Striking while the iron is hot: Iron metabolism and ferroptosis in neurodegeneration

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Abstract

Perturbations in iron homeostasis and iron accumulation are a feature of several neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ALS). Proteins such as α-synuclein, tau and amyloid precursor protein that are pathologically associated with neurodegeneration are involved in molecular crosstalk with iron homeostatic proteins. Quantitative susceptibility mapping, an MRI based non-invasive technique, offers proximal evaluations of iron load in regions of the brain and powerfully predicts cognitive decline. Further, small molecules that target elevated iron have shown promise against PD and AD in preclinical studies and clinical trials. Despite these strong links between altered iron homeostasis and neurodegeneration the molecular biology to describe the association between enhanced iron levels and neuron death, synaptic impairment and cognitive decline is ill defined. In this review we discuss the current understanding of brain iron homeostasis and how it may be perturbed under pathological conditions. Further, we explore the ramifications of a novel cell death pathway called ferroptosis that has provided a fresh impetus to the "metal hypothesis" of neurodegeneration. While lipid peroxidation plays a central role in the execution of this cell death modality the removal of iron through chelation or genetic modifications appears to be sufficient to completely extinguish the ferroptotic pathway. Conversely, tissues that harbour elevated iron may be predisposed to ferroptotic damage. These emerging findings are of relevance to neurodegeneration where ferroptotic signalling may offer new targets to mitigate cell death and dysfunction.

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Introduction

Iron is the most abundant transition metal on Earth and essential for life. Iron availability in primordial oceans allowed for its incorporation in living organisms. Metabolic processes catalysed by iron or by iron-sulfur clusters that could be generated in prebiotic settings may be among the first of such processes to evolve on Earth and essential for the emergence of carbon-based life (Bonfio et al., 2017, Varma et al., 2018). The photolysis of water by the process of photosynthesis around 2.45 billion years ago introduced a new global poison *i.e.* oxygen, causing what is described as the Great Oxygenation Event (Sessions et al., 2009). The resultant oxidising environment transformed iron into a limiting factor for life processes due to the limited solubility of the oxidised iron cation.

The ability of iron to cycle through its oxidation states and form coordination bonds is utilised by many enzymes to carry out their catalytic function. Iron has thus emerged as an indispensable co-factor for proteins involved in essential (respiration, DNA replication, cell division) and specialised (oxygen transport, neurotransmission) cellular functions. Iron can serve as a potent oxidant that can wreak havoc on biomolecules, ironically endangering the life that it helps facilitate. This conundrum necessitated the evolution of homeostatic mechanisms to ensure the availability of this critical element while mitigating potential oxidative damage. In the body iron levels are maintained through the precise uptake of iron from the diet. However, the body has no specific physiological mechanism for iron excretion. Iron thus tends to accumulate in certain tissues with age.

The brain is a major organ where iron accumulates with age, especially in regions of pathological relevance. The study of monogenic genetic disorders that affect iron homeostasis, and indications from dietary studies, have established that brain iron homeostasis is mostly independent of systemic iron homeostasis (Belaidi and Bush, 2016). Furthermore, indicators of systemic iron levels are weakly correlated with iron in the brain. Several neurodegenerative conditions including Alzheimer's disease (AD) and Parkinson's disease (PD) are associated with increased iron levels in affected region of the brain with levels of iron corresponding to disease severity (Belaidi and Bush, 2016). However, the iron-mediated events that may promote neurodegeneration appear to be more intricate than iron-associated oxidative damage. Here we review the development of the “iron-

hypothesis" of neurodegeneration, shifting our focus beyond iron toxicity to consider the recently (re)discovered iron-dependent programmed cell death pathway called ferroptosis.

Iron homeostasis in the brain

Overview

Iron is essential for brain health and development. Iron-dependent enzymes and proteins are required for development of synapses, myelination, and production and turnover of neurotransmitters (Carpenter et al., 2016). Further, the brain is a highly metabolically active organ and energetically reliant on iron-dependent proteins involved in cellular respiration. Iron deficiency during early development is deleterious to normal brain development and negatively impacts brain function including IQ, cognition, motor skills, and social behaviour (Beard and Connor, 2003). Dietary iron deficiency during early development leads to severe iron deficit in the brain as iron stores are primarily utilised to maintain haemoglobin levels in the blood. While impaired brain development during childhood, resulting from iron deprivation, are reversible by providing an iron-replete diet, changes during early infancy tend to persist despite a correction in iron status (Beard and Connor, 2003).

Iron in the paediatric brain is lower than that in the adult brain. Iron accumulates in the adult brain largely as a function of age but is also reportedly influenced by BMI and habits such as smoking (Pirpamer et al., 2016). Iron accumulation in the brain is spatially and temporally heterogeneous. Higher concentrations of iron within the brain are preferentially found in the nucleus accumbens, substantia nigra (SN), deep cerebellar nuclei and parts of the hippocampus (Drayer et al., 1986, Griffiths and Crossman, 1993, Haacke et al., 2005, Singh et al., 2014). However, the rate of brain iron accumulation varies with the stage of brain development. Interestingly, brain iron accumulation is a characteristic feature in several neurodegenerative disorders such as AD, PD, motor neuron disease (MND)/amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and a group of disorders categorised as Neurodegeneration with Brain Iron Accumulation (NBIA). This iron accumulation is

associated with regions implicated in disease pathology and may accompany oxidative stress, inflammation, and cell death.

Iron import in the brain

The blood-brain barrier (BBB) mounts a formidable defence for the brain against foreign molecules and separates the iron homeostasis of the brain from that of the periphery. This compartmentalisation can be clearly observed in conditions of iron excess, such as haemochromatosis, where iron-associated cellular damage occurs in several peripheral organs but not the brain (Crowe and Morgan, 1992, Moos et al., 2000, Russo et al., 2004). Brain capillary endothelial cells (BCECs) maintain the integrity of the BBB. Their function is in turn facilitated, at their abluminal surface, by astrocytes that serve to detoxify/neutralise inbound solutes that may perturb the extracellular balance of the brain (Moos et al., 2007). This protective mechanism allows for a highly regulated uptake of iron in the brain (Taylor et al., 1991, Crowe and Morgan, 1992). Iron import into the brain starts by binding of blood-circulating transferrin (Tf) to transferrin receptor 1 (TfR1) on the surface of BCECs and internalisation of the Tf/TfR1 complex (Taylor et al., 1991). However, the mechanism of iron transport across the BCECs is unresolved and two likely scenarios have emerged. In the classical model iron is released from Tf inside endosomal compartment of BCECs under a low pH (around 5.5) achieved by the activity of proton pumps. This is followed by reduction of iron to its ferrous state in the presence of ferrireductases, such as STEAP3 (Burkhart et al., 2016). Subsequent transport of iron across the endosomal membrane into the cytosol is mediated by the divalent metal transporter 1 (DMT1). Ferrous iron may then be used by neurons, stored into ferritin or effluxed across the cell membrane by the iron exporter ferroportin 1 (FPN). Iron export through FPN is accompanied by oxidation of iron, which can be accomplished by ferroxidases such as ceruloplasmin (Cp) and hephaestin and binding to extracellular transferrin (Burkhart et al., 2016). An alternate model (transcytosis model) arose due to confounding evidence regarding the expression of DMT1 and FPN in BCECs (Burkhart et al., 2016, Moos et al., 2007, Moos et al., 2006). According to this model the iron-loaded Tf is transported through the BCEC cytosol to the abluminal site and is directly released in the brain (Raub and Newton, 1991). This model is supported by the observation that cultured

bovine BCECs cycle Tf-TfR1 complexes and that Tf-Fe transported across these cells may not undergo intraendothelial degradation (Raub and Newton, 1991, Descamps et al., 1996). However no evidence has emerged to demonstrate the transport of Tf from the systemic circulation across BCECs into the brain (Crowe and Morgan, 1992, Moos et al., 2006, Strahan et al., 1992).

Iron homeostasis in glial cells

Astrocytes, at least *in vivo*, appear to be devoid of TfR1 and steps involved in the import of iron in these cells are unclear (Wong et al., 2014a, Belaidi and Bush, 2016). DMT1 may facilitate iron import but its expression in astrocytes is uncertain (Moos and Morgan, 2004, Huang et al., 2004a, Huang et al., 2006). Astrocytes are essential support cells in the neurovascular unit and serve to release the iron supplied by BCECs to the neurons while mitigating iron toxicity (Abbott et al., 2006, Burkhart et al., 2016). To this end astrocytes are specialised for iron export. The export of iron from astrocytes is reliant on Cp (Klomp et al., 1996, Jeong and David, 2006), which they express both as secreted protein and in a membrane bound glycosylphosphatidylinositol (GPI)-anchored form (Patel and David, 1997, Patel et al., 2000).

Oligodendrocytes are macroglial cells responsible for the synthesis of myelin and are the principal cells in the brain that stain for iron (Belaidi and Bush, 2016, Todorich et al., 2011, Benkovic and Connor, 1993). Iron is essential for myelin formation and iron deficiency leads to hypomyelination and associated disorders (Todorich et al., 2009). Oligodendrocytes may acquire iron in a TfR1-independent manner (Moos et al., 2007, Todorich et al., 2011). Ferritin, the high capacity iron storage protein in the cytosol of most mammalian cells, has been suggested as a possible source of iron for oligodendrocytes (Connor et al., 1995a, Qi et al., 1995, Sanyal et al., 1996, Zhang et al., 2006). Indeed, Tim2 (T-cell immunoglobulin mucin domain 2 protein) has been identified as a specific receptor mediating ferritin uptake and internalisation by oligodendrocytes (Todorich et al., 2011, Hulet et al., 2000). The expression of Tim2, but not TfR1, can be demonstrated on oligodendrocytes *in vivo*, suggesting ferritin endocytosis to be the predominant mode of iron uptake in oligodendrocytes (Todorich et al., 2011). However, Tim2 has been identified only in rodents and the evidence of an analogous receptor in humans is still lacking. Iron and ferritin content in

oligodendrocytes increase with age (Benkovic and Connor, 1993). Oligodendrocytes express Tf and FPN possibly for intracellular transport and efflux of iron, respectively (Moos et al., 2007).

Microglia originate as circulating monocytes that migrate to the brain during the developmental stage and then differentiate into quiescent microglial cells (Milligan et al., 1991). The migrating monocytes are laden with iron and consequently possess abundant ferritin, both of which are gradually lost during the transformation to microglial state (Moos, 1995, Cheepsunthorn et al., 1998). In rodents, cultured microglial cells have been shown to release ferritin that increases survival of oligodendrocytes (Zhang et al., 2006). Further, lipopolysaccharide-induced oligodendrocyte genesis in the rat spinal cord occurs following a robust infiltration of ferritin-positive microglia (Schonberg and McTigue, 2009, Schonberg et al., 2007). Microglia may thus serve as a source of iron-laden ferritin for oligodendrocytes (Todorich et al., 2011).

Iron homeostasis in neurons

Iron uptake in neurons occurs via TfR1 which binds and internalises Tf-Fe (**Figure 1**). Following endosomal acidification, dissociation of iron for Tf-Fe and subsequent reduction, DMT1 facilitates the transfer of iron across the endosomal membrane into the cytosol. This available iron can then be used for neuronal function and metabolism. Excess iron may be stored or exported across the plasma membrane. Ferritin can be expressed in neurons to store and detoxify excess iron (Hansen et al., 1999). FPN exports ferrous iron from neurons which is then bound by Tf, following oxidation by circulating or astrocyte-tethered Cp in the interstitium. The amyloid precursor protein (APP) stabilises FPN at the neuronal cell surface facilitating iron efflux. The trafficking of APP to the cell membrane is supported by the microtubule-associated protein tau (**Figure 1**) (Lei et al., 2012).

Iron homeostasis is regulated in all mammalian cells by the activities of cytosolic iron response proteins (IRP1/2) that is modulated by intracellular iron levels. In neurons IRP2 serves as the main sensor of labile (available intracellular) iron (Meyron-Holtz et al., 2004). Under condition of low intracellular iron IRPs bind to the iron-regulatory elements (IREs) located on the stem-loop structures of the untranslated region (UTR) of mRNA of iron-responsive proteins. The mRNAs that harbour

IREs in their 3'-UTR (e.g. TfR1 and DMT1) are stabilised by IRP binding leading to enhanced translation while on the contrary, the translation of mRNA that harbour IREs in their 5'-UTR (e.g. ferritin, FPN, APP, α-synuclein) is repressed (Anderson et al., 2012). In this way, IRP/IRE system upregulates TfR1 and DMT1 in iron limiting conditions while in condition of iron excess, the lack of IRP binding to IREs allows for an increase in iron storage (ferritin) and export (FPN). Elevated iron directly impacts on the translation of APP and α-synuclein that are implicated in several neurodegenerative conditions including Alzheimer's disease (AD) and Parkinson's disease (PD) (Rogers et al., 1999, Rogers et al., 2002).

Role of iron in neurodegeneration

Iron is essential for normal brain development and cognitive function. The deficiency of iron thus adversely impacts on neurological development and function, especially in prenatal or early postnatal stages, where the dysfunction may affect memory and learning ability (Radlowski and Johnson, 2013). Iron progressively accumulates in the brain with age with a greater accumulation observed in the cortex and the nuclei of the basal ganglia *viz.* SN, putamen, globus pallidus and caudate nucleus; iron accumulation in these regions is associated with neurodegenerative disorders (Belaidi and Bush, 2016).

Neurodegenerative disorders that are associated with high brain-iron include AD, PD, HD, MND/ALS, infantile neuroaxonal dystrophy (INAD), Schindler disease, and other neuroaxonal dystrophies termed NBIA disorders (Morgan et al., 2006, Hayflick, 2006, Belaidi and Bush, 2016, Veyrat-Durebex et al., 2014, Gajowiak et al., 2016). Further, iron overload is associated with a subset of psychiatric diseases (Heidari et al., 2016, Cutler, 1994, Feifel and Young, 1997). While the deleterious effects of enhanced iron in the brain have been ascribed to an oxidative damage component, the recently (re)discovered iron-mediated regulated cell death pathway, ferroptosis, is now under scrutiny for its role in neurodegeneration and cognitive impairment.

Iron in AD pathology

AD is the leading cause of dementia and is a progressive neurodegenerative disorder affecting cortical and hippocampal neurons. AD is characterised by the accumulation of extracellular senile plaques composed primarily of aggregated amyloid beta peptide (Aβ), and intracellular neurofibrillary tangles (NFTs) formed by hyper-phosphorylated microtubule-associated protein tau. While the pathology of AD is largely ascribed to a toxic accumulation of Aβ, clinical strategies that have reduced Aβ burden have not been successful in limiting pathological progression (Morris et al., 2014). Several other pathological hallmarks that have been implicated in the progression of AD which include altered metal homeostasis, inflammation, oxidative stress, defects in autophagy and lysosomal function, mitochondrial dysfunction, and impaired glial function (Nixon, 2013, Belaidi and Bush, 2016, Bush and Curtain, 2008). While some of these pathways are amenable to therapeutic targeting these avenues have been largely neglected. (Belaidi and Bush, 2016, Bush and Curtain, 2008). The strongest genetic risk factor for AD, apolipoprotein E (ApoE) 4 allele exerts a gene dosage effect on the age of onset of AD however the underlying mechanism of pathogenesis remains unclear (Huang and Mahley, 2014).

Iron accumulates in the AD brain and is associated with senile plaques and NFTs (Goodman, 1953, Smith et al., 1997, Smith et al., 2007, Lovell et al., 1998, Connor et al., 1992). Using magnetic resonance imaging (MRI) iron accumulation is preferentially observed in the AD affected regions of the brain *viz.* parietal cortex, motor cortex, and hippocampus (Bartzokis et al., 1994, Bartzokis and Tishler, 2000, Ding et al., 2009, Pfefferbaum et al., 2009, Bilgic et al., 2012, Luo et al., 2013, Langkammer et al., 2014, Tao et al., 2014, Ghadery et al., 2015). Systemic changes in iron homeostasis accompany AD such as decreased iron in plasma resulting from Tf desaturation and decreased cellular iron export indicated by decreased expression of aconitase 1, Cp and APP in peripheral blood mononuclear cells of AD patients *vs*. normal subjects (Faux et al., 2014, Guerreiro et al., 2015, Hare et al., 2013, Hare et al., 2015). Several studies implicate iron in the aggregation, oligomerisation, amyloidosis, and toxicity of Aβ peptides (Mantyh et al., 1993, Schubert and Chevion, 1995, Huang et al., 2004b, Liu et al., 2011). Studies suggest that Aβ lacks toxicity by itself and the

oxidative damage associated with its aggregation is due to its affinity for binding redox active metals, *viz.* iron and copper, leading to production of potent oxidants such as hydrogen peroxide (Huang et al., 1999, Jomova et al., 2010). Further, iron binds to tau and can mediate its hyper-phosphorylation and aggregation (Yamamoto et al., 2002, Lovell et al., 2004, Chan and Shea, 2006). These events can be mitigated through iron chelation (Amit et al., 2008). Tau accumulation in NFTs is associated with an induction of heme oxygenase-1 which may further exacerbate oxidative stress through the release of ferrous iron by the catabolism of haem (Wang et al., 2015, Perry et al., 2002, Ward et al., 2014, Schipper et al., 2006).

Iron status may directly impact the translation of APP that harbours an IRE in its 5'-UTR mRNA (Rogers et al., 1999, Rogers et al., 2002). APP is a transmembrane protein that through amyloidogenic processing results in its cleavage product Aβ (Caldwell et al., 2013, Huang et al., 2017). Cellular iron levels may thus impact on the production of Aβ. Neuronal APP is normally processed thorough a non-amyloidogenic pathway involving cleavage by α-secretase followed by cleavage by γ-secretase. Amyloidogenesis occurs when APP is first cleaved by β-secretase and then γ-secretase. Iron modulates the α-secretase mediated cleavage of APP (Bodovitz et al., 1995). Further, the activation of α-secretase and β-secretase is modulated proteolytically by furin; the levels of furin protein are reduced in condition of excess iron, which favours β-secretase activity, thus promoting amyloidogenesis (Silvestri and Camaschella, 2008, Ward et al., 2014). APP can also facilitate the efflux of iron from neurons by stabilizing FPN on the cell membrane and APP depletion leads to iron accumulation in cultured neurons and in mouse models (McCarthy et al., 2014, Wan et al., 2012, Wong et al., 2014b, Duce et al., 2010). Further, tau deficiency leads to iron accumulation, which is associated with impaired APP trafficking to the cell membrane (Lei et al., 2012).

The association of iron with AD pathology is supported by the finding that CSF ferritin levels positively correlate with cognitive decline and can predict the transition from mild cognitive impairment to AD in a longitudinal study (Ayton et al., 2015a). Ferritin levels also correlated with ApoE protein levels suggesting a possible bearing of iron homeostasis on the mechanism by which ApoE isoforms may influence AD pathogenesis (Ayton et al., 2015a). CSF ferritin level is strongly

associated with cognitive decline in carriers of *ApoE4* allele (Ayton et al., 2017a) Further, iron treatment in cultured neurons and astrocytes upregulates the transcription of *ApoE* (Xu et al., 2016). Higher magnetic susceptibility, a proxy for tissue iron, in the hippocampus predicted an accelerated decline in cognition in amyloid positive subjects in over 6 years in another longitudinal study (Ayton et al., 2017b). This indicates involvement of a combinatorial effect of iron and Aβ to affect cognitive decline (Ayton et al., 2017b).

Iron in PD pathology

PD is a neurodegenerative disease that is characterised by loss of motor automatic function, muscle stiffness (rigidity), slowness of movement (bradykinesia) in patients, and in advanced stages leads to dementia and severe axial disorders. Most cases of PD are sporadic (90%) with several risk factors (Farrer, 2006, De Lau and Breteler, 2006). PD is associated with a loss of dopaminergic neurons in the SN pars compacta (pc) and the appearance of aggregated α-synuclein inclusions, called Lewy bodies, in neurons (Fearnley and Lees, 1991, Moore et al., 2005). Overexpression of α-synuclein leads to PD and mutations in α-synuclein are linked to familial PD (Decressac et al., 2012, Polymeropoulos et al., 1997). Iron accumulation in neurons and glia of the SN is an established feature of PD with iron concentrations correlated with severity of disease (Dexter et al., 1987, Dexter et al., 1989c, Hirsch et al., 1991, Pyatigorskaya et al., 2015). Further, iron can promote conformational change of α-synuclein from α-helical to the β-sheet structure that is observed in Lewy bodies (el-Agnaf and Irvine, 2002). However, iron alone does not seem to be sufficient to induce neuronal death. This is supported by the observation that mouse SNpc, which contains 25% less iron than adjoining SN pars reticulata, is selectively vulnerable to neurodegeneration (Hare et al., 2014). This selective degeneration of SNpc despite relatively lower iron accumulation compared to unaffected adjoining tissue could be attributed to the colocalisation of dopamine and iron in the SNpc or on additional factors such as the presence of neuromelanin in the region that may exacerbate iron-mediated oxidative damage (Hare et al., 2014, Enochs et al., 1994, Zecca et al., 1994, Zucca et al., 2014). The vulnerability of SNpc could be also explained by the high-energy demands due to the neurons autonomous pace-making activity. A higher

energy demand renders the SNpc more susceptible to imbalances in labile iron levels and ensuing reactive oxygen species production (Guzman et al., 2010).

In addition to elevated iron in the SN of PD patients there is sufficient evidence of dysregulation of iron homeostasis. Levels of several key iron homeostasis proteins are aberrantly altered in PD. In post-mortem PD brains a sustained activity of IRP1 has been reported in the SN which may be sufficient to limit ferritin levels, and increase neuronal iron uptake by increasing TfR1, and sensitise melanised neurons of the SNpc to iron-associated oxidative damage (Faucheux et al., 2002). Indeed, ferritin levels are reported to be significantly lower in the SN of PD patients compared to those in healthy controls (Dexter et al., 1991, Connor et al., 1995b). Elevated levels of DMT1 along with diminished ferroxidase activity of Cp have been observed in PD patients and animal models of PD the effect of which may lead to the reported increase in iron levels (Salazar et al., 2008, Ayton et al., 2013, Boll et al., 1999, Olivieri et al., 2011). Interestingly, in tau knockout mice, where the loss of function of tau leads to impaired APP-mediated iron export, a concomitant increase in iron is seen in the SN accompanied by marked neuronal loss, cognitive deficit and parkinsonism; these can be prevented through iron chelation (Lei et al., 2010, Lei et al., 2012, Lei et al., 2014, Lei et al., 2015). Further, APP levels are decreased in SN dopaminergic neurons in human PD patients and APP knockout in mice models results in iron-dependent neuronal cell death in the SN (Ayton et al., 2015b). Taken together these data suggest iron dysregulation as a common feature in AD and PD pathology.

Clinical assessment of iron chelation

Regarding the promising effects obtained in neurodegenerative animal models with iron chelators, clinical trials were conducted to examine the effect of iron removal therapy.

Iron chelation and metal targeted strategies in AD

In 1991, McLachlan and collaborators have been first to suggest that iron chelation therapy could be used as a treatment of AD (Crapper McLachlan et al., 1991). Indeed, a two-year single-blind study on 48 patients treated intramuscularly with deferoxamine (DFO) showed that low dose administration of this iron chelator slowed the clinical progression of the dementia associated with AD compared with

controls. There was no further confirmation, but a decade later, a pilot phase 2 clinical trial in patients with moderately severe AD using clioquinol (BPT1), a drug inhibiting zinc and copper ions from binding to Aβ was conducted on 36 patients (Ritchie et al., 2003). A positive clinical effect, corresponding to a slow progression of the cognitive decline, has only been seen for the more severely affected patients. Moreover, a biological effect, corresponding to a decline of the plasma Aβ levels, was observed. PBT2, a clioquinol derivative, has been administrated for 12 weeks to 78 patients in a recent phase IIa double-blind, randomized, placebo-controlled trial. They received either 50 mg PBT2, or 250 mg PBT2 or placebo. No serious adverse effect was reported. A benefit on cognition was observed on two executive functions among the cognitive tests (Lannfelt et al., 2008). The PBT2 effect on the classical AD biomarker, $A\beta_{42}$ concentration, was significant in CSF but not in plasma or serum. A further *post-hoc* analysis indicated that cognitive improvement was significantly higher in the PBT2 250 mg group than in placebo group (Faux et al., 2010). There was no correlation between changes in CSF Aβ or tau species and cognitive change.

A phase 2, randomized, placebo-controlled clinical trial using deferiprone is currently progress (NCT03234686). The primary objective of this trial is to investigate the safety and efficacy of deferiprone, 15 mg/kg (twice a day, orally) in participants with prodromal AD and mild AD.

Iron chelation in PD

The first pilot chelation therapy in PD was a year double-blind, randomized, placebo-controlled clinical trial, in early-stage PD patients on stabilised dopamine regimens treated with deferiprone (30 mg/kg/day). A slower disease progression was observed for the early start group (patients who started the iron chelator 6 month earlier). Indeed, at 12 months these 'early start' patients retained a significantly lower motor handicap [1 point on the motor Unified Parkinson's Diseases Rating Scale (UPDRS): 21.3±8] compared to the delayed start group (22.8±6), signifying a disease modifying effect. Concomitantly, iron content of the SN and markers of oxidative stress was significantly decreased compared to baseline (Devos et al., 2014). Positive clinical outcomes were recently confirmed by another randomized double-blind, placebo-controlled trial in early-onset PD patients. In

this smaller sized trial, deferiprone reduced dentate and caudate nucleus iron content and indicated a trend for improvement in motor-UPDRS scores and quality of life (Martin-Bastida et al., 2017). In both trials deferiprone had a good safety profile; despite the requirement of weekly blood counts during the first 6 months to monitor reversible neutropenia that may occur in 1-3% (agranulocytosis in 0.8%) of patients treated with deferiprone. It has also been demonstrated that patients with a lower Cp activity in the CSF and serum appeared to responded better to iron chelation (Grolez et al., 2015).

A large phase 2, randomized clinical trial with PD patients receiving either deferiprone (30 mg/kg/day) or placebo is currently under investigation (NCT02655315). The aim is to evaluate the effect of iron chelation as a therapeutic strategy on motor and non-motor handicap. Another phase 2 clinical trial under investigation (NCT02728843) is to evaluate the effects of deferiprone at four different dosages in patients with PD and to assess the motor score with the Movement Disorder Society (MDS)-UPDRSIII.

Iron chelation in ALS

The first evidence of the iron chelation potential for ALS was recently described in a single-center, one-year pilot clinical trial (Moreau et al., 2018a). Twenty-three patients were enrolled and received deferiprone treatment (30 mg/kg/day) for a year. The results showed a good safety, a significant decrease in the ALS Functional Rating Scale and the body mass index after 3 months of treatment compared to previous 3 months of treatment-free period. Moreover, iron levels measured by magnetic resonance imaging, cerebrospinal fluid levels of oxidative stress and neurofilament light chains were lower after deferiprone treatment. The efficacy of this new therapeutic modality is now under investigation in a randomized, double-blind, placebo-controlled, multicenter study (NCT03293069).

Iron chelation in other neurological conditions

In Friedreich Ataxia, tolerance and efficacy of iron chelator was first demonstrated in a monocentric open label small phase 2 trial in nine adolescent patients treated 6 months with deferiprone (20 to 30 mg/kg/day). Iron content of the dentate nuclei was significantly decreased in patients and a moderate neurological improvement observed in the youngest ones (Boddaert et al., 2007). Few years later,

same results were obtained in an open-label clinical trial combining idebenone and deferiprone (Velasco-Sanchez et al., 2011). Then, a 6 month randomized, double-blind, placebo-controlled study was conducted in 72 patients treated with 3 different dosages of deferiprone (20, 40 or 60 mg/kg/day) or placebo (Pandolfo et al., 2014). Investigators concluded that the lower dose of deferiprone was well tolerated by Friedreich Ataxia patients and, for those with less severe disease, a possible benefit on ataxia and neurological function observed with this lower dose.

In 2015, safety, tolerability and efficacy of PBT2 was conducted in a phase 2, randomized, double-blind, placebo-controlled trial in HD patients. This iron chelator was administered for 26 weeks to adults with early stage to mid-stage HD daily at two different doses (250 and 100 mg). This clinical trial demonstrates a good safety and well tolerability of this drug in HD patients (NCT 01590888, Huntington study group).

Among the NBIA disorders, a six-month phase 2 pilot trial was conducted in nine patients with pantothenate kinase-associated neurodegeneration (PKAN) treated with deferiprone (25 mg/kg/day). No clinical changes were observed whereas a significant reduction of iron content in the globus pallidus was observed by magnetic resonance imaging (Zorzi et al., 2011). In contrast, a four years follow up clinical trial on 6 PKAN patients treated with deferiprone at 15 mg/kg (twice a day, orally) confirmed the iron content reduction but also reported an improvement or stabilization of motor symptoms (Cossu et al., 2014). This clinical improvement was also observed after 12 months of treatment in a recent pilot trial with 5 PKAN patients enrolled and treated with deferiprone at 30 mg/kg/day (Rohani et al., 2017). A large phase II trial is in progress.

Toward the new concept of conservative iron chelation: iron scavenging and redeployment

For any chelator to be of clinical value in disorders of regional siderosis they ought to be endowed with a requisite accessibility to the relevant sites and differential specificity so as to spare unaffected areas of the organism from scavenging an essential element (Cabantchik et al., 2013). Different agents with iron chelating features [e.g. DFO, clioquinol, VK28, M30 and natural plant-derived polyphenol

flavonoids] have been assessed but not progressed to clinical trial testing for PD (Moreau et al., 2018b).

Deferiprone is exceptional among iron chelators in its ability to cross membranes, including the, and to chelate components of the cellular labile iron pool in brain tissue. Deferiprone has the remarkable ability to rescue transfusional hemosiderosis in the heart of β-thalassemia patients without inducing anaemia. This ability of deferiprone is largely attributable to the redeployment of captured iron to extracellular iron free Tf, and subsequent distribution (e.g. for uptake to iron-sulfur cluster and haem biosynthetic machineries) (Cabantchik et al., 2013). Thus, this conservative repositioning strategy to subserve iron scavenging and redeployment is under assessment using deferiprone at the relatively low oral dose of 30 mg/kg/day in AD (NCT03234686), PD (NCT02728843 and NCT02655315) and ALS (NCT03293069).

Ferroptosis: a regulated cell death that harnesses the potential of iron

Overview

Ferroptosis is a newly described mode of regulated cell death that is triggered by a build-up of lipid peroxides and is prevented by iron chelation (Dixon et al., 2012, Yagoda et al., 2007, Yang and Stockwell, 2008, Reed and Pellecchia, 2012, Galluzzi et al., 2018). While an inceptive pathway describing known key players of ferroptosis is now available, the exact role played by iron in the execution of ferroptosis is yet to be ascertained (**Figure 1**).

Glutathione peroxidase 4 (GPX4), a selenoprotein enzyme, is regarded as a critical enzyme that regulates ferroptosis. GPX4 catalyses the reduction of lipid peroxides in a reaction dependent on the availability of its cofactor, reduced glutathione (GSH). Molecules that can impede GPX4 activity directly (e.g. RSL3) or indirectly, through depleting levels of GSH (e.g. erastin), are potent inducers of ferroptosis (**Table 1**) (Shimada et al., 2016, Dixon et al., 2012, Yang and Stockwell, 2008, Gaschler et al., 2018). Inhibition of GPX4 results in a build-up of fatty acid radicals ultimately leading to ferroptotic death (Yang et al., 2016). Correspondingly, molecules that mitigate lipid peroxides (e.g.

vitamin E, liproxstatin-1, zileuton, and ferrostatin-1) serve as protective agents against ferroptosis (Xie et al., 2016). Acyl-CoA synthetase long-chain family member 4 (ACSL4) is implicated as amajor driver of ferroptosis (Doll et al., 2017). ACSL4 generates the acyl Co-A derivatives of arachidonic acid (AA) or adrenic acid (AdA) which are esterified with phosphoethanolamine (PE) by the action of lysophosphotidylcholine acyltransferase 3 (LPCAT3). These AA-PE and Ada-PE esters may then be oxidised to generate lipid hydroperoxides (LOOH) through the enzymatic action of lipoxygenases and/or through autoxidation reactions (**Figure 1**) (Kagan et al., 2017, Doll et al., 2017, Shah et al., 2018).

Ferroptotic susceptibility is contingent upon cellular status of GSH, lipid antioxidants, polyunsaturated fatty acids (PUFA) and lipids, selenium availability, and aspects of molecular crosstalk such as activation of mitogenic pathways (Ras-Raf-MEK-ERK) and tumour suppressor p53 protein (Ingold et al., 2017, Kagan et al., 2017, Xie et al., 2016, Jiang et al., 2015, Tarangelo et al., 2018, Gnanapradeepan et al., 2018, Li et al., 2012, Wang et al., 2016, Masaldan et al., 2018a, Masaldan et al., 2018b).

Ferroptosis is reliant on the availability of iron either imported or liberated through autophagic/lysosomal degradation of ferritin (ferritinophagy) or through catabolism of haem (Dixon et al., 2012, Reed and Pellecchia, 2012, Yang and Stockwell, 2008, Gao et al., 2016, Masaldan et al., 2018a, Kwon et al., 2015). Cells can be made refractory to ferroptosis through genetic ablation of iron uptake (*TfR1*), metabolism (*IRP2* and *ISCU*), and storage (*ferritin H*) genes (Yang and Stockwell, 2008, Dixon et al., 2012, Cao and Dixon, 2016), or through depleting Tf in the extracellular milieu (Gao et al., 2015). Further, ferroptosis can be prevented by iron chelators (e.g. DFO, 2,2-bipyridyl or compound 311) (Dixon et al., 2012, Reed and Pellecchia, 2012, Yang and Stockwell, 2008). Ferroptosis initiated through repression of the p53-upregulated target, SLC7A11, which encodes a component of the cysteine/glutamate antiporter (xCT), results in iron-dependent accumulation of ROS and GSH depletion (Dixon et al., 2012, Cao and Dixon, 2016, Jiang et al., 2015). Erastin induces ferroptosis through inhibiting the activity of SLC7A11 (Dixon et al., 2014) and was recently demonstrated to enhance iron bioavailability through autophagic/lysosomal degradation of ferritin and

its cargo receptor NCOA4 (Gao et al., 2016). Thus ferroptosis appears to depend on the bioavailable iron pool with evidence of changes in iron flux prior to the execution of ferroptosis (Aron et al., 2016) **(Figure 1)**.

The exact function of iron in ferroptosis remains elusive. One possibility is that iron catalyses the formation of lipid peroxides directly through its oxidative potential involving Fenton chemistry (Shah et al., 2018). Alternatively/additionally, iron may facilitate generation of lipid peroxides through iron-dependent oxidases. Lipoxygenases are non-haem iron-containing enzymes that can catalyse dioxygenation of PUFAs in lipids generating the proximate inducers of ferroptosis (Kagan et al., 2017). Another possibility is that iron may play multiple roles in the ferroptosis pathway both upstream and downstream of effector molecules and some of these functions may be independent of its redox activity (Gao and Jiang, 2018, Kagan et al., 2017).

Implications of ferroptosis in disease and physiology

The initial discovery of ferroptotic cell death in a subset of cells that harbour oncogenic *Hras* highlighted the relevance of ferroptosis in certain cancer types (Dixon et al., 2012, Dolma et al., 2003, Yang and Stockwell, 2008). Ferroptosis is implicated in the onco-suppressive function of p53, in the context of failing antioxidant defences, by negatively regulating SLC7A11, a ferroptosis regulator that is highly expressed in human cancers and is implicated in resistance to chemotherapeutics (Galluzzi et al., 2015, Huang et al., 2005, Jiang et al., 2015, Chen et al., 2015b, Yu et al., 2015, Roh et al., 2016). ACSL4, a central ferroptosis executor, is found elevated in malignancies such as liver (Sung et al., 2003), prostate (Monaco et al., 2010, Wu et al., 2015) and breast cancers (Monaco et al., 2010) which may therefore be susceptible to ferroptotic chemotherapeutics (Doll et al., 2017, Ma et al., 2016). Additionally, renal cell carcinomas and large B cell lymphomas may be particularly sensitive to ferroptosis in a GPX4-dependent manner (Yang et al., 2014). Therapy-resistant cancer cells show a unique sensitivity to ferroptosis and may be a target for ferroptosis based therapies (Doll et al., 2017, Viswanathan et al., 2017).

Aberrant ferroptosis activation may lead to pathological consequences. Ischemia-reperfusion injury (IRI) of the liver and kidneys has been shown to involve ferroptosis; these can be rescued by ferroptosis inhibitors *in vivo* (Friedmann Angeli et al., 2014, Linkermann et al., 2014, Martin-Sanchez et al., 2017). Ferroptosis may also contribute to myocardial infarction and IRI (Baba et al., 2017, Magni et al., 1994). We have recently shown that ferroptosis has a bearing on ischemic stroke which can be mitigated by ferroptosis inhibitors (Tuo et al., 2017). In addition, dysregulated ferroptosis may lead to degenerative disorders of photoreceptor cells (Ueta et al., 2012), abnormal skin phenotypes (Sengupta et al., 2013), and a host of disorders related to improper development of vasculature (Wortmann et al., 2013). Aberrant ferroptosis activation in T cells of the immune system has been shown to affect their ability to mount a response to *Leishmania* parasite infection (Matsushita et al., 2015).

While the significance of ferroptosis in various pathological settings has been determined, a specific physiological role, in addition to a general anti-proliferative function, is yet to be ascribed. However, recently ferroptosis has been indicated as a possible arm of the innate immune response that is specialized against intracellular pathogens, such as the malarial pathogen *Plasmodium* (Kain et al., 2018).

Role of ferroptosis in AD

AD results from loss of synapses and neuronal cell death in the brain. The chronic inflammation and degeneration that accompanies AD and the absence of downstream indicators of apoptotic death suggest that an alternate cell death mechanisms may be involved (Raina et al., 2001, Hambright et al., 2017, Khandelwal et al., 2011). Elevated brain iron is associated with increased risk of AD and affected regions in the brain show elevated iron. Interestingly, levels of CSF ferritin that match cognitively normal subjects can strongly indicate the progression of mild cognitive impairment to AD, with higher CSF ferritin predictive of earlier conversion to AD (Ayton et al., 2015a). Further, quantitative susceptibility mapping (QSM) value, a measure of magnetic susceptibility of tissue determined by MRI and used as a proxy for iron level in tissue, of the hippocampus indicated that abnormal levels of elevated iron may not be required for AD progression. However, individuals with

Aβ pathology that show higher iron, but within normal range, deteriorate faster than those with lower iron (Ayton et al., 2017b). Furthermore, α-lipoic acid, which can stabilize cognitive function of AD patients through controlling Tau hyper-phosphorylation, was recently shown to mitigate Tau-induced iron overload and accompanied lipid peroxidation in P301S Tau transgenic mice (Zhang et al., 2018). These observations implicate a possible involvement of ferroptotic processes as iron appears to accelerate disease progression by enhancing susceptibility towards neurodegeneration rather than through direct toxicity.

Lipid peroxidation, a hallmark feature of ferroptosis, is considered an early event in the pathology of AD (Pratico and Sung, 2004, Reed et al., 2009). Proteins involved in antioxidant, neuronal communication, neurite outgrowth and energy metabolism are modified thorough extensive binding to 4-hydroxy-2-nonenal (HNE) which is a proximal marker for lipid peroxidation (Reed et al., 2009). Deuterated PUFAs (D-PUFA), which are known to block ferroptosis, have been used to mitigate lipid peroxidation in brain tissue and also reduce Aβ in a mouse model of AD (APP/PS1 transgenic mice) (Raefsky et al., 2018, Yang et al., 2016). Recently, the conditional ablation of GPX4 in the forebrain (cerebral cortex and hippocampus) of mice (Gpx4BIKO) was shown to result in AD-like cognitive impairment (special learning and memory) accompanied by hippocampal neurodegeneration, elevated lipid peroxidation (enhanced HNE adducts observed in the cerebral cortex) and neuro-inflammation (Hambright et al., 2017). These phenotypes were further exacerbated in mice fed with a diet deficient in tocopherol, a lipid soluble antioxidant that serves as a natural anti-ferroptotic compound in the body (Hambright et al., 2017). Further, AD is accompanied by depletion of GSH in the frontal cortex and hippocampus which correlates with decline in cognitive function (Mandal et al., 2015). Taken together these data suggest an important role of ferroptosis in AD and thus, targeting aspects of ferroptosis may be sufficient to alleviate AD.

Role of ferroptosis in PD

Pathological progression of PD displays features that may facilitate ferroptosis induction such as elevated iron in the SNpc (Lei et al., 2012, Do Van et al., 2016, Guiney et al., 2017, Dexter et al., 1989a), depleted GSH (Sian et al., 1994) and lipid peroxidation (Dexter et al., 1989b). Application of

iron chelation has been shown to mitigate the motor impairment in mouse models of PD (Ayton et al., 2014, Lei et al., 2015, Lei et al., 2012), and in a human clinical trial (Devos et al., 2014). Further iron chelation was found to enhance GPX activity in the CSF (Devos et al., 2014). Similarly, N-acetylcysteine (NAC), an antioxidant that can enhance brain GSH, offers partial protection against neurodegeneration in PD mouse models (Park et al., 2004, Perry et al., 1985, Coles et al., 2018). Further, a recent short term (3 months) phase II clinical trial (NCT02445651) indicated protection of dopaminergic neurons in the caudate and putamen in PD patients receiving NAC with concomitant significant improvement in clinical symptoms (Monti et al., 2016).

A recent study characterized erastin-induced ferroptosis in a cell culture model of PD [Lund human mesencephalic cells (LUHMES)] and *ex vivo* using organotypic slice cultures. Further, the study showed that the ferroptosis inhibitor, ferrostatin 1 can prevent neuron loss and behavioural impairment in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse PD model (Do Van et al., 2016). Further, cell death initiated in LUHMES cells using environmental neurotoxins such as rotenone and paraquat, that are causally associated to sporadic PD, was rescued by the iron chelator deferiprone, ferrostatin-1 and liproxstatin-1 (Do Van et al., 2016). Taken together, these studies indicate that ferroptosis inhibitors may be effective in alleviating/preventing PD (including sporadic PD).

Ferroptosis in other neurological conditions

Cell death mechanisms associated with neurological impairment remain poorly understood. However, conditions that may favour ferroptosis, such as elevated brain iron and diminished GSH, conspicuously appear across multiple neurodegenerative and certain psychiatric disorders (Belaidi and Bush, 2016, Mandal et al., 2015, Gawryluk et al., 2011, Cutler, 1994, Feifel and Young, 1997, Serata et al., 2012).

ALS/MND

ALS/MND is a progressive neurodegenerative disorder characterized by a selective dysfunction of the cortical and spinal motor neurons (Gajowiak et al., 2016). While genetic factors account for up to

50% of familial cases of ALS (FALS), adult-onset motor neuron disease leading to sporadic ALS (SALS) has an unknown aetiology (Veyrat-Durebex et al., 2014). However, several condition that may predispose cells to ferroptotic death have emerged as possible biomarkers of ALS hinting at a causal role of ferroptosis (Gajowiak et al., 2016, Simpson et al., 2004, Choi et al., 2015, Chen et al., 2015a, Moreau et al., 2018a). These conditions include aberrant iron homeostasis that leads to iron accumulation in mouse models of ALS as well as in SALS and FALS (Moreau et al., 2018a, Gajowiak et al., 2016, Veyrat-Durebex et al., 2014). Iron chelation using deferiprone has been recently shown to enhance mean lifespan in a mouse model of ALS ($SOD1^{G86R}$) and has shown positive effects (e.g. stabilized BMI) in a small cohort (n=23) of ALS patients (Moreau et al., 2018a). Further, ALS patients display enhanced lipid peroxidation in the CSF and sera, as well as, reduced GSH in their motor cortex suggesting that conditions in ALS are conducive to ferroptosis (Choi et al., 2015, Simpson et al., 2004). In a mouse model of ferroptosis (Gpx4NIKO) the ablation of *Gpx4* in neurons led to a rapid paralysis, severe muscle atrophy and death which was associated with the ferroptotic degeneration of motor neurons of the spinal cord; thus recapitulating ALS (Chen et al., 2015a). Further, no overt neurodegeneration in the cerebral cortex was observed in the Gpx4NIKO mice or in another mouse model [Gpx4(f/f);Camk2α-creERT] where *Gpx4* was selectively ablated in cortical neurons suggesting that spinal motor neurons are especially susceptible to GPX4-dependent ferroptosis (Chen et al., 2015a). Thus, ferroptosis may be involved in ALS pathology.

Stroke

Stroke is a major cause for morbidity and disability resulting from interruption of blood supply to the brain. Ischemic stroke, accounting for a majority of stroke cases (~85%) result from vascular occlusion (Tuo et al., 2017, Langhorne et al., 2011). Spontaneous intracerebral haemorrhage (ICH) which leads to 15% of all strokes is a cause of great morbidity and mortality with few therapeutic avenues (Donnan et al., 2010). Ischemic and haemorrhagic stroke may lead to ferroptotic death of neurons (Li et al., 2017, Tuo et al., 2017, Zille et al., 2017). Acute focal cerebral ischemia induced in mice through transient middle cerebral artery occlusion (MCAO) leads to enhanced brain iron and reperfusion damage following MCAO is exacerbated in aged mice and those fed on high-iron feed

(Tuo et al., 2017, Lei et al., 2012, Castellanos et al., 2002). Recently ferroptosis inhibitors, ferrostatin-1 and liproxstatin-1, were shown to mitigate neuronal damage when administered intranasally immediately following MCAO/reperfusion (Tuo et al., 2017). These agents were also effective to a lesser extent when administered 6 hours following MCAO/reperfusion. Other ferroptosis mitigating interventions, such as limiting brain iron through administration of Cp or APP ectodomain, or the use of 15-lipoxygenase-1 inhibitor, ML351, inhibited ischemia-reperfusion mediated brain damage (Tuo et al., 2017). Similarly, ferrostatin-1 administration in the affected region of an induced ICH mouse model reduced neurodegeneration and neurological deficit and also rescues haem/haemoglobin induced cell death in cultured primary neurons and organotypic hippocampal slice cultures (Li et al., 2017, Zille et al., 2017). Taken together ferroptosis signalling may be involved in neuronal cell death following stroke and targeting this cell death pathway may be a therapeutic option to mitigate stroke associated pathology.

Conclusion and future perspectives

AD and PD continue to be major health challenges with the situation set to worsen as global populations continue to age. With little to no progress in treatment modalities new hypothesis are required to treat, if not explain, neurodegenerative process associated with these debilitating conditions. Iron dysregulation in the brain implicated in these and several other neurodegenerative disorders coupled with deeper understanding of iron-mediated/dependent cell death pathways, such as ferroptosis, may offer interesting and new therapeutic avenues. While anti-ferroptotic molecules show remarkable potency *in vitro* their clinical use is limited due to their inability to cross the BBB. Thus, there is a potential to develop the next class of molecules that may breach this barrier. Iron chelators that can cross the BBB are under investigation in clinical settings now. In some ways, while this may not be specific anti-ferroptotic agents, therapies based on iron chelation may be advantageous as they may mitigate a broad range of neurodegenerative processes. In the future a better understanding of the effector arm of ferroptosis may offer a range of theranostic opportunities.

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Figure legend

Figure 1. **Crosstalk between ferroptosis and iron homeostasis.** Lipid peroxides (L-OOH) that ignite ferroptotic death are produced through auto-oxidation and/or enzymatic activity of lipoxygenases on lipid esters generated from lipids via the activity of ACSL4 and LPCAT3. Pharmacological inhibitors of lipoxygenases (e.g. zileuton) and genetic ablation of ACSL4 can limit the process of generation of LOOH thus limiting ferroptosis. Glutathione peroxidase 4 (GPX4) plays a central role in the ferroptotic pathway though its ability to convert L-OOH to lipid alcohols (L-OH). Pharmacological inhibition of GPX4 can execute ferroptosis in a variety of cell types. This is achieved through direct inactivation of GPX4 (e.g. RSL3), or by limiting cellular stores of its cofactor, glutathione (GSH). GSH levels in cells can be limited though pharmacological inhibition of γGCL (e.g. by BSO) or through blocking of xCT (e.g. by erastin) thus limiting uptake of cysteine, a required substrate for GSH synthesis. Iron exacerbates ferroptosis potentially by catalysing/facilitating lipid peroxidation. Iron chelators (e.g. deferiprone, deferoxamine) are potent inhibitors of ferroptosis. Iron uptake in neurons is facilitated through circulating transferrin bound iron (Tf-Iron) which is endocytosed by binding to transferrin receptor 1 (TfR1). Endosomal iron is released to the cytosol through the activity of divalent metal transporter 1 (not shown) following which iron is either utilised (e.g. synthesis of haeme and iron-sulfur clusters in mitochondria), stored in ferritin, or exported through the activity of ferroportin (FPN). The amyloid precursor protein (APP) facilitates the iron export function of FPN by maintaining it at the cell membrane. This "tethering" of APP and FPN is facilitated by normal cytosolic levels of tau. Autophagic degradation of ferritin (ferritinophagy) and catabolism of haem that may enhance labile iron (LIP) can enhance ferroptosis sensitivity in cells. Conversely, silencing of TfR1 and depletion of Tf may enhance resistance to ferroptotic death.

Table

Ferroptosis inducer	Mechanism(s) of action	References
Erastin	a) Indirect inhibition of GPX4: Inhibits cystine uptake through xCT → GSH depletion b) VDAC-dependant mitochondrial involvement	(Dolma et al., 2003, Dixon et al., 2012, Yang et al., 2014, Yang and Stockwell, 2008, Yagoda et al., 2007, Dixon et al., 2014)
Sulfasalazine	Indirect inhibition of GPX4: Inhibits cystine uptake through xCT → GSH depletion	(Dixon et al., 2012)
Sorafenib	Indirect inhibition of GPX4: Inhibits cystine uptake through xCT → GSH depletion	(Dixon et al., 2014, Lachaier et al., 2014)
L-buthionine sulfoximine (BSO)	Indirect inhibition of GPX4: Inhibits γ-GCS → GSH depletion	(Seiler et al., 2008, Yang et al., 2014)
RSL5	VDAC-dependant mitochondrial involvement	(Yang and Stockwell, 2008)
RSL3	Direct inhibition of GPX4	(Dixon et al., 2012, Yang and Stockwell, 2008, Yang et al., 2014)
Altretamine	Direct inhibition of GPX4	(Woo et al., 2015)
DPI7 (ML162), DPI10 (ML210), DPI12, DPI13, etc.	Direct inhibition of GPX4	(Yang et al., 2014)
FIN56	a) Decreases GPX4 levels b) Coenzyme Q10 depletion	(Shimada et al., 2016)
FINO2	a) GPX4 inactivation b) Iron oxidation	(Gaschler et al., 2018)
***t*-butyl hydroperoxide (TBH)**	Enhanced ROS/lipid peroxidation	(Wenz et al., 2018, Jiang et al., 2015)

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Figure
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