Regulation of the p53-mediated ferroptosis signaling pathway in cerebral ischemia stroke (Review)

SHUANGLI XU[1], XUEWEI LI[2] and YANQIANG WANG[3]

Departments of [1]Emergency, [2]Rheumatology and Immunology, and [3]Neurology, Affiliated Hospital of Weifang Medical University, Weifang, Shandong 261031, P.R. China

Received July 15, 2022; Accepted December 16, 2022

DOI: 10.3892/etm.2023.11812

Abstract. Stroke is one of the most threatening diseases worldwide, particularly in countries with larger populations; it is associated with high morbidity, mortality and disability rates. As a result, extensive research efforts are being made to address these issues. Stroke can include either hemorrhagic stroke (blood vessel ruptures) or ischemic stroke (blockage of an artery). Whilst the incidence of stroke is higher in the elderly population (≥65), it is also increasing in the younger population. Ischemic stroke accounts for ~85% of all stroke cases. The pathogenesis of cerebral ischemic injury can include inflammation, excitotoxic injury, mitochondrial dysfunction, oxidative stress, ion imbalance and increased vascular permeability. All of the aforementioned processes have been extensively studied, providing insights into the disease. Other clinical consequences observed include brain edema, nerve injury, inflammation, motor deficits and cognitive impairment, which not only cause disabilities obstructing daily life but also increase the mortality rates. Ferroptosis is a type of cell death that is characterized by iron accumulation and increased lipid peroxidation in cells. In particular, ferroptosis has been previously implicated in ischemia-reperfusion injury in the central nervous system. It has also been identified as a mechanism involved in cerebral ischemic injury. The tumor suppressor p53 has been reported to modulate the ferroptotic signaling pathway, which both positively and negatively affects the prognosis of cerebral ischemia injury. The present review summarizes the recent findings on the molecular mechanisms of ferroptosis under the regulation of p53 underlying cerebral ischemia injury. Understanding of the p53/ferroptosis signaling pathway may provide insights into developing methods for improving the diagnosis, treatment and even prevention of stroke.

Correspondence to: Dr Yanqiang Wang, Department of Neurology, Affiliated Hospital of Weifang Medical University, 2,428 Yuhe Road, Kuiwen, Weifang, Shandong 261031, P.R. China
E-mail: wangqiangdoctor@126.com

Key words: ferroptosis, stroke, p53, iron metabolism, middle cerebral artery occlusion

Contents

1. Introduction

The aging population combined with the burden of various risk factors, including atrial fibrillation, hypertension, diabetes, hyperlipidemia, smoking, lack of physical activity, unhealthy diets, abdominal obesity and alcohol consumption, all lead to an increased lifelong risk of stroke (1,2). Ischemic stroke is a result of either permanent or transient regional reduction in brain blood supply in the brain, resulting in motor difficulties, such as in movement or speech. Through advances in pharmacological and mechanical thrombolysis, significant progress has been made in the field of therapeutic interventions for ischemic stroke. However, these methods are only effective during a narrow window of time (1,2). To the best of our knowledge, the pathogenesis of ischemic stroke remains unclear, meaning that further studies are needed to identify valuable therapeutic targets for improving treatment methods. The identification of genes associated with the progression of ischemic stroke is currently being studied to identify relevant targets (1,2).

Ferroptosis is caused by an increase in iron-dependent toxic lipid reactive oxygen species (ROS) levels, particularly when the oxidation of membrane polyunsaturated fatty acids (PUFAs) cannot be performed due to the inactivation of lipid hydroperoxide glutathione (GSH) peroxidase 4 (GPX4) (3,4). Ferroptosis is a regulated process that differs from apoptosis and other forms of non-apoptotic cell death, which are typically caspase-dependent (5). In addition, it has been found to be associated with neuronal cell death during a stroke (4).

Upregulated mutant p53 was first discovered in cancer. Subsequent research has demonstrated other functions of

p53 and it is now considered to be the most important tumor suppressor, known as 'guardian of the genome (3). p53 is essential for growth and development; mice treated with a p53 inhibitor or that had p53 expression knocked out were found to have manifestations of extracerebral malformation, spina bifida, ocular abnormalities, embryonic brain malformations and other developmental issues (6). The earlier the loss of genes regulating p53, such as mouse double minute (MDM)2 and MDM4, the more serious the phenotypic abnormalities (7). Physiologically, ubiquitination and E3 ubiquitin ligases strictly controls p53 and keeps its functional levels low during cell and embryonic development (8). Therefore, p53 is controlled precisely under physiological condition and it regulates growth and development.

Recently, it has been found that p53 is involved in the pathogenesis of ischemic stroke and the ferroptotic signaling pathway (9). As a transcription factor, p53 can directly activate or inhibit the transcription of a long list of genes, several of which serve a key role in ferroptosis (6). In the present review, the primary signaling pathways and relationship between p53, ferroptosis and ischemic stroke are discussed.

2. Potential pathogenesis and treatment status of cerebral ischemia injury

Ischemia stroke is the result of ischemia/reperfusion (I/R) or ischemia in the brain, in which the damaged region of the brain can be divided into two following different regions: Ischemic core region and the penumbra region (10). In the core area, if the cerebral blood flow decreases below the end-stage depolarization threshold, it would irreversibly destroy the structure and function of neurons residing here (11). The penumbra area refers to the neuronal regions around the infarcted core, which maintains its structural integrity but has impaired neuronal function due to the decrease in cerebral blood flow (12,13). Compared with those in the core area, neurons with normal structure and activity in the penumbra area can be preserved if treated in the appropriate window of time. This area can maintain a potential viability for 16-48 h, which provides a treatment window for clinical intervention (11). Therefore, this is a potential therapeutic target for the treatment of acute ischemic brain injury in a clinical setting.

It has been previously reported through experimental data that the recovery of the blood supply will not restore the function of injured neurons, but instead aggravate the damage, in a phenomenon known as cerebral I/R injury (14). This typically occurs in various blood flow occlusion conditions in the clinic, such as cardiopulmonary resuscitation, which causes delayed/prolonged neuronal damage, impairing the function of the central nervous system (15). To the best of our knowledge, the underlying mechanism remains unclear, although several theories have been proposed (2). The cessation of arterial blood flow leads to hypoxia in neurons, which in turn disrupts electron transport chain function in the mitochondria due to the loss of oxygen and glucose supply. This results in reduced ATP production, promoting anaerobic metabolism and dysfunctional Na^+-K^+-ATPase and Ca^{2+}-ATPase pump activity (4). A series of intracellular signaling cascades are activated as a result of decreased ATP and antioxidant levels. Insufficient blood supply results in irreversible injury and necrosis in the core area, but the peri-infarcted area is potentially salvageable. Following the reperfusion phase, blood flow to the ischemic tissue is restored, which also restores the oxygen supply (14). However, this results in increased production of ROS, coupled with an insufficient quantity of antioxidants due to the cellular dysfunction caused by ischemia (14). Therefore, the reperfusion continues to aggravate the oxidative stress (2), promoting endothelial dysfunction, DNA damage and a local inflammatory cascade. This cascade of inflammation then activates the microglia in the brain, increasing the permeability of the blood-brain barrier (BBB) and infiltration by peripheral immune cells (2), further aggravating the injury.

Patients who have experienced an ischemic stroke are typically treated with thrombolytic therapy [tissue plasminogen activator (tPA)] within 4.5 h of the onset of stroke symptoms (4,10,16,17). tPA treatment outside of this specific time window can result in a hemorrhagic performance, which causes additive but unnecessary damage to the brain (10). Other treatment strategies, including thrombectomy and preventive drug treatments, such as the early application of blood pressure- and cholesterol-lowering drugs, have also been used for treating ischemic stroke (16). Since a second stroke frequently occurs immediately after the initial stroke, timely treatment is of importance for reducing the depth of disabilities caused by the initial and/or secondary stroke (16). Patients with disabilities caused by ischemic strokes, such as hemiparesis, facial paresis, dysarthria, unconsciousness, language and speech disorders or impaired vision, have a significantly reduced quality of life (16). Several factors, such as diet, smoking habits, high blood pressure and diabetes, have been shown to increase the risk of stroke (18). In recent years, the incidence rate of stroke and the prevalence of younger patients with stroke have both increased (10). Therefore, adequate control of manageable risk factors, such as diabetes and hypertension, can prevent ischemic stroke in high-risk groups (19). In addition, further research into the accurate mechanism of ischemic stroke can facilitate the identification of novel avenues for the management of this disease.

3. Ferroptosis

Features of ferroptosis. A primary cause of ferroptosis is the increase in iron-dependent toxic lipid ROS levels through the peroxidation of PUFAs, which is caused by the dysregulation of the endogenous antioxidant network (5,20-23). Another key condition for ferroptosis is the overload of Fe^{2+} in cells (5,17,21,24). These two aforementioned elements are hypothesized to induce irreversible lipid damage and increased membrane permeability.

During mammalian development, caspase-dependent apoptosis is the most common method of regulated cell death (4,25). Ferroptosis was initially identified in oncogenic Ras-expressing human foreskin fibroblast cell cells treated with the synthetic small molecule erastin or RAS-selective lethal 3 (RSL3), which did not induce caspase activation but was significantly reversed by specific antioxidants and iron chelators (26). These results suggest that the molecular mechanism of ferroptosis differed from that of classical apoptosis.

Intracellular ATP consumption, caspases and lysosome activation are not necessary for ferroptosis, in stark contrast

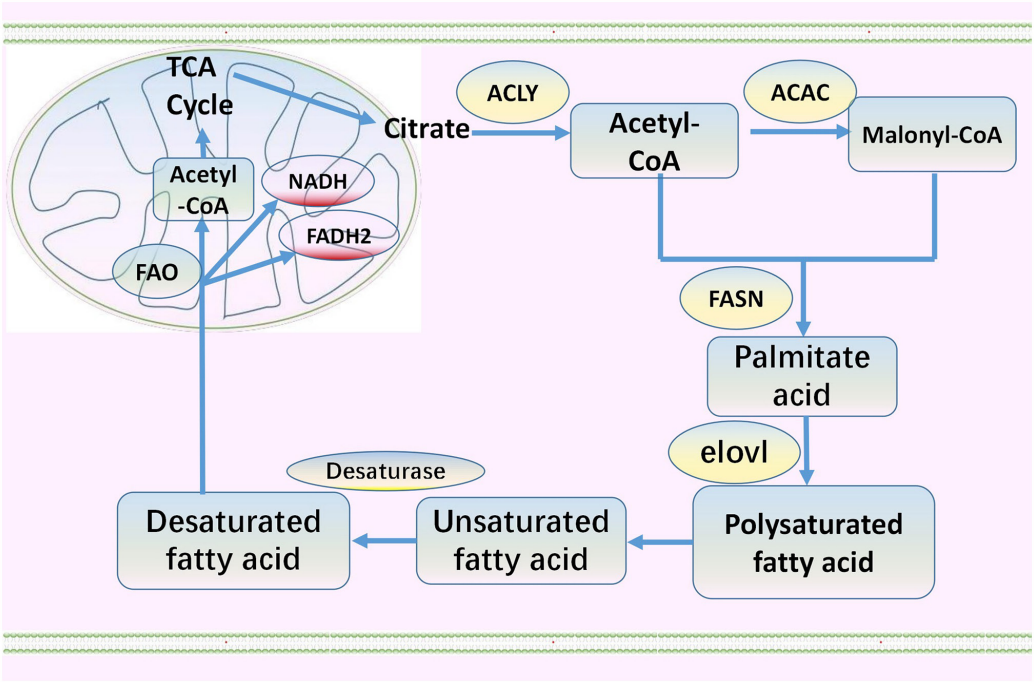

Figure 1. Brief process of lipid metabolism. TCA, tricarboxylic acid cycle; ACLY, ATP-citrate lyase; ACAC, acetyl coenzyme A carboxylase; FASN, fatty acid synthase; elovl, elovl fatty acid elongation enzyme; FAO, fatty acid oxidation; CoA, coenzyme A.

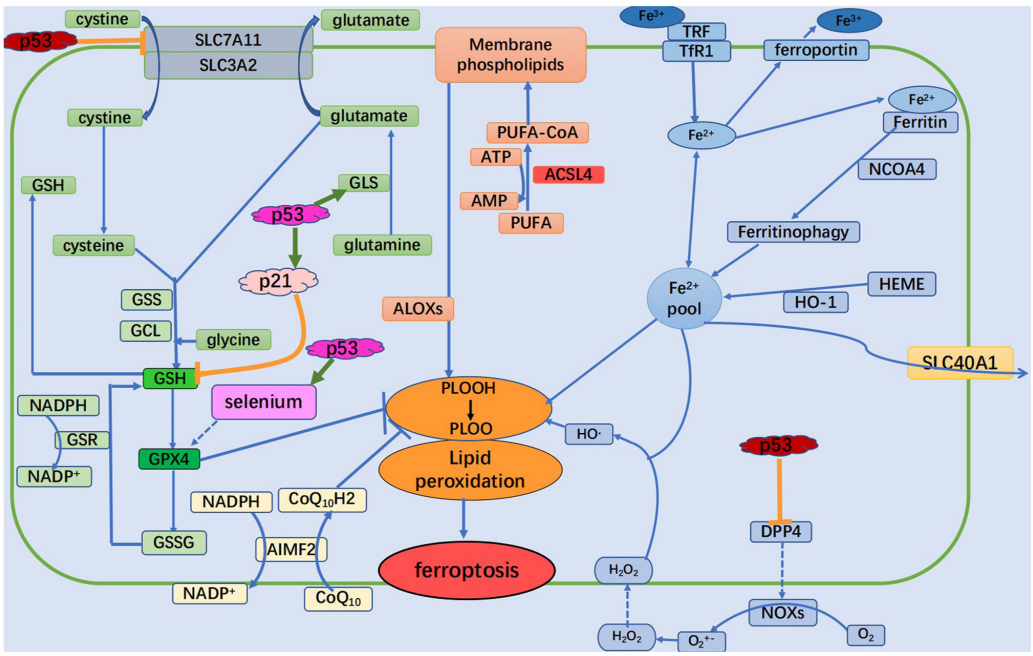

Figure 2. Summary points of previous studies on ferroptosis signaling pathways that are potentially regulated by p53. The dual role of p53 in the control of ferroptosis is shown. p53 can enhance ferroptosis through the inhibition of SLC7A11 expression or the promotion of SAT1 and GLS2 expression. However, p53 can also suppress ferroptosis through the inhibition of DPP4 activity or the induction of CDKN1A/p21 expression. GSS, glutathione synthetase; GCL, glutamate-cysteine ligase; SLC, soluble carrier family; GPX4, glutathione peroxidase 4; GSH, glutathione; GSSG, oxidized glutathione; GSR, glutathione-disulfide reductase; GLS, glutaminase; ALOXs, lipoxygenases; CoA, coenzyme A; CoQ10, coenzyme Q10; CoQ10H2, ubiquinol; H2O2, hydrogen peroxide; HO-1, heme oxygenase 1; NCOA4, nuclear receptor co-activator 4; PUFA, polyunsaturated fatty acid; PUFA-CoA, PUFA-coenzyme; DPP4, dipeptidyl peptidase 4; NOXs, NADPH oxidases; ACSL4, acyl-CoA synthetase long chain family member 4; PLOOH, phospholipid hydroperoxides; TRF, transferrin; TfR1, transferrin receptor.

to apoptosis, necrosis and autophagy (27). The characteristics and morphological features of ferroptosis can be used to identify ferroptotic cells. Typically, ferroptotic mitochondria will exhibit disordered cristae and increases in mitochondrial membrane density (4,22,28,29). By contrast, nuclear changes, such as nuclear condensation or chromatin marginalization and condensation, do not occur in ferroptosis (30). Instead, lipid peroxidation is observed, such that the condition of the

cells after treatment with iron chelators or lipid peroxidation inhibitors can be used to determine whether ferroptosis is occurring (26). Recently, antibodies against transferrin (TF) receptor 1 (TfR1), including 3F3 iron body membrane antibody, have also been used to detect ferroptosis (31).

Ferroptosis has been observed in several processes, including the inhibition of different types of human cancer (32-34), tissue I/R injury (24,25,33,35-37), neurodegenerative diseases (21,24,25,33,36,38,39), intracerebral hemorrhage (40) and both the innate and adaptive immune responses (30,41,42). In addition, ferroptosis can form part of the process downstream of different molecular signaling pathways (43). Recent studies have also confirmed that it is involved in solute carrier family 7 member 11 (SLC7A11)/GSH/GPX4 signaling pathways and can be controlled by specific factors, such as p53, in cells in the central nervous system (43).

Iron metabolism. In mammalian cells, iron is normally bound and absorbed by various transporters or receptors, through non-heme and heme-dependent absorption pathways. Fe^{2+} is involved in the regulation of various cellular processes, including oxygen transport, cell proliferation, cell division, energy production and DNA synthesis. In addition, Fe^{2+} can also serve as a key co-factor in regulating various activities, such as cell size, inflammatory response and cell death (36).

In the non-heme-dependent iron absorption pathway, extracellular iron (Fe^{3+}) binds to TF/TFR1 on the cell membrane, resulting in membrane infiltration and localization in the specialized endosomes formed (4). Fe^{3+} in the endosomes is then released from TF and reduced to Fe^{2+} by the ferric reductase steam 3, which is specifically located in endosomes (24,25,27). Fe^{2+} can be released into the labile iron pool (LIP) in the cytoplasm through the endosomal membrane by solute carrier family 11 member 2 (SLC11A2) (44). When the iron load exceeds the carrying capacity of TF in certain pathological states, Fe^{3+} are present as non-TF-bound iron, Fe^{3+} is reduced to Fe^{2+} by ferrireductases on the cell surface or released cellular reductants and Fe^{2+} is moved into cells through transmembrane transporters, such as SLC11A2 (44). In the heme-dependent iron absorption pathway, Fe^{2+} present in hemoglobin or heme in plasma is internalized into endosomes after binding to various transporters, such as Feline leukemia virus subgroup C receptor heme transporter 2, solute carrier family 48 member 1 and solute carrier family 46 member 1 (45). In cells, heme treated with cytoplasmic heme oxygenase 1 (HO-1), releases Fe^{2+} into the labile iron pool.

Solute carrier family 40 member 1 (SLC40A1) is the only known iron export protein in mammalian cells (45). Iron oxidases ceruloplasmin (CP), hephaestin (HEPH) and HEPH like 1 (bacteriophage like 1) can all regulate iron balance through SLC40A1-dependent iron output (45,46). In addition, there is evidence that overexpression of SLC40A1 can improve ferroptosis through reduction of the level of Fe^{2+} in cells and knockdown of SLC40A1 can promote ferroptosis through the accumulation of Fe^{2+} (47). The overexpression of SLC40A1 could reduce the levels of Fe^{2+} in cells, which could reduce the risk of ferroptosis; however, Fe^{2+} will accumulate in cells when SLC40A1 is knocked-down, which contributes to ferroptosis (45).

Ferritin is an iron storage protein that can store 70-80% of the internalized iron ions (4,28); it is primarily localized in the cytoplasm but can also be found in the mitochondria. Ferritin can be divided into two subtypes: H type and L type. The H subtype, also known as ferritin heavy chain 1 (FTH1), oxidizes Fe^{2+} into Fe^{3+}. By contrast, the L subtype, also known as ferritin light chain (FTL), contributes to iron nucleation and mineralization (35).

In the central nervous system, iron ions need to be transferred from the circulating blood to the brain parenchyma through TF/TFR at the endothelial layer of the BBB (28). Since iron ions can accept and donate electrons, pathological iron accumulation leads to oxidative damage or even cell death (45). Excessive Fe^{2+} can react with hydrogen peroxide (H_2O_2) and organic peroxide to produce hydroxyl radicals ($HO^{\bullet}$), which can attack DNA, proteins and lipid membranes to disrupt cellular function and cause neuronal death. In addition, lipid alkoxy radicals can be produced, which are the primary sources of ROS produced by iron metabolism through the Fenton reaction (27,30,48-51). The increase in ROS levels not only reduces GSH consumption but also reacts with PUFAs at the lipid membrane to induce lipid peroxidation (52). Neuronal membranes are rich in cholesterol and PUFAs, which are readily oxidized by ROS. This renders neuronal membranes highly vulnerable to damage, because superoxide dismutase and GPX activity in the brain is decreased and the neurons there cannot clear ROS adequately following an ischemic stroke (53).

Alterations in iron metabolism, including iron deficiency and iron overload, can adversely affect the central nervous system (33). Iron deficiency can reduce the activity of cytochrome oxidase in the brain, which can lead to developmental disorders in the hippocampus and prefrontal lobe, such as motor development and cognitive memory impairment (4). Intracellular iron accumulation is generally caused by iron export impairments, where increases in the brain iron load can increase sensitivity to ferroptosis and aggravate brain injury (49). The middle cerebral artery occlusion (MCAO) animal model has revealed increased iron levels in the affected brain hemisphere (10). Treatment with lipostatin-1 or ferristatin-1 can protect against neurotoxicity induced by glutamate, reduce the size of the infarcted area and reduce the degree of brain edema and behavioral disorders caused by cerebral ischemic injury (33,54).

Ferroptosis observed during the MCAO model caused by iron metabolism disorders can be managed by iron inhibitors, such as lipstatin-1 and iron statin-1, which are both used in research settings. However, they are not yet approved for human use (55).

Lipid metabolism. Fatty acids are not only a source of energy but are also important precursors of all bio-membrane lipids (56). Acetyl coenzyme A in the cytoplasm is typically catalyzed to malonyl coenzyme A by the key enzyme acetyl coenzyme A carboxylase (Fig. 1). Subsequently, through fatty acid synthase, malonyl CoA and acetyl CoA are condensed to produce the 16-carbon fatty acid palmitate (C16:0; Fig. 1) (33,45). C16:0 is then extended by the elovl fatty acid elongation enzyme and desaturated by fatty acid desaturase (Fig. 1). Fatty acids are catabolized by fatty acid oxidation in

the mitochondria (Fig. 1). This process produces acetyl CoA, NADH and FADH2. Acetyl CoA then enters the Krebs cycle, whilst NADH and FADH2 enter the electron transport chain to produce ATP (45,53,57). Fatty acids are stored in the form of lipid droplets, which can buffer and store excess lipids. The formation of lipid droplets prevents palmitic acid-induced lipotoxicity by separating damaged membranes. Therefore, increasing lipid storage through the formation of lipid droplets can limit ferroptosis. In addition, monitoring the dynamic balance between lipid droplet formation and degradation is important for evaluating the progress of ferroptosis (57).

Under pathological conditions, ROS, including superoxide anions, H_2O_2 and $OH^{\bullet}$, are formed due to the incomplete reduction of oxygen (41,42). These oxidants can attack the carbon-carbon double bonds of lipids, especially PUFAs, to cause lipid peroxidation (45). Free PUFAs from neuronal membranes are particularly sensitive to lipid peroxidation; they can be esterified into membrane phospholipids and oxidized to transmit ferroptotic signals (42). Acyl-Coenzyme A (Acyl-CoA) synthase long-chain family member 4 (ACSL4) and lysophosphatidylcholinyl transferase 3 participate in the biosynthesis of phosphatidylethanolamine, activate PUFA and regulate the transmembrane properties of PUFA upstream of iron/lipid signaling transduction (28,36,52,58). Lipids can also be oxidized directly by oxygenase. Under these conditions, lipid peroxidation occurs, which alters the electric potential, fluidity and permeability of membranes, resulting in an imbalance in osmotic potential and membrane breakage, and ultimately ferroptosis (59).

Abnormal lipid metabolism is an important cause of ferroptosis (4,53). Ferrostatin-1 has been shown to reduce the infarct size in the brain of a mouse MCAO model of stroke, by inhibiting the accumulation of ROS induced by glutamate in neurons (33). Small molecule lipoxygenase inhibitors can also capture free radicals and produce antioxidants, which inhibit the production of ROS and lipid peroxidation to block ferroptosis in neurons (33,52,60).

Ferroptosis observed during MCAO induced by ROS accumulation as a result of lipid metabolism disorders can be directly regulated by the small molecule inhibitor of ACSL4, which prevents the accumulation of lipid ROS (45,61). This includes natural products tricystin C and synthetic thiazolidinediones, such as rosiglitazone, which can reduce the load of PUFA (61).

Participants in the ferroptosis signaling pathway. Substrate-specific subunit SLC7A11 and auxiliary regulatory subunit SLC3A2 form the main pump of the cysteine glutamate antiporter (system xc-), which imports cystine into cells whilst pumping out glutamate (4,36,38,43,49,62). This cycle is driven by high concentrations of intracellular glutamate but does not require ATP (27). Erastin, sulfasalazine, sorafenib, RAS-selective lethal 3 and related molecular proteins such as p53 can all target system xc- to inhibit ferroptosis (23,33,63). In addition, it has been reported that the deficiency of SLC7A11 can lead to liver injury as a result of ferroptosis caused by an overload of iron (62).

Cystine is reduced to cysteine by GSH and thioredoxin reductase 1. Cysteine combines with glutamic forms GSH, which can in turn bind to cytotoxic lipid peroxides (64). Glutamic acid cysteine ligase (GCL) is comprised of a catalytic subunit and a modified subunit; it is the rate-limiting enzyme for the *de novo* synthesis of GSH by combining glutamic acid, cysteine and glycine in cells (49). However, cysteine content is low across the whole cell and is therefore considered to be a limiting factor in GSH synthesis. Therefore, the availability of cystine is important for maintaining the levels of GSH for preventing ferroptosis (30,49,65).

RAS-selective lethal 3 can also inhibit the GSH-dependent enzyme GPX4. GPX4 is an isozyme member of the GPX family (4). Compared with other GPX members, GPX4 is the only known key cellular enzyme involved in the regulation of ferroptosis, which can directly reduce lipid peroxide to non-toxic alcohols (L-OH) in the membrane to prevent ferroptosis during lipid oxidation (22,23,25,33,36,39,49,59,65,66). GPX4-knockout mice have been shown to exhibit ferroptosis in the brain and early embryonic lethality. The absence of GPX4 in the mouse forebrain neurons can promote ferroptosis and lead to cognitive impairments and neurodegeneration (63). GPX4 selenocysteine, the active site of GPX4, can form a catalytic quadruplex with tryptophan, glutamine and asparagine to catalyze the reduction of lipid hydroperoxide to inhibit ferroptosis (42). ROS inhibitors, such as ferrostatin-1 and liproxstatin-1, in addition to GPX4 promoters, such as dopamine and selenium, have all been reported to be effective in preventing ferroptosis in various animal models (49,67).

PUFA is converted to acyl-coA by the fatty acid-activating enzyme ACSL4. Acyl-CoA is involved in the lipid peroxidation of membrane phospholipids (24,65). After a series of complex dynamic processes, PUFA lipid peroxide (L-OOH) is converted to oxidized glutathione and non-toxic alcohol (L-OH) (23,25,27,30). In cells, L-OOH can be oxidized by Fe^{2+} to produce highly active alkoxy radicals ($L\text{-}O^{\bullet}$) (41). Under physiological conditions, L-OOH and L-OH levels are held in equilibrium due to the activity of GPX4. When GPX4 is absent or becomes inactivated, L-OOH accumulates above physiological levels, resulting in the increased production of $L\text{-}O^{\bullet}$ and cell membrane damage (50).

Coenzyme Q (CoQ). CoQ with 10 isoprene units at the tail of its side chain is called CoQ10. The main function of CoQ10 is to transfer electrons in the mitochondrial electron transport chain. In addition, the reduced form of CoQ10, namely panthenol, is an effective lipophilic antioxidant. CoQ10 is an endogenous inhibitor of ferroptosis, which can neutralize free radicals produced during the I/R damage process (33).

Iron and ferrous chelators, such as deferoxamine (DFO), VK-28, deferiprone, minocycline, nitrilotriacetic acid, ethylenediaminetetraacetic acid and clioquinol, are effective inhibitors of ferroptosis, as previously shown in several different animal models (30,38); they deplete Fe^{2+} in the LIP to prevent iron-dependent lipid peroxidation to inhibit ferroptosis (49). Ferroxidase is another type of iron metabolism inhibitor, such as CP, which oxidizes the toxic Fe^{2+} to the less toxic Fe^{3+} to inhibit ferroptosis (49). Inhibition of TF *in vitro* can block ferroptosis, whereas TF supplementation can restore this process (49). In cancer cells, silencing of the expression of key genes associated with iron metabolism such as Transferrin Receptor (TFRC) has been shown to reduce iron uptake and susceptibility to ferritin disease (45). However, the function of the lipid oxygenase family is iron-dependent (23,61,68).

Overall, iron is an indispensable factor in ferroptosis. Any molecules or factors that can modulate the unstable LIP in cells can either reduce or promote ferroptosis (33,44). TF, TFR1, ferroportin (SLC11A3) and HO-1 have all been found to weaken ferroptosis (69).

However, other factors, such as the transcription factors nuclear factor erythroid 2-related factor 2 and p53, have also been reported to regulate ferroptosis by transcriptionally inducing the expression of GPX4, SLC7A11 and HO-1 (3).

4. p53

In total, p53 has six major protein domains and is negatively regulated by two homologous proteins, MDM2 and MDM4 (5,43,70,71).

A total of two intrinsically disordered N-terminal domains, tRNA-specific adenosine deaminase (TAD)1 and TAD2, form the binding sites of its negative regulator MDM2 (43,72,73). Abrogation of TAD1 can inhibit the p53 response, whilst abrogation of TAD2 does not notably alter p53 function, since p53 can continue to induce the expression of target genes in addition to retaining the ability to induce cell cycle arrest and apoptosis (74,75). By contrast, deletion of both TAD1 and TAD2 results in the complete abrogation of p53 function (74). The proline-rich domain, also known as the polyproline region of the PXXP repeat sequence (P stands for proline, whilst X can be any amino acid), is of great significance for the stability of p53 and for p53-mediated apoptosis (74). Deletion of this region results in the nuclear export of p53, which then becomes prone to ubiquitination and degradation mediated by MDM2 (76). Residues 102-292 in the central core region of p53 contain the DNA binding domain (DBD) of p53, which allows p53 to function as a transcription factor in a sequence-specific manner, by recognizing the p53 response element (77). The tetramerization domain (TD) allows four p53 proteins to form a tetramer, facilitating the acquisition of the appropriate protein conformation when bound with DNA for sequence recognition (78). p53 as a tetramer can typically bind to the target gene element or interact with other proteins. TD has also been shown to be necessary for the post-translational modifications of p53, such as phosphorylation and ubiquitination (78). Only after post-translational modification, namely acetylation and phosphorylation, can the intrinsically disordered C-terminal regulatory domain (CTD) change from an inactive conformation to an active conformation, after which it binds with the DBD to exert its function (79,80). This process requires the p53 cofactor P300 to participate in its acetylation or phosphorylation (79,80). The CTD also contains nuclear export and localization signals, which regulates intracellular location (79,80).

Each domain of p53 has unique properties and contributes to the overall function of p53 (79,80). These domains not only interact with other proteins and regulate signal transduction, but can also be affected by extensive post-translational modifications to regulate protein stability, turnover and cell localization (3).

p53 is mainly regulated by two negative regulators, MDM2 and MDM4, which are homologous proteins. p53, MDM4 and MDM2 constitute a highly dynamic regulatory core, consisting of complex positive and negative feedback loops, ensuring the precise regulation of p53 under physiological conditions and rapid responses to stress (1,70,81). MDM2 is an oncogene that encodes an E3 ubiquitin ligase. p53 oligomerizes to form a tetramer, which then regulates cell proliferation, apoptosis, cell cycle arrest, DNA repair and metabolism upstream of oxidative stress, aging, autophagy and ferroptosis (82). MDM4 is another important negative regulator of p53. Although MDM4 does not have E3 ligase activity and does not directly control p53, it does form a heterodimer with MDM2, stabilizing MDM2 E3 ligase activity and promoting MDM2-mediated ubiquitination and p53 degradation (73). In addition, p53 transcription can also induce MDM2 and MDM4, forming a negative feedback pathway to strictly regulate p53 activity. Exogenous MDM2-MDM4 dimers are maintained at a low level under physiological conditions, reducing promotion of p53, which reduces the amount of p53. However, with the increase in the half-life of the p53 protein in cells, it can regulate the subsequent cell response by binding with p53 response elements of its target genes (80). When the expression of MDM2, a negative regulator of p53, was specifically ablated in the central neuron system, the mice developed hydrated brain malformations between embryonic days 12 and 5 due to apoptosis. By contrast, the deletion of MDM4 expression, another negative regulator of p53, resulted in multiple brain malformations between embryonic days 17 and 5 due to cell cycle arrest and apoptosis (73). Both phenotypes can be completely rescued by p53 deletion. In general, p53 is involved in apoptosis, DNA repair, cell cycle arrest, DNA replication stress response, autophagy, ferritin-based diseases (such as neuroferritinopathy), the pathological process of cancer inhibition, anti-infection, immune response, tissue I/R injury, neurodegenerative diseases (81), maternal reproduction, development and aging (43,83,84).

5. p53-mediated regulation of iron and lipid metabolism involved in ferroptosis

p53-mediated iron metabolism. p53 can directly activate the expression of iron sensors, such as iron regulatory hormone, to regulate the intracellular iron pool. p53 has been previously shown to upregulate hepcidin antimicrobial peptide (HAMP), which encodes ferritin (3), through a putative p53-responsive element, to reduce iron export (3). In addition, p53 can directly transcribe the mitochondrial iron-binding protein XN (frataxin) to regulate mitochondrial iron homeostasis (3). Furthermore, p53 can mediate the expression of iron oxidoreductase (FDXR) to form a FDXR/p53 loop to upregulate FDXR, which prevents mitochondrial iron overload, whilst FDXR deficiency inhibits p53 mRNA translation (43). p53 can also upregulate the translation of *fth1* mRNA and abrogates the stability of TF mRNA, which results in increased cellular iron storage and reduced cellular iron import. Ferritin is a protein, composed of both light (FTL) and heavy chain (FTH1) subunits, which can store iron ions. When FTH is upregulated, the balance between FTH1 and FTL is broken and ferritin cannot maintain its balance. Cellular iron storage is increasing and the iron ions are balanced inside and outside the cells. Cells will reduce the import of iron ions when there are enough iron ions inside the cell. Functionally, the

p53 protein can transactivate ferritin directly in response to ferroptosis and inflammation (43). Ferritin can reduce serum iron by chelation in reticuloendothelial macrophages (43). In addition, the heme-p53 interaction can regulate iron metabolism and ferroptosis. p53 was also found to be associated with hypoxia-inducible factor 1α to increase p53 protein stability and expression levels during iron deficiency (73). Iron overload can reduce the levels and function of the p53 protein. Iron porphyrin heme directly binds to p53 to interfere with p53-DNA interactions, which promotes the nuclear export and degradation of p53 (73). The MDM2 antagonist, nutlin-3, has been previously shown to delay the occurrence of ferroptosis in liver and liver cancer cells in a p53-dependent manner, to promote cell survival under metabolic stress (44).

p53 regulates lipid metabolism. p53 is also involved in the transcription of genes associated with lipid metabolism through various mechanisms (6); it can bind directly to the promoter region of the transcription factor for sterol regulatory element-binding transcription factor 1 to inhibit its expression, which in turn regulates the expression of a group of genes involved in lipid metabolism (5). p53 also regulates the transcription of two enzymes involved in fatty acid oxidation, namely carnitine palmitoyl transferase 1C and phosphatidylate phosphatase, which regulates the transport of activated fatty acids to the mitochondria to enhance the oxidation of fatty acids in cells (5,85). p53 also promotes the transcription of malonyl-CoA decarboxylase, which catalyzes intracellular fatty acid oxygenation to prevent lipid accumulation in cells (5). In terms of protein-protein binding, glucose-6-phosphate dehydrogenase can bind to p53 and is directly inhibited by p53, leading to a decrease in NADPH production. p53 also serves a role in lipid transport (5). Apolipoprotein B (APOB) and APOB editing enzyme complex 1 have been found to serve a role in atherosclerotic lipoproteins by the regulation of p53 transcription (85).

6. p53-mediated ferroptosis

It has been found that, in H1299 cells with the p53 gene silenced and then treated with ROS, the cell activity remained unchanged. However, when treated with ROS after p53 activation, 90% of the cells died. This suggests that p53 activation reduced the antioxidant capacity of these cells. In addition, after treatment with the ferroptosis inhibitor fer-1, the cell death rate decreased significantly. This also suggests that p53 serves an important role in ferroptosis (3).

p53 has been previously found to promote ferroptosis (36,43). p53 can downregulate the expression of SLC7A11 (Fig. 2) (85). System xc- operates by transforming cystine to intracellular cysteine. After the reaction between cysteine and glutamate, glutathione is produced as the substrate of GPX4 activity, which is required to inhibit ferroptosis (3). p53 can inhibit SLC7A11 to reduce cystine uptake and intracellular glutathione production, which in turn leads to an increase in intracellular ROS (5,36). Ferroptosis is regulated by glutamine metabolism. During glutamine catabolism, glutamine is first converted to glutamic acid by glutaminase (GLS) 1 and GLS2, which is then converted into α-ketoglutarate, an important substrate for the Kreb's cycle (29). GLS2 is a hepatic glutaminase present in the mitochondria. p53 induces GLS2 transcription, which in turn mediates oxygen consumption and ATP production in cells (Fig. 2) (5,85). GLS2 can also promote antioxidant function by increasing the production of GSH and NADH in cells (3,43). In male C57BL/6J mice, the knockdown of GLS2 expression has been found to inhibit serum-dependent ferroptosis induced by amino acid deprivation (3,85). Spermidine/spermine N1 acetyltransferase 1 (SAT1) has also been reported to be a direct p53 target, which catalyzes the acetylation of spermidine and spermine, serving as a key enzyme for polyamine catabolism (5,36,85). SAT1 can be activated by nutlin-3, a small molecule MDM2 inhibitor, in a p53-dependent manner, to promote the formation of ROS-dependent lipid peroxides and render cells sensitive to ferroptosis (43). In addition, ROS-induced cell death can only be inhibited by ferriprotease-1 in SAT1-overexpressing cells (86). However, SAT1 did not affect the expression or activity of SLC7A11 and GPX4. Following SAT1 induction, the expression levels of arachidonic acid (ALOX)15 lipoxygenase, a member of the lipoxygenase family, increased (43). Its oxygenates PUFAs which is one of the necessary conditions for ferroptosis (43). Ferroptosis induced by SAT1 can be effectively blocked by the specific inhibitor of ALOX15, pd146176, suggesting that ALOX15 is a mediator of p53 in the pathway between SAT1 induction and ferroptosis (86). ALOX12 is another important positive regulator of p53 in the lipoxygenase family to mediate ferroptosis. ALOX12 inactivation can inhibit ROS stress-induced p53-mediated ferroptosis, independent of GPX4 and ACSL4 activation (87). In conclusion, these observations suggest that p53 serves an important role in regulating ferroptosis by regulating the expression of its targets.

p53 can not only promote but also inhibit ferroptosis. p21 is a major p53 target that inhibits glutathione degradation, where its ability to induce cell cycle arrest and aging allows it to respond to stress signals (Fig. 2) (3). p53 transactivates p21 to inhibit glutathione degradation and promote GPX4 activity, which results in the reduction of ROS accumulation from toxic lipids to inhibit ferroptosis (88).

7. p53-mediated ferroptosis in the MCAO model

p53 activation is induced by various types of cellular stress, including DNA damage, oncogene activation, ribosomal stress or hypoxia. Ischemia and hypoxia caused by the occurrence of brain MCAO provide the conditions for the activation of p53. The brain contains the highest PUFA content, providing abundant precursor material for lipid peroxidation. In addition, the neurons in the brain are prone to downstream molecular events, such as Ca^{2+} influx, which accumulates iron ions and increases lipid ROS, increasing the risk of ferroptosis (89). After severe ischemic and hypoxic brain injury, the accumulation of Fe^{2+} in the basal ganglia, thalamus, periventricular and subcortical white matter areas, increases lipid oxide levels and reduces GPX4 function, supporting their involvement in ferroptosis (4,24,25,41,89). The expression of ROS, GPX4, GSH, GSSH, SLC7A11, TFRC (31), FTH1 and FTL can be used as indicators of ferroptosis (31). A previous study reported that the use of ferroptosis inhibitors significantly improve the prognosis of ischemic stroke (4).

p53 transcription can promote ferroptosis after MCAO in the cerebrum. SLC7A11 expression promotes ferroptosis, whilst in p53-deficient cells, SLC7A11 upregulation promotes sensitivity to ferroptosis (70,90,91). It has been previously shown that p53 can promote the nuclear translocation of ubiquitin specific peptidase 7, a nuclear deubiquitinase, to remove ubiquitin from histone 2B ubiquitination (H2BUB) at lysine 120. This removes H2BUB from the SLC7A11 promoter, inhibiting SLC7A11 transcription (92). Ferroptotic cells in the MCAO model may undergo irreversible cell death through this pathway (4). p53 regulation of SLC7A11 expression can also regulate ALOX12-dependent lipid peroxidation to regulate ferroptosis (87). Glutamine is transformed into glutamic acid by GLS1 and GLS2 after entering the cell (3). Glutamic acid serves two important functions, acting as a precursor for GSH synthesis and as an intermediate for its conversion to α-ketoglutarate (3). It is widely known that GSH synthesis is a key process in ferroptosis. GSL2 expression could promote GSH production to increase cellular antioxidant function (3). p53 can induce GLS2 transcription to regulate glutamate levels in cells and then induce ferroptosis (70). Glutamate can also be exported from cells through SLC7A11 in exchange for extracellular cystine (62). Therefore, SLC7A11 can mediate glutamate export, leading to reduction in intracellular glutamate. Subsequently, SLC7A11 absorbs additional glutamine through negative feedback and activates the glutamate enzymes to increase glutamate production, leading to SLC7A11/glutamine-dependent ferroptosis. Therefore, p53 transcription-mediated SLC7A11 activity can interact with p53-mediated glutamine-dependent ferroptosis to jointly regulate cell death (3). In addition, p53 transcription promotes the expression of spermidine/SAT1 to regulate ALOX15-dependent lipid peroxidation and increase the sensitivity of cells to ferroptosis (70).

p53 directly participates in the regulation of HAMP, frataxin, FDXR, ferritin, heme and ferroptosis by regulating iron metabolism (93). p53 also regulates the expression of lipid metabolism genes and transporters upstream of ferroptosis. Following cerebral ischemia and hypoxia, the regulation of various genes regulating iron and lipid metabolism is altered (53,56). In cerebral ischemic reperfusion process, both iron and lipid metabolism will change under the influence of regulators such as p53. However, the mechanism underlying the regulation of iron and lipid metabolism by p53 remains unclear and requires further verification.

p53 can also inhibit ferroptosis (42). p53 can bind to dipeptidyl peptidase 4 (DDP4) in the nucleus, inhibiting the binding of DPP4 to NADPH oxidase 1 to mediate the production of ROS in the cell membrane (43). p53 also promotes the expression of p21, which induces the production of GSH to inhibit lipid peroxidation (3). The tumor suppressor p21 is a primary p53 target that inhibits glutathione degradation to inhibit ferroptosis (70,94). Although p53 can inhibit ferroptosis, its value in the MCAO model remains unclear.

8. Conclusions and future perspectives

The present review aimed to discuss the role of p53-mediated ferroptosis in ischemic stroke. Ferroptosis is a unique regulatory form of cell death that was first discovered in 2012 and involves a complex network of gene sets and signaling pathways that is distinct from apoptosis, necrosis, autophagy and oxygenation (42). p53 was first found in cancer cells. However, p53 can act on numerous proteins involved in ferroptotic signaling pathways and has been shown to serve an important role in the progression of several nervous system diseases. The iron overload that occurs during MCAO is caused by several mechanisms, such as system xc-, GSH depletion, GPX4 inactivity, lipoxygenase inactivation and/or intracellular iron accumulation. These mechanisms can be either promoted or inhibited by known inducers of ferroptosis (glutamate, erastine and rsl3) and inhibitors (fer-1, lipstatin-1, DFO and vitamin E). Based on the above, we hypothesized that p53-mediated ferroptosis is potentially an important form of cell death in the MCAO model. Further studies on ferroptosis will provide novel opportunities for the diagnosis and treatment of ischemic stroke.

Although p53 has been found to act directly on proteins involved in ferroptosis signaling, it is not clear if p53 interacts with each target protein. The majority of associated previous studies have focused on the effect of ferroptosis on brain function. However, to the best of our knowledge, relatively few studies have been performed regarding brain immune infiltration, immune cell activation and circulatory function following the establishment of the MCAO model. Therefore, future research should not only reveal the therapeutic effect of inhibiting ferroptosis in brain cells, but should also pay attention to the comprehensive treatment of all aspects of ischemic stroke.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 81870943).

Availability of data and materials

Not applicable.

Authors' contributions

SX and XL conceived the topic of review. YW was responsible for reviewing and editing the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Zhang T, Wang H, Li Q, Fu J, Huang J and Zhao Y: MALAT1 Activates the P53 signaling pathway by regulating MDM2 to promote ischemic stroke. Cell Physiol Biochem 50: 2216-2228, 2018.
2. Sun MS, Jin H, Sun X, Huang S, Zhang FL, Guo ZN and Yang Y: Free radical damage in ischemia-reperfusion injury: An obstacle in acute ischemic stroke after revascularization therapy. Oxid Med Cell Longev 2018: 3804979, 2018.
3. Kang R, Kroemer G and Tang D: The Tumor Suppressor protein p53 and the ferroptosis network. Free Radic Biol Med 133: 162-168, 2019.
4. Bu ZQ, Yu HY, Wang J, He X, Cui YR, Feng JC and Feng J: Emerging role of ferroptosis in the pathogenesis of ischemic stroke: A new therapeutic target? ASN Neuro 13: 17590914211037505, 2021.
5. Liu J, Zhang C, Hu W and Feng Z: Tumor suppressor p53 and metabolism. J Mol Cell Biol 11: 284-292, 2019.
6. Laubach K, Zhang J and Chen X: The p53 Family: A role in lipid and iron metabolism. Front Cell Dev Biol 9: 715974, 2021.
7. Filichia E, Shen H, Zhou X, Qi X, Jin K, Greig N, Hoffer B and Luo Y: Forebrain neuronal specific ablation of p53 gene provides protection in a cortical ischemic stroke model. Neuroscience 295: 1-10, 2015.
8. Hernández Borrero LJ and El-Deiry WS: Tumor suppressor p53: Biology, signaling pathways, and therapeutic targeting. Biochim Biophys Acta Rev Cancer 1876: 188556, 2021.
9. Zhao J, Dong Y, Chen X, Xiao X, Tan B, Chen G, Hu J, Qi D, Li X and Xie R: p53 inhibition protects against neuronal ischemia/reperfusion injury by the p53/PRAS40/mTOR pathway. Oxid Med Cell Longev 2021: 4729465, 2021.
10. Paul S and Candelario-Jalil E: Emerging neuroprotective strategies for the treatment of ischemic stroke: An overview of clinical and preclinical studies. Exp Neurol 335: 113518, 2021.
11. Kanazawa M, Takahashi T, Ishikawa M, Onodera O, Shimohata T, del Zoppo GJ: Angiogenesis in the ischemic core: A potential treatment target?. J Cereb Blood Flow Metab 39: 753-769, 2019.
12. del Zoppo GJ, Sharp FR, Heiss WD and Albers GW: Heterogeneity in the penumbra. J Cereb Blood Flow Metab 31: 1836-1851, 2011.
13. Sommer CJ: Ischemic stroke: Experimental models and reality. Acta Neuropathol 133: 245-261, 2017.
14. Granger DN and Kvietys PR: Reperfusion injury and reactive oxygen species: The evolution of a concept. Redox Biol 6: 524-551, 2015.
15. Abrams D, MacLaren G, Lorusso R, Price S, Yannopoulos D, Vercaemst L, Bělohlávek J, Taccone FS, Aissaoui N, Shekar K, *et al*: Extracorporeal cardiopulmonary resuscitation in adults: evidence and implications. Intensive Care Med 48: 1-15, 2022.
16. Barthels D and Das H: Current advances in ischemic stroke research and therapies. Biochim Biophys Acta Mol Basis Dis 1866: 165260, 2020.
17. Wang P, Cui Y, Ren Q, Yan B, Zhao Y, Yu P, Gao G, Shi H, Chang S and Chang YZ: Mitochondrial ferritin attenuates cerebral ischaemia/reperfusion injury by inhibiting ferroptosis. Cell Death Dis 12: 447, 2021.
18. Przykaza L: Understanding the connection between common stroke comorbidities, their associated inflammation, and the course of the cerebral ischemia/reperfusion cascade. Front Immunol 12: 782569, 2021.
19. Boehme AK, Esenwa C, Elkind MS: Stroke risk factors, genetics, and prevention. Circ Res 120: 472-495, 2017.
20. Guo H, Zhu L, Tang P, Chen D, Li Y, Li J and Bao C: Carthamin yellow improves cerebral ischemia-reperfusion injury by attenuating inflammation and ferroptosis in rats. Int J Mol Med 47: 52, 2021.
21. Xie BS, Wang YQ, Lin Y, Mao Q, Feng JF, Gao GY and Jiang JY: Inhibition of ferroptosis attenuates tissue damage and improves long-term outcomes after traumatic brain injury in mice. CNS Neurosci Ther 25: 465-475, 2019.
22. Hirschhorn T and Stockwell BR: The development of the concept of ferroptosis. Free Radic Biol Med 133: 130-143, 2019.
23. Magtanong L and Dixon SJ: Ferroptosis and brain injury. Dev Neurosci 40: 382-395, 2018.
24. She X, Lan B, Tian H and Tang B: Cross talk between ferroptosis and cerebral ischemia. Front Neurosci 14: 776, 2020.
25. Mao H, Zhao Y, Li H and Lei L: Ferroptosis as an emerging target in inflammatory diseases. Prog Biophys Mol Biol 155: 20-28, 2020.
26. Yang WS and Stockwell BR: Ferroptosis: Death by lipid peroxidation. Trends Cell Biol 26: 165-176, 2016.
27. Hu X, Xu Y, Xu H, Jin C, Zhang H, Su H, Li Y, Zhou K and Ni W: Progress in understanding ferroptosis and its targeting for therapeutic benefits in traumatic brain and spinal cord injuries. Front Cell Dev Biol 9: 705786, 2021.
28. Yan N and Zhang JJ: The emerging roles of ferroptosis in vascular cognitive impairment. Front Neurosci 13: 811, 2019.
29. Gao M, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB and Jiang X: Role of mitochondria in ferroptosis, Mol Cell 73: 354-363 e3, 2019.
30. Han C, Liu Y, Dai R, Ismail N, Su W and Li B: Ferroptosis and its potential role in human diseases. Front Pharmacol 11: 239, 2020.
31. Feng H, Schorpp K, Jin J, Yozwiak CE, Hoffstrom BG, Decker AM, Rajbhandari P, Stokes ME, Bender HG, Csuka JM, *et al*: Transferrin receptor is a specific ferroptosis marker. Cell Rep 30: 3411-3423 e7, 2020.
32. Hassannia B, Wiernicki B, Ingold I, Qu F, Van Herck S, Tyurina YY, Bayır H, Abhari BA, Angeli JPF, Choi SM *et al*: Nano-targeted induction of dual ferroptotic mechanisms eradicates high-risk neuroblastoma. J Clin Invest 128: 3341-3355, 2018.
33. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascón S, Hatzios SK, Kagan VE, *et al*: Ferroptosis: A regulated cell death nexus linking metabolism, Redox Biology, and Disease. Cell 171: 273-285, 2017.
34. Fan Z, Wirth AK, Chen D, Wruck CJ, Rauh M, Buchfelder M and Savaskan N: Nrf2-Keap1 pathway promotes cell proliferation and diminishes ferroptosis. Oncogenesis 6: e371, 2017.
35. Lin W, Zhang T, Zheng J, Zhou Y, Lin Z and Fu X: Ferroptosis is Involved in Hypoxic-ischemic brain damage in neonatal rats. Neuroscience 487: 131-142, 2022.
36. Li J, Cao F, Yin HL, Huang ZJ, Lin ZT, Mao N, Sun B and Wang G: Ferroptosis: Past, present and future. Cell Death Dis 11: 88, 2020
37. Guo P, Jin Z, Wu H, Li X, Ke J, Zhang Z and Zhao Q: Effects of irisin on the dysfunction of blood-brain barrier in rats after focal cerebral ischemia/reperfusion. Brain Behav 9: e01425, 2019.
38. Proneth B and Conrad M: Ferroptosis and necroinflammation, a yet poorly explored link. Cell Death Differ 26: 14-24, 2019.
39. Hambright WS, Fonseca RS, Chen L, Na R and Ran Q: Ablation of ferroptosis regulator glutathione peroxidase 4 in forebrain neurons promotes cognitive impairment and neurodegeneration. Redox Biol 12: 8-17, 2017.
40. Li Q, Weiland A, Chen X, Lan X, Han X, Durham F, Liu X, Wan J, Ziai WC, Hanley DF and Wang J: Ultrastructural characteristics of neuronal death and white matter injury in mouse brain tissues after intracerebral hemorrhage: Coexistence of ferroptosis, autophagy, and necrosis. Front Neurol 9: 581, 2018.
41. Bayir H, Anthonymuthu TS, Tyurina YY, Patel SJ, Amoscato AA, Lamade AM, Yang Q, Vladimirov GK, Philpott CC and Kagan VE: Achieving life through death: Redox biology of lipid peroxidation in ferroptosis. Cell Chem Biol 27: 387-408, 2020.
42. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, Kang R and Tang D: Ferroptosis: Process and function. Cell Death Differ 23: 369-379, 2016.
43. Liu J, Zhang C, Wang J, Hu W and Feng Z: The regulation of ferroptosis by tumor suppressor p53 and its pathway. Int J Mol Sci 21: 8387, 2020.
44. Vogt AS, Arsiwala T, Mohsen M, Vogel M, Manolova V and Bachmann MF: On iron metabolism and its regulation. Int J Mol Sci 22: 4591, 2021.
45. Chen X, Li J, Kang R, Klionsky DJ and Tang D: Ferroptosis: Machinery and regulation. Autophagy 17: 2054-2081, 2021.
46. Nemeth E and Ganz T: Hepcidin-Ferroportin interaction controls systemic iron homeostasis. Int J Mol Sci 22: 6493, 2021.
47. Li J, Liu J, Xu Y, Wu R, Chen X, Song X, Zeh H, Kang R, Klionsky DJ, Wang X and Tang D: Tumor heterogeneity in autophagy-dependent ferroptosis. Autophagy 17: 3361-3374, 2021.
48. Zhang Y, Khan S, Liu Y, Zhang R, Li H, Wu G, Tang Z, Xue M and Yong WV: Modes of brain cell death following intracerebral hemorrhage. Front Cell Neurosci 16: 799753, 2022.
49. Zhou SY, Cui GZ, Yan XL, Wang X, Qu Y, Guo ZN and Jin H: Mechanism of ferroptosis and its relationships with other types of programmed cell death: insights for potential interventions after intracerebral hemorrhage. Front Neurosci 14: 589042, 2020.
50. Cepelak I, Dodig S and Dodig DC: Ferroptosis: Regulated cell death. Arh Hig Rada Toksikol 71: 99-109, 2020.
51. Kerins MJ and Ooi A: The Roles of NRF2 in modulating cellular iron homeostasis. Antioxid Redox Signal 29: 1756-1773, 2018.

52. Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS and Stockwell BR: Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. Proc Natl Acad Sci USA 113: E4966-E4975, 2016.
53. Lee JY, Kim WK, Bae KH, Lee SC and Lee EW: Lipid metabolism and ferroptosis. Biology (Basel) 10: 184, 2021.
54. Wan J, Ren H and Wang J: Iron toxicity, lipid peroxidation and ferroptosis after intracerebral haemorrhage. Stroke Vasc Neurol 4: 92-95, 2019.
55. Yan HF, Tuo QZ, Yin QZ and Lei P: The pathological role of ferroptosis in ischemia/reperfusion-related injury. Zool Res 41: 220-230, 2020.
56. Magtanong L, Ko PJ and Dixon SJ: Emerging roles for lipids in non-apoptotic cell death. Cell Death Differ 23: 1099-1109, 2016.
57. Bian X, Liu R, Meng Y, Xing D, Xu D and Lu Z: Lipid metabolism and cancer. J Exp Med 218: e20201606, 2021.
58. Cao Y, Li Y, He C, Yan F, Li JR, Xu HZ, Zhuang JF, Zhou H, Peng YC, Fu XJ, *et al*: Selective ferroptosis inhibitor liproxstatin-1 attenuates neurological deficits and neuroinflammation after subarachnoid hemorrhage. Neurosci Bull 37: 535-549, 2021.
59. Zou Y and Schreiber SL: Progress in understanding ferroptosis and challenges in its targeting for therapeutic benefit. Cell Chem Biol 27: 463-471, 2020.
60. Karuppagounder SS, Alin L, Chen Y, Brand D, Bourassa MW, Dietrich K, Wilkinson CM, Nadeau CA, Kumar A, Perry S, *et al*: N-acetylcysteine targets 5 lipoxygenase-derived, toxic lipids and can synergize with prostaglandin E2 to inhibit ferroptosis and improve outcomes following hemorrhagic stroke in mice. Ann Neurol 84: 854-872, 2018.
61. Shah R, Shchepinov MS and Pratt DA: Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. ACS Cent Sci 4: 387-396, 2028.
62. Koppula P, Zhuang L and Gan B: Cystine transporter SLC7A11/xCT in cancer: Ferroptosis, nutrient dependency, and cancer therapy. Protein Cell 12: 599-620, 2021.
63. Zhang Z, Yao Z, Wang L, Ding H, Shao J, Chen A, Zhang F and Zheng S: Activation of ferritinophagy is required for the RNA-binding protein ELAVL1/HuR to regulate ferroptosis in hepatic stellate cells. Autophagy 14: 2083-2103, 2018.
64. Wenzel SE, Tyurina YY, Zhao J, St Croix CM, Dar HH, Mao G, Tyurin VA, Anthonymuthu TS, Kapralov AA, Amoscato AA, *et al*: PEBP1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. Cell 171: 628-641, e26, 2017.
65. Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, *et al*: ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. Nat Chem Biol 13: 91-98, 2017.
66. Kagan VE, Mao G, Qu F, Angeli JP, Doll S, Croix CS, Dar HH, Liu B, Tyurin VA, Ritov VB, *et al*: Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. Nat Chem Biol 13: 81-90, 2017.
67. Wang GX, Tu HC, Dong Y, Skanderup AJ, Wang Y, Takeda S, Ganesan YT, Han S, Liu H, Hsieh JJ and Cheng EH: DeltaNp63 inhibits oxidative stress-induced cell death, including ferroptosis, and cooperates with the BCL-2 family to promote clonogenic survival. Cell Rep 21: 2926-2939, 2017.
68. Anthonymuthu TS, Kenny EM, Lamade AM, Kagan VE and Bayir H: Oxidized phospholipid signaling in traumatic brain injury. Free Radic Biol Med 124: 493-503, 2018.
69. Li Q, Han X, Lan X, Gao Y, Wan J, Durham F, Cheng T, Yang J, Wang Z, Jiang C, *et al*: Inhibition of neuronal ferroptosis protects hemorrhagic brain. JCI Insight 2: e90777, 2017.
70. Venkatesh D, O'Brien NA, Zandkarimi F, Tong DR, Stokes ME, Dunn DE, Kengmana ES, Aron AT, Klein AM, Csuka JM, *et al*: MDM2 and MDMX promote ferroptosis by PPARα-mediated lipid remodeling. Genes Dev 34: 526-543, 2020.
71. Liu Y, Tavana O and Gu W: p53 modifications: Exquisite decorations of the powerful guardian. J Mol Cell Biol 11: 564-577, 2019.
72. Ho T, Tan BX and Lane D: How the other half lives: What p53 does when it is not being a transcription factor. Int J Mol Sci 21: 13, 2019.
73. Karni-Schmidt O, Lokshin M and Prives C: The Roles of MDM2 and MDMX in Cancer. Annu Rev Pathol 11: 617-644, 2016.
74. He F, Borcherds W, Song T, Wei X, Das M, Chen L, Daughdrill GW and Chen J: Interaction between p53 N terminus and core domain regulates specific and nonspecific DNA binding. Proc Natl Acad Sci USA 116: 8859-8868, 2019.
75. Levine AJ: The many faces of p53: Something for everyone. J Mol Cell Biol 11: 524-530, 2019.
76. Nagpal I annd Yuan ZM: The basally expressed p53-mediated homeostatic function. Front Cell Dev Biol 9: 775312, 2021.
77. Lou J, Hao Y, Lin K, Lyu Y, Chen M, Wang H, Zou D, Jiang X, Wang R, Jin D, *et al*: Circular RNA CDR1as disrupts the p53/MDM2 complex to inhibit Gliomagenesis. Mol Cancer 19: 138, 2020.
78. Gencel-Augusto J and Lozano G: p53 tetramerization: At the center of the dominant-negative effect of mutant p53. Genes Dev 34: 1128-1146, 2020.
79. Hamard PJ, Lukin DJ and Manfredi JJ: p53 basic C terminus regulates p53 functions through DNA binding modulation of subset of target genes. J Biol Chem 287: 22397-22407, 2012.
80. Laptenko O, Shiff I, Freed-Pastor W, Zupnick A, Mattia M, Freulich E, Shamir I, Kadouri N, Kahan T, Manfredi J, *et al*: The p53 C terminus controls site-specific DNA binding and promotes structural changes within the central DNA binding domain. Mol Cell 57: 1034-1046, 2015.
81. Xiong Y, Zhang Y, Xiong S and Williams-Villalobo AE: A glance of p53 functions in brain development, neural stem cells, and brain cancer. Biology (Basel) 9: 285, 2020.
82. Kastenhuber ER and Lowe SW: Putting p53 in Context. Cell 170: 1062-1078, 2017.
83. Li Y, Cao Y, Xiao J, Shang J, Tan Q, Ping F, Huang W, Wu F, Zhang H and Zhang X: Inhibitor of apoptosis-stimulating protein of p53 inhibits ferroptosis and alleviates intestinal ischemia/reperfusion-induced acute lung injury. Cell Death Differ 27: 2635-2650, 2020.
84. Zhao Y, Wu L, Yue X, Zhang C, Wang J, Li J, Sun X, Zhu Y, Feng Z and Hu W: A polymorphism in the tumor suppressor p53 affects aging and longevity in mouse models. Elife 7: e34701, 2018.
85. Gnanapradeepan K, Basu S, Barnoud T, Budina-Kolomets A, Kung CP and Murphy ME: The p53 tumor suppressor in the control of metabolism and ferroptosis. Front Endocrinol (Lausanne) 9: 124, 2018.
86. Ou Y, Wang SJ, Li D, Chu B and Gu W: Activation of SAT1 engages polyamine metabolism with p53-mediated ferroptotic responses. Proc Natl Acad Sci USA 113: E6806-E6812, 2016.
87. Chu B, Kon N, Chen D, Li T, Liu T, Jiang L, Song S, Tavana O and Gu W: ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway. Nat Cell Biol 21: 579-591, 2019.
88. Venkatesh D, Stockwell BR and Prives C: p21 can be a barrier to ferroptosis independent of p53. Aging (Albany NY) 12: 17800-17814, 2020.
89. Kenny EM, Fidan E, Yang Q, Anthonymuthu TS, New LA, Meyer EA, Wang H, Kochanek PM, Dixon CE, Kagan VE and Bayir H: Ferroptosis contributes to neuronal death and functional outcome after traumatic brain injury. Crit Care Med 47: 410-418, 2019.
90. Liu DS, Duong CP, Haupt S, Montgomery KG, House CM, Azar WJ, Pearson HB, Fisher OM, Read M, Guerra GR, *et al*: Inhibiting the system xC⁻/glutathione axis selectively targets cancers with mutant-p53 accumulation. Nat Commun 8: 14844, 2017.
91. Jiang L, Kon N, Li T., Wang SJ, Su T, Hibshoosh H, Baer R and Gu W: Ferroptosis as a p53-mediated activity during tumour suppression. Nature 520: 57-62, 2015.
92. Snyder NA and Silva GM: Deubiquitinating enzymes (DUBs): Regulation, homeostasis, and oxidative stress response. J Biol Chem 297: 101077, 2021.
93. Shen J, Sheng X, Chang Z, Wu Q, Wang S, Xuan Z, Li D, Wu Y, Shang Y, Kong X, *et al*: Iron metabolism regulates p53 signaling through direct heme-p53 interaction and modulation of p53 localization, stability, and function. Cell Rep 7: 180-193, 2014.
94. Chaudhary R and Lal A: Long noncoding RNAs in the p53 network. Wiley Interdiscip Rev RNA 8: 10.1002/wrna.1410, 2017.