iron export. *Developmental Cell*, **51**(5): 575−586.

Bulluck H, Rosmini S, Abdel-Gadir A, White SK, Bhuva AN, Treibel TA, Fontana M, Ramlall M, Hamarneh A, Sirker A, Herrey AS, Manisty C, Yellon DM, Kellman P, Moon JC, Hausenloy DJ. 2016. Residual myocardial iron following intramyocardial hemorrhage during the convalescent phase of reperfused st-segment-elevation myocardial infarction and adverse left ventricular remodeling. *Circulation: Cardiovascular Imaging*, **9**(10): e004940.

Cadenas S. 2018. Ros and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection. *Free Radical Biology and Medicine*, **117**: 76−89.

Cardoso BR, Hare DJ, Bush AI, Roberts BR. 2017. Glutathione peroxidase 4: a new player in neurodegeneration?. *Molecular Psychiatry*, **22**(3): 328−335.

Castaneda MP, Swiatecka-Urban A, Mitsnefes MM, Feuerstein D, Kaskel FJ, Tellis V, Devarajan P. 2003. Activation of mitochondrial apoptotic pathways in human renal allografts after ischemia-reperfusion injury. *Transplantation*, **76**(1): 50−54.

Castellanos M, Puig N, Carbonell T, Castillo J, Martinez JM, Rama R, Dávalos A. 2002. Iron intake increases infarct volume after permanent middle cerebral artery occlusion in rats. *Brain Research*, **952**(1): 1−6.

Chan W, Taylor AJ, Ellims AH, Lefkovits L, Wong C, Kingwell BA, Natoli A, Croft KD, Mori T, Kaye DM, Dart AM, Duffy SJ. 2012. Effect of iron chelation on myocardial infarct size and oxidative stress in ST-elevation-myocardial infarction. *Circulation: Cardiovascular Interventions*, **5**(2): 270−278.

Chang LC, Chiang SK, Chen SE, Yu YL, Chou RH, Chang WC. 2018. Heme oxygenase-1 mediates bay 11-7085 induced ferroptosis. *Cancer*

Letters, **416**: 124−137.

Choi DW. 1988. Glutamate neurotoxicity and diseases of the nervous system. *Neuron*, **1**(8): 623−634.

Conrad M, Pratt DA. 2019. The chemical basis of ferroptosis. *Nature Chemical Biology*, **15**(12): 1137−1147.

Conrad M, Sato H. 2012. The oxidative stress-inducible cystine/glutamate antiporter, system : cystine supplier and beyond. *Amino Acids*, **42**(1): 231−246.

Dabkowski ER, Williamson CL, Hollander JM. 2008. Mitochondria-specific transgenic overexpression of phospholipid hydroperoxide glutathione peroxidase (*GPx4*) attenuates ischemia/reperfusion-associated cardiac dysfunction. *Free Radical Biology and Medicine*, **45**(6): 855−865.

Dare AJ, Bolton EA, Pettigrew GJ, Bradley JA, Saeb-Parsy K, Murphy MP. 2015. Protection against renal ischemia-reperfusion injury in vivo by the mitochondria targeted antioxidant mitoq. *Redox Biology*, **5**: 163−168.

Davis S, Helfaer MA, Traystman RJ, Hurn PD. 1997. Parallel antioxidant and antiexcitotoxic therapy improves outcome after incomplete global cerebral ischemia in dogs. *Stroke*, **28**(1): 198−204.

Dietrich RB, Bradley WG Jr. 1988. Iron accumulation in the basal ganglia following severe ischemic-anoxic insults in children. *Radiology*, **168**(1): 203−206.

Ding H, Yan CZ, Shi HL, Zhao YS, Chang SY, Yu P, Wu WS, Zhao CY, Chang YZ, Duan XL. 2011. Hepcidin is involved in iron regulation in the ischemic brain. *PLoS One*, **6**(9): e25324.

Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison III B, Stockwell BR. 2012. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*, **149**(5): 1060−1072.

Dixon SJ, Winter GE, Musavi LS, Lee ED, Snijder B, Rebsamen M, Superti-Furga G, Stockwell BR. 2015. Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. *ACS Chemical Biology*, **10**(7): 1604−1609.

Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, Prokisch H, Trümbach D, Mao GW, Qu F, Bayir H, Füllekrug J, Scheel CH, Wurst W, Schick JA, Kagan VE, Angeli JPF, Conrad M. 2017. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nature Chemical Biology*, **13**(1): 91−98.

Doll S, Freitas FP, Shah R, Aldrovandi M, da Silva MC, Ingold I, Grocin AG, Xavier da Silva TN, Panzilius E, Scheel CH, Mourão A, Buday K, Sato M, Wanninger J, Vignane T, Mohana V, Rehberg M, Flatley A, Schepers A, Kurz A, White D, Sauer M, Sattler M, Tate EW, Schmitz W, Schulze A, O'Donnell V, Proneth B, Popowicz GM, Pratt DA, Angeli JPF, Conrad M. 2019. Fsp1 is a glutathione-independent ferroptosis suppressor. *Nature*, **575**(7784): 693−698.

Dolma S, Lessnick SL, Hahn WC, Stockwell BR. 2003. Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. *Cancer Cell*, **3**(3): 285−296.

Drossos G, Lazou A, Panagopoulos P, Westaby S. 1995. Deferoxamine cardioplegia reduces superoxide radical production in human myocardium. *The Annals of Thoracic Surgery*, **59**(1): 169−172.

Eltzschig HK, Eckle T. 2011. Ischemia and reperfusion-from mechanism to translation. *Nature Medicine*, **17**(11): 1391−1401.

Fang KM, Cheng FC, Huang YL, Chung SY, Jian ZY, Lin MC. 2013. Trace element, antioxidant activity, and lipid peroxidation levels in brain cortex of gerbils after cerebral ischemic injury. *Biological Trace Element Research*, **152**(1): 66−74.

Fang XX, Wang H, Han D, Xie EJ, Yang X, Wei JY, Gu SS, Gao F, Zhu NL, Yin XJ, Cheng Q, Zhang P, Dai W, Chen JH, Yang FQ, Yang HT, Linkermann A, Gu W, Min JX, Wang FD. 2019. Ferroptosis as a target for protection against cardiomyopathy. *Proceedings of the National Academy of Sciences of the United States of America*, **116**(7): 2672−2680.

Farmer EE, Mueller MJ. 2013. ROS-mediated lipid peroxidation and res-activated signaling. *Annual Review of Plant Biology*, **64**: 429−450.

Forcina GC, Dixon SJ. 2019. GPX4 at the crossroads of lipid homeostasis and ferroptosis. *Proteomics*, **19**(18): 1800311.

Frei B, Kim MC, Ames BN. 1990. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proceedings of the National Academy of Sciences of the United States of America*, **87**(12): 4879−4883.

Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, 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, Yefremova O, Heinrichmeyer M, Bornkamm GW, Geissler EK, Thomas SB, Stockwell BR, O'Donnell VB, Kagan VE, Schick JA, Conrad M. 2014. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nature Cell Biology*, **16**(12): 1180−1191.

Fujiki K, Inamura H, Sugaya T, Matsuoka M. 2019. Blockade of *ALK*4/5 signaling suppresses cadmium- and erastin-induced cell death in renal proximal tubular epithelial cells via distinct signaling mechanisms. *Cell Death & Differentiation*, **26**(11): 2371−2385.

Galaris D, Barbouti A, Korantzopoulos P. 2006. Oxidative stress in hepatic ischemia-reperfusion injury: The role of antioxidants and iron chelating compounds. *Current Pharmaceutical Design*, **12**(23): 2875−2890.

Gao MH, Monian P, Quadri N, Ramasamy R, Jiang XJ. 2015. Glutaminolysis and transferrin regulate ferroptosis. *Molecular Cell*, **59**(2): 298−308.

Gao MH, Monian P, Pan QH, Zhang W, Xiang J, Jiang XJ. 2016. Ferroptosis is an autophagic cell death process. *Cell Research*, **26**(9): 1021−1032.

Gill I, Valivety R. 1997a. Polyunsaturated fatty acids, part 1: occurrence, biological activities and applications. *Trends in Biotechnology*, **15**(10): 401−409.

Gill I, Valivety R. 1997b. Polyunsaturated fatty acids, part 2: biotransformations and biotechnological applications. *Trends in Biotechnology*, **15**(11): 470−478.

Gores GJ, Nieminen AL, Fleishman KE, Dawson TL, Herman B, Lemasters JJ. 1988. Extracellular acidosis delays onset of cell death in atp-depleted hepatocytes. *American Journal of Physiology Cell Physiology*, **255**(3): C315−C322.

Granger DN, Stokes KY, Shigematsu T, Cerwinka WH, Tailor A, Krieglstein CF. 2001. Splanchnic ischaemia-reperfusion injury: mechanistic insights provided by mutant mice. *Acta Physiologica Scandinavica*, **173**(1): 83−91.

Grivennikova VG, Vinogradov AD. 2006. Generation of superoxide by the mitochondrial complex I. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, **1757**(5−6): 553−561.

Guan XY, Li XL, Yang XJ, Yan JW, Shi PL, Ba LN, Cao YG, Wang P. 2019. The neuroprotective effects of carvacrol on ischemia/reperfusion-induced hippocampal neuronal impairment by ferroptosis mitigation. *Life Sciences*,

235: 116795.

Gudipaty SA, Conner CM, Rosenblatt J, Montell DJ. 2018. Unconventional ways to live and die: Cell death and survival in development, homeostasis, and disease. *Annual Review of Cell and Developmental Biology*, **34**: 311−332.

Halestrap AP. 2006. Calcium, mitochondria and reperfusion injury: a pore way to die. *Biochemical Society Transactions*, **34**(2): 232−237.

Halliwell B, Cross CE. 1994. Oxygen-derived species: their relation to human disease and environmental stress. *Environmental Health Perspectives*, **102**(S10): 5−12.

Hanson LR, Roeytenberg A, Martinez PM, Coppes VG, Sweet DC, Rao RJ, Marti DL, Hoekman JD, Matthews RB, Frey WH, Panter SS. 2009. Intranasal deferoxamine provides increased brain exposure and significant protection in rat ischemic stroke. *Journal of Pharmacology and Experimental Therapeutics*, **330**(3): 679−686.

Hassannia B, Wiernicki B, Ingold I, Qu F, Van Herck S, Tyurina YY, Bayır H, Abhari BA, Angeli JPF, Choi SM, Meul E, Heyninck K, Declerck K, Chirumamilla CS, Lahtela-Kakkonen M, Van Camp G, Krysko DV, Ekert PG, Fulda S, De Geest BG, Conrad M, Kagan VE, Vanden Berghe W, Vandenabeele P, Vanden Berghe T. 2018. Nano-targeted induction of dual ferroptotic mechanisms eradicates high-risk neuroblastoma. *The Journal of Clinical Investigation*, **128**(8): 3341−3355.

Hassannia B, Vandenabeele P, Vanden Berghe T. 2019. Targeting ferroptosis to iron out cancer. *Cancer Cell*, **35**(6): 830−849.

Hess ML, Manson NH. 1984. Molecular oxygen: friend and foe: the role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. *Journal of Molecular and Cellular Cardiology*, **16**(11): 969−985.

Hou W, Xie YC, Song XX, Sun XF, Lotze MT, Zeh III HJ, Kang R, Tang DL. 2016. Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy*, **12**(8): 1425−1428.

Huang LL, Liao XH, Sun H, Jiang X, Liu Q, Zhang L. 2019. Augmenter of liver regeneration protects the kidney from ischaemia-reperfusion injury in ferroptosis. *Journal of Cellular and Molecular Medicine*, **23**(6): 4153−4164.

Iadecola C, Anrather J. 2011. Stroke research at a crossroad: asking the brain for directions. *Nature Neuroscience*, **14**(11): 1363−1368.

Ingold I, Berndt C, Schmitt S, Doll S, Poschmann G, Buday K, Roveri A, Peng XX, Porto Freitas F, Seibt T, Mehr L, Aichler M, Walch A, Lamp D, Jastroch M, Miyamoto S, Wurst W, Ursini F, Arnér ESJ, Fradejas-Villar N, Schweizer U, Zischka H, Friedmann Angeli JP, Conrad M. 2018. Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. *Cell*, **172**(3): 409−422.

Jiang Y, Li LL, Tan XD, Liu B, Zhang YH, Li CQ. 2015. miR-210 mediates vagus nerve stimulation-induced antioxidant stress and anti-apoptosis reactions following cerebral ischemia/reperfusion injury in rats. *Journal of Neurochemistry*, **134**(1): 173−181.

Kagan VE, Mao G, Qu F, Angeli JPF, Doll S, Croix CS, Dar HH, Liu B, Tyurin VA, Ritov VB, Kapralov AA, Amoscato AA, Jiang JF, Anthonymuthu T, Mohammadyani D, Yang Q, Proneth B, Klein-Seetharaman J, Watkins S, Bahar I, Greenberger J, Mallampalli RK, Stockwell BR, Tyurina YY, Conrad M, Bayır H. 2017. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nature Chemical Biology*, **13**(1): 81−90.

Kakhlon O, Cabantchik ZI. 2002. The labile iron pool: characterization, measurement, and participation in cellular processes. *Free Radical Biology and Medicine*, **33**(8): 1037−1046.

Ke BW, Tian M, Li JJ, Liu B, He G. 2016. Targeting programmed cell death using small-molecule compounds to improve potential cancer therapy. *Medicinal Research Reviews*, **36**(6): 983−1035.

Kimura S, Bassett AL, Gaide MS, Kozlovskis PL, Myerburg RJ. 1986. Regional changes in intracellular potassium and sodium activity after healing of experimental myocardial infarction in cats. *Circulation Research*, **58**(2): 202−208.

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 & Metabolism*, **15**(2): 216−226.

Kondo Y, Asanuma M, Nishibayashi S, Iwata E, Ogawa N. 1997. Late-onset lipid peroxidation and neuronal cell death following transient forebrain ischemia in rat brain. *Brain Research*, **772**(1−2): 37−44.

Krishnamurthi RV, Feigin VL, Forouzanfar MH, Mensah GA, Connor M, Bennett DA, Moran AE, Sacco RL, Anderson LM, Truelsen T, O'Donnell M, Venketasubramanian N, Barker-Collo S, Lawes CMM, Wang WZ, Shinohara Y, Witt E, Ezzati M, Naghavi M, Murray C. 2013. Global and regional burden of first-ever ischaemic and haemorrhagic stroke during 1990-2010: findings from the global burden of disease study 2010. *The Lancet Global Health*, **1**(5): e259−e281.

Kruszewski M. 2003. Labile iron pool: the main determinant of cellular response to oxidative stress. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **531**(1−2): 81−92.

Lagarde M, Calzada C, Jouvène C, Bernoud-Hubac N, Létisse M, Guichardant M, Véricel E. 2015. Functional fluxolipidomics of polyunsaturated fatty acids and oxygenated metabolites in the blood vessel compartment. *Progress in Lipid Research*, **60**: 41−49.

Lei P, Ayton S, Finkelstein DI, Spoerri L, Ciccotosto GD, Wright DK, Wong BXW, Adlard PA, Cherny RA, Lam LQ, Roberts BR, Volitakis I, Egan GF, McLean CA, Cappai R, Duce JA, Bush AI. 2012. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. *Nature Medicine*, **18**(2): 291−295.

Lei P, Ayton S, Appukuttan AT, Moon S, Duce JA, Volitakis I, Cherny R, Wood SJ, Greenough M, Berger G, Pantelis C, McGorry P, Yung A, Finkelstein DI, Bush AI. 2017. Lithium suppression of tau induces brain iron accumulation and neurodegeneration. *Molecular Psychiatry*, **22**(3): 396−406.

Lesnefsky EJ, Hedlund BE, Hallaway PE, Horwitz LD. 1990. High-dose iron-chelator therapy during reperfusion with deferoxamine-hydroxyethyl starch conjugate fails to reduce canine infarct size. *Journal of Cardiovascular Pharmacology*, **16**(4): 523−528.

Li L, Hao Y, Zhao Y, Wang HJ, Zhao XJ, Jiang Y, Guo FL. 2018. Ferroptosis is associated with oxygen-glucose deprivation/reoxygenation-induced sertoli cell death. *International Journal of Molecular Medicine*, **41**(15): 3051−3062.

Li Y, Feng DC, Wang ZY, Zhao Y, Sun RM, Tian DH, Liu DS, Zhang F, Ning SL, Yao JH, Tian XF. 2019. Ischemia-induced ACSL4 activation contributes to ferroptosis-mediated tissue injury in intestinal ischemia/reperfusion. *Cell Death & Differentiation*, **26**(11): 2284−2299.

Liang C, Zhang XL, Yang MS, Dong XC. 2019. Recent progress in ferroptosis inducers for cancer therapy. *Advanced Materials*, **31**(51): 1904197.

Linkermann A, Bräsen JH, Darding M, Jin MK, Sanz AB, Heller JO, De Zen F, Weinlich R, Ortiz A, Walczak H, Weinberg JM, Green DR, Kunzendorf U, Krautwald S. 2013. Two independent pathways of regulated necrosis mediate ischemia-reperfusion injury. *Proceedings of the National Academy of Sciences of the United States of America*, **110**(29): 12024−12029.

Linkermann A, Skouta R, Himmerkus N, Mulay SR, Dewitz C, De Zen F, Prokai A, Zuchtriegel G, Krombach F, Welz PS, Weinlich R, Vanden Berghe T, Vandenabeele P, Pasparakis M, Bleich M, Weinberg JM, Reichel CA, Bräsen JH, Kunzendorf U, Anders HJ, Stockwell BR, Green DR, Krautwald S. 2014. Synchronized renal tubular cell death involves ferroptosis. *Proceedings of the National Academy of Sciences of the United States of America*, **111**(47): 16836−16841.

Linkermann A. 2016. Nonapoptotic cell death in acute kidney injury and transplantation. *Kidney International*, **89**(1): 46−57.

Maiorino M, Conrad M, Ursini F. 2018. GPx4, lipid peroxidation, and cell death: Discoveries, rediscoveries, and open issues. *Antioxidants & Redox Signaling*, **29**(1): 61−74.

McBean GJ. 2012. The transsulfuration pathway: a source of cysteine for glutathione in astrocytes. *Amino Acids*, **42**(1): 199−205.

Meister A, Anderson ME. 1983. Glutathione. *Annual Review of Biochemistry*, **52**: 711−760.

Mullen PJ, Yu R, Longo J, Archer MC, Penn LZ. 2016. The interplay between cell signalling and the mevalonate pathway in cancer. *Nature Reviews Cancer*, **16**(11): 718−731.

Müller T, Dewitz C, Schmitz J, Schröder AS, Bräsen JH, Stockwell BR, Murphy JM, Kunzendorf U, Krautwald S. 2017. Necroptosis and ferroptosis are alternative cell death pathways that operate in acute kidney failure. *Cellular and Molecular Life Sciences*, **74**(19): 3631−3645.

Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT. 1989. Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. *Neuron*, **2**(6): 1547−1558.

Nayler WG. 1981. The role of calcium in the ischemic myocardium. *American Journal of Pathology*, **102**(2): 262−270.

Ni DL, Wei H, Chen WY, Bao QQ, Rosenkrans ZT, Barnhart TE, Ferreira Carolina A, Wang YP, Yao HL, Sun TW, Jiang DW, Li SY, Cao TY, Liu ZF, Engle JW, Hu P, Lan XL, Cai WB. 2019. Ceria nanoparticles meet hepatic ischemia-reperfusion injury: the perfect imperfection. *Advanced Materials*, **31**(40): 1902956.

Olzmann JA, Carvalho P. 2019. Dynamics and functions of lipid droplets. *Nature Reviews Molecular Cell Biology*, **20**(3): 137−155.

Park UJ, Lee YA, Won SM, Lee JH, Kang SH, Springer JE, Lee YB, Gwag BJ. 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.

Patt A, Horesh IR, Berger EM, Harken AH, Repine JE. 1990. Iron depletion or chelation reduces ischemia/reperfusion-induced edema in gerbil brains. *Journal of Pediatric Surgery*, **25**(2): 224−228.

Pefanis A, Ierino FL, Murphy JM, Cowan PJ. 2019. Regulated necrosis in kidney ischemia-reperfusion injury. *Kidney International*, **96**(2): 291−301.

Perico N, Cattaneo D, Sayegh MH, Remuzzi G. 2004. Delayed graft function in kidney transplantation. *The Lancet*, **364**(9447): 1814−1827.

Prass K, Ruscher K, Karsch M, Isaev N, Megow D, Priller J, Scharff A, Dirnagl U, Meisel A. 2002. Desferrioxamine induces delayed tolerance against cerebral ischemia *in vivo* and *in vitro*. *Journal of Cerebral Blood Flow & Metabolism*, **22**(5): 520−525.

Raat NJ, Shiva S, Gladwin MT. 2009. Effects of nitrite on modulating ros generation following ischemia and reperfusion. *Advanced Drug Delivery Reviews*, **61**(4): 339−350.

Rouzer CA, Marnett LJ. 2003. Mechanism of free radical oxygenation of polyunsaturated fatty acids by cyclooxygenases. *Chemical Reviews*, **103**(6): 2239−2304.

Scindia Y, Dey P, Thirunagari A, Huang LP, Rosin DL, Floris M, Okusa MD, Swaminathan S. 2015. Hepcidin mitigates renal ischemia-reperfusion injury by modulating systemic iron homeostasis. *Journal of the American Society of Nephrology*, **26**(11): 2800−2814.

Scindia Y, Leeds J, Swaminathan S. 2019. Iron homeostasis in healthy kidney and its role in acute kidney injury. *Seminars in Nephrology*, **39**(1): 76−84.

Shimada K, Skouta R, Kaplan A, Yang WS, Hayano M, Dixon SJ, Brown LM, Valenzuela CA, Wolpaw AJ, Stockwell BR. 2016. Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. *Nature Chemical Biology*, **12**(7): 497−503.

Skouta R, Dixon SJ, Wang JL, Dunn DE, Orman M, Shimada K, Rosenberg PA, Lo DC, Weinberg JM, Linkermann A, Stockwell BR. 2014. Ferrostatins inhibit oxidative lipid damage and cell death in diverse disease models. *Journal of the American Chemical Society*, **136**(12): 4551−4556.

Sogabe K, Roeser NF, Venkatachalam MA, Weinberg JM. 1996. Differential cytoprotection by glycine against oxidant damage to proximal tubule cells. *Kidney International*, **50**(3): 845−854.

Song EQ, Su CY, Fu JL, Xia XM, Yang SY, Xiao CX, Lu B, Chen HJ, Sun ZY, Wu SM, Song Y. 2014. Selenium supplementation shows protective effects against patulin-induced brain damage in mice via increases in gsh-related enzyme activity and expression. *Life Sciences*, **109**(1): 37−43.

Stamenkovic A, Pierce GN, Ravandi A. 2019. Phospholipid oxidation products in ferroptotic myocardial cell death. *American Journal of Physiology-Heart and Circulatory Physiology*, **317**(1): H156−H163.

Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascón S, Hatzios SK, Kagan VE, Noel K, Jiang XJ, Linkermann A, Murphy ME, Overholtzer M, Oyagi A, Pagnussat GC, Park J, Ran QT, Rosenfeld CS, Salnikow K, Tang DL, Torti FM, Torti SV, Toyokuni S, Woerpel KA, Zhang DD. 2017. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell*, **171**(2): 273−285.

Sun XF, Ou ZH, Chen RC, Niu XH, Chen D, Kang R, Tang DL. 2016. Activation of the p62-keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology*, **63**(1): 173−184.

Sun ZX, Zhao TY, Lv SJ, Gao Y, Masters J, Weng H. 2018. Dexmedetomidine attenuates spinal cord ischemia-reperfusion injury through both anti-inflammation and anti-apoptosis mechanisms in rabbits. *Journal of Translational Medicine*, **16**(11): 209.

Terman A, Kurz T. 2013. Lysosomal iron, iron chelation, and cell death. *Antioxidants & Redox Signaling*, **18**(8): 888−898.

Thomas JP, Geiger PG, Maiorino M, Ursini F, Girotti AW. 1990. Enzymatic reduction of phospholipid and cholesterol hydroperoxides in artificial bilayers and lipoproteins. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, **1045**(3): 252−260.

Tosatto SCE, Bosello V, Fogolari F, Mauri P, Roveri A, Toppo S, Flohé L, Ursini F, Matilde M. 2008. The catalytic site of glutathione peroxidases. *Antioxidants & Redox Signaling*, **10**(9): 1515−1526.

Tuo QZ, Lei P, Jackman KA, Li XL, Xiong H, Li XL, Liuyang ZY, Roisman L, Zhang ST, Ayton S, Wang Q, Crouch PJ, Ganio K, Wang XC, Pei L, Adlard PA, Lu YM, Cappai R, Wang JZ, Liu R, Bush AI. 2017. Tau-mediated iron export prevents ferroptotic damage after ischemic stroke. *Molecular Psychiatry*, **22**(11): 1520−1530.

Weisfeldt ML, Zweier J, Ambrosio G, Becker LC, Flaherty JT. 1988. *In*: Simic MG, Taylor KA, Ward JF, von Sonntag C. Evidence that free radicals result in reperfusion injury in heart muscle. Oxygen Radicals in Biology and Medicine. Boston, MA: Springer, 911−919.

Wenzel SE, Tyurina YY, Zhao J, St Croix CM, Dar HH, Mao G, Tyurin VA, Anthonymuthu TS, Kapralov AA, Amoscato AA, Mikulska-Ruminska K, Shrivastava IH, Kenny EM, Yang Q, Rosenbaum JC, Sparvero LJ, Emlet DR, Wen XY, Minami Y, Qu F, Watkins SC, Holman TR, Van Demark AP, Kellum JA, Bahar I, Bayır H, Kagan VE. 2017. Pebp1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. *Cell*, **171**(3): 628−641.

WHO. 2016. Global burden of stroke. https://www.who.int/cardiovascular_diseases/en/cvd_atlas_15_burden_stroke.pdf.

Wu JR, Tuo QZ, Lei P. 2018. Ferroptosis, a recent defined form of critical cell death in neurological disorders. *Journal of Molecular Neuroscience*, **66**(2): 197−206.

Wu M, Xu LG, Li XY, Zhai ZH, Shu HB. 2002. Amid, an apoptosis-inducing factor-homologous mitochondrion-associated protein, induces caspase-independent apoptosis. *Journal of Biological Chemistry*, **277**(28): 25617−25623.

Xu XM, Turanov AA, Carlson BA, Yoo MH, Everley RA, Nandakumar R, Sorokina I, Gygi SP, Gladyshev VN, Hatfield DL. 2010. Targeted insertion of cysteine by decoding uga codons with mammalian selenocysteine machinery. *Proceedings of the National Academy of Sciences of the United States of America*, **107**(50): 21430−21434.

Yagoda N, von Rechenberg M, Zaganjor E, Bauer AJ, Yang WS, Fridman DJ, Wolpaw AJ, Smukste I, Peltier JM, Boniface JJ, Smith R, Lessnick SL, Sahasrabudhe S, Stockwell BR. 2007. RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. *Nature*, **447**(7146): 864−868.

Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, Brown LM,, Girotti AW, Cornish VW, Schreiber SL, Stockwell BR. 2014. Regulation of ferroptotic cancer cell death by GPX4. *Cell*, **156**(1−2): 317−331.

Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS, Stockwell BR. 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.

Yellon DM, Hausenloy DJ. 2007. Myocardial reperfusion injury. *New England Journal of Medicine*, **357**(11): 1121−1135.

Yeo LL, Paliwal P, Teoh HL, Seet RC, Chan BPL, Liang S, Venketasubramanian N, Rathakrishnan R, Ahmad A, Ng KW, Loh PK, Ong JJ, Wakerley BR, Chong VF, Bathla G, Sharma VK. 2013. Timing of recanalization after intravenous thrombolysis and functional outcomes after acute ischemic stroke. *JAMA Neurology*, **70**(3): 353−358.

Yu LL, Huang B, Po SS, Tan TT, Wang ML, Zhou LP, Meng GN, Yuan SX, Zhou XY, Li XF, Wang Z, Wang SY, Jiang H. 2017. Low-level tragus stimulation for the treatment of ischemia and reperfusion injury in patients with st-segment elevation myocardial infarction: a proof-of-concept study. *JACC: Cardiovascular Interventions*, **10**(15): 1511−1520.

Yu Y, Song J, Guo X, Wang S, Yang X, Chen L, Wei JY. 2014. Characterization and structural analysis of human selenium-dependent glutathione peroxidase 4 mutant expressed in *Escherichia coli*. *Free Radical Biology and Medicine*, **71**: 332−338.

Zhang C, Zheng L, Li L, Wang LY, Li LP, Huang S, Gu CL, Zhang LX, Yang C, Zhu TY, Rong RM. 2014. Rapamycin protects kidney against ischemia reperfusion injury through recruitment of NKT cells. *Journal of Translational Medicine*, **12**: 224.

Zhao YS, Xin Z, Li NN, Chang SY, Chen YD, Geng LN, Chang HR, Shi HL, Chang YZ. 2018. Nano-liposomes of lycopene reduces ischemic brain damage in rodents by regulating iron metabolism. *Free Radical Biology and Medicine*, **124**: 1−11.

Zhou H, Ma Q, Zhu PJ, Ren J, Reiter RJ, Chen YD. 2018. Protective role of melatonin in cardiac ischemia-reperfusion injury: from pathogenesis to targeted therapy. *Journal of Pineal Research*, **64**(3): e12471.

Zou YL, Li HX, Graham ET, Deik AA, Eaton JK, Wang WY, Sandoval-Gomez G, Clish CB, Doench JG, Schreiber SL. 2020. Cytochrome P450 oxidoreductase contributes to phospholipid peroxidation in ferroptosis. *Nature Chemical Biology*, **16**(3): 302−309.

Zweier JL, Flaherty JT, Weisfeldt ML. 1987. Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proceedings of the National Academy of Sciences of the United States of America*, **84**(5): 1404−1407.