# Role of ferroptosis in the occurrence and progression of acute ischemic stroke

Haoxiu Li[1], Meng Bi[1], Zhongqiang Cheng[1], Miao He[1], and Weirong Li[2]

[1]Shanxi Medical University
[2]Shanxi Cardiovascular Hospital

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**Abstract**

Ferroptosis is a new non-apoptotic form of regulatory cell death, which is characterized by intracellular iron overload and excessive accumulation of lipid peroxides and reactive oxygen species (ROS). Ferroptosis is closely related to intracellular iron, amino acid, and lipid metabolism disorders. Ferroptosis is increasingly recognized as an important process mediating the pathogenesis and progression of acute ischemic stroke, and it can be involved in influencing acute ischemic stroke and acute ischemic stroke risk factors atherosclerosis, atrial fibrillation, hypertension, diabetes mellitus, and obstructive sleep apnea. Therefore, understanding the mechanisms of ferroptosis regulation in different diseases may have significant implications for the preventive treatment and improvement of prognosis in patients with acute ischemic stroke and patients with risk factors for acute ischemic stroke. This article reviews not only the specific important mechanisms of ferroptosis in the development of acute ischemic stroke, but also the relevant associations between risk factors for acute ischemic stroke and ferroptosis, and describes the current limitations and future directions of ferroptosis in the pathogenesis of acute ischemic stroke and its risk factors.

**Role of ferroptosis in the occurrence and progression of acute ischemic stroke**

**Haoxiu Li [1] [+],Meng Bi[1+],Zhongqiang Cheng[1],Miao He[1],Weirong Li[2*]**

[1]Department of Neurology, Taiyuan Central Hospital, Ninth Clinical College of Shanxi Medical University, Taiyuan, Shanxi, China

[2]Department of Neurology, Shanxi Cardiovascular Disease Hospital, Taiyuan, Shanxi, China

***Correspondence:** Weirong Li*weironglee@163.com*

**[+] Correspondence:**

**Haoxiu Li [1+]and Meng Bi [1+]contributed equally to this work and share first authorship.**

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factors for acute ischemic stroke and ferroptosis, and describes the current limitations and future directions of ferroptosis in the pathogenesis of acute ischemic stroke and its risk factors.

**Keywords:** Ferroptosis, Acute ischemic stroke, Atherosclerosis, Atrial fibrillation,Hypertension.

## Introduction

Acute ischemic stroke (AIS) is a disease caused by the insufficient blood supply to the brain resulting in neuronal necrosis and brain dysfunction, and is one of the leading causes of death and disability, affecting millions of people worldwide[1]. Ferroptosis is a new form of non-apoptotic cell death first proposed by Dixon in 2012, which is characterized by intracellular iron overload and excessive accumulation of lipid peroxides and reactive oxygen species (ROS), and its occurrence is related to the three major metabolic disorders of intracellular iron, amino acid, and lipid[2,3]. In recent years, more and more studies have shown that ferroptosis is not only involved in the development of AIS but also in the development of AIS risk factors such as atherosclerosis, atrial fibrillation, hypertension, diabetes mellitus, obstructive sleep apnea, and so on[4-9]. The use of ferroptosis inhibitors such as ferrostatin-1 and deferoxamine can not only prevent neuronal cell death and reduce secondary brain injury after stroke to improve the prognosis of patients but also slow the pathological progression of AIS risk factors[8,10-14], which suggests that regulating ferroptosis may become a new method for the prevention and treatment of AIS. This article mainly reviews the research progress of the mechanism of ferroptosis in AIS and its risk factor-related diseases, and by understanding the specific mechanism of ferroptosis in stroke and its risk factor-related diseases, it is expected to provide a reference for the prevention and treatment of acute ischemic stroke by targeting ferroptosis, to significantly improve the prognosis and survival of patients.

## 2. Ferroptosis and acute ischemic stroke

### 2.1 Disorder of iron metabolism leads to ferroptosis

Iron is an important participant and regulator in the normal physiological activity of the brain and is abundant in brain tissue[15,16]. Physiologically, the brain is protected from whole-body iron fluctuations due to the protection provided by the blood-brain barrier (BBB) [17]. Under normal circumstances, BBB endothelial cells through the transferrin receptor - transferrin (TFR - TF) compounds mediated endocytosis of inactive iron intake ($Fe^{3+}$)[18], then through the Six-transmembrane epithelial antigen of prostate 3 (STEAP3) convert $Fe^{3+}$ to free iron ($Fe^{2+}$). $Fe^{2+}$ is transported from endosomes by divalent metal transporter 1 (DMT1) and exported to the extracellular space of the brain by active transport or exocytosis mediated by ferroportin (FPN)[19,20], and can also be stored in ferritin or the labile iron pool (LIP)[21], which is involved in lipid ROS production. Studies have shown that BBB capillary endothelial cells can store and release iron in a regulated manner, thus acting as an iron reservoir for the brain[22]. Under acute ischemic conditions, microglia are overactivated by ischemic stimuli, resulting in disruption of the BBB[23], and free iron and ferritin enter the brain parenchyma when the BBB is disrupted. Studies have shown that the expressions of TFR1 and DMT1 can be significantly increased, and the expression level of FPN can be decreased in the middle cerebral artery ischemia model of MCAO rats[24,25], indicating that the ability of intracellular iron uptake is enhanced and the ability of iron efflux is weakened after cerebral ischemia, which will eventually lead to intracellular iron overload. In addition, in the MCAO rat model, the assembly of cytoplasmic NCOA4 protein in neurons is significantly increased to recognize ferritin and transport it to lysosome for degradation, resulting in a significant increase in the concentration of free iron in neurons[26]. Knockdown of the *NCOA4* gene can reduce the uptake of ferritin, avoid the accumulation of free iron and eliminate the accumulation of reactive oxygen species, thereby interfering with ferroptosis [27]. This suggests that NCOA4-mediated ferritin autophagy is one of the additional key mechanisms mediating ferroptosis in AIS. Therefore, any form of induced iron transport imbalance, autophagic degradation of ferritin, and impairment of the blood-brain barrier will lead to iron overload in the brain. Excessive intracellular $Fe^{2+}$ can not only generate ROS through the Fenton reaction but also participate in the synthesis of lipoxygenase and then catalyze lipid peroxidation[28,29]. Lethal accumulation of intracellular lipid peroxides as well as reactive oxygen species promotes nuclear, protein, and membrane damage that ultimately mediates cell death. Studies

have shown that the increase of intracellular free iron is positively correlated with the degree of neuronal damage after ischemic stroke[30], which directly affects the recovery of cerebral nerve function. In the early stages of ischemia-reperfusion, iron overload increases the risk of bleeding transformation after early tPA administration, accelerates ischemia-induced elevation of serum matrix metalloproteinase-9, and enhances lipid peroxidation, thereby increasing the likelihood of adverse outcomes[31]. A clinical trial reports that the use of the iron-chelating agent deferoxamine to reduce systemic iron levels within 1-3 days of ischemic stroke may be beneficial for short-term outcomes in patients with acute ischemic stroke[10]. It has also been proposed that the use of ferroptosis inhibitors liproxstatin-1 and ferrostatin-1 can reduce neurological dysfunction and cerebral infarct size in MCAO mice[32]. Therefore, rational application of ferroptosis inhibitors such as deferoxamine to reduce the iron content in the brain after AIS can reduce neuronal death and promote the recovery of neurological function after ischemic stroke.

### 2.2 Disorder of amino acid metabolism leads to ferroptosis

System Xc- is a cystine/glutamic acid anti-transporter, which is composed of light chain SLC7A11 and heavy chain SLC3A2, and is an important intracellular antioxidant element. System Xc- exchanges glutamate and cystine inside and outside the cell in a 1:1 ratio[33]. Extracellular cystine is introduced into cells via SLC7A11, the light chain of System Xc-. The cystine that enters the cells is reduced to cysteine, and glutamic acid and glycine are added to produce glutathione (GSH) under the catalyst of cysteine ligase (GCL) and glutathione synthase (GSS), respectively. GSH is an important antioxidant, which can enhance the anti-lipid peroxidation activity of glutathione peroxidase 4 (GPX4). GPX4 can catalyze reduced GSH into oxidized glutathione (GSSG). Normally, reduced GSH accounts for the majority, and GPX4 takes GSH as the substrate to react with lipid peroxides to play an antioxidant role. Ultimately, it reduces the occurrence of lipid peroxidation and ferroptosis and protects cells from oxidative damage[34].

After acute ischemic stroke, excess extracellular glutamate inhibits the activity of System Xc- by restricting cystine absorption, thereby affecting GSH synthesis[35]. In addition, acute ischemia and hypoxia can increase the expression of BACH1, ATF3, and *p53* , and these factors can reduce the uptake of cystine by neurons by inhibiting the expression of System Xc- light chain SLC7A11, leading to a decrease in GSH synthesis[36-38]. Other studies have suggested that GPX4 protein expression and activity decreased after acute ischemic stroke [39]. The abnormal function of these specific factors can lead to the accumulation of lipid peroxides in cells and eventually ferroptosis. The above results suggest that ferroptosis of cells caused by amino acid metabolism disorder after AIS is manifested in the following three aspects: (1) inhibition of System Xc-; (2) decreased glutathione synthesis; (3) The expression and activity of GPX4 were decreased. This suggests that ferroptosis can be inhibited by intervening in the above process, and related studies have used some drugs to intervene in the above process to inhibit the occurrence of ferroptosis. Some studies have shown that galangin and Naotaifang extracts can inhibit ferroptosis and reduce neuronal cell death by enhancing the expression of SLC7A11 and GPX4[24,40]. Edaravone is an antioxidant protective agent that effectively inhibits ferroptosis caused by decreased GSH content caused by cystine deficiency[41]. Furthermore, in the rat ischemic stroke model, it was found that edaravone treatment reduced $Fe^{2+}$, MDA, and LPO in the brain tissue, increased GSH content, and up-regulated GPX4 expression, suggesting that edaravone could inhibit ferroptosis and attenuate cerebral ischemia-reperfusion injury[25]. Edaravone is currently used in the treatment of acute ischemic stroke. Research shows that post-tMCAO selenium treatment significantly reduces cerebral infarct volume, oxidative stress, and ferroptosis and enhances post-tMCAO motor performance in the acute phase after stroke[42]. Carvacrol can inhibit ferroptosis by enhancing GPX4 expression, thereby rescuing hippocampal neuronal injury induced by reperfusion [43]. Icariin has been shown to have a neuroprotective effect against acute ischemic stroke[44]. In a study of the action and mechanism of icariin as a synovitis therapeutic agent, it was found that icariin enhances cell survival in lipopolysaccharide-induced synoviocytes by suppressing ferroptosis via the Xc-/GPX4 axis[45]. However, there is still a lack of relevant studies on the effect of icariin on anti-ferroptosis to improve the prognosis of AIS. Therefore, regulating amino acid metabolism and GPX4 expression can reduce neuronal ferroptosis and promote brain injury recovery after AIS. In the future, more sensitive drugs can be developed to inhibit ferroptosis according to the specific mechanism of amino acid metabolism disorders, to improve the treatment and prognosis of AIS.

### 2.3 Dysregulation of lipid metabolism leads to ferroptosis

Neuronal cells are prone to ferroptosis caused by iron-dependent lipid ROS accumulation, mainly because the brain is rich in polyunsaturated fatty acids (PUFAs), which are susceptible to lipoxygenases and reactive oxygen species (ROS) to generate lipid peroxides[46]. PUFAs, especially arachidonic acid (AA) and adrenic acid (ADA) are highly susceptible to oxidation and subsequently lead to the accumulation of lipid peroxides (LOOH) and ROS [47]. Acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase-3 (LPCAT3), as promoters of the esterification of AA and ADA into PE, play an important role in the formation of AA/ADA-PE[48,49]. AA/AdA-PE is converted to the harmful PE-AA-OOH or PE-ADA-OOH by lipoxygenase (LOXs) through enzymatic or non-enzymatic lipid peroxidation, resulting in the formation of large amounts of lipid peroxides, which leads to the destruction of membrane integrity and ferroptosis [50,51].

ACSL4 is widely expressed in brain tissue, and as a potential target of miR-347 after cerebral ischemia, it is upregulated with over-expression of miR-347 after cerebral ischemia in mice, inducing neuronal death[52]. Some studies have proposed that upregulation of thrombin after acute cerebral ischemia reperfusion can promote arachidonic acid mobilization by not affecting intracellular Fe2+ level, and then catalyzed esterification by ACSL4 stimulates ferroptosis signal transduction, leading to ferroptosis of neuronal cells. The results of this study strongly suggest that antithrombin therapy may be beneficial to post-stroke reperfusion by inhibiting ferroptosis[4]. It is worth further study. ACSL4 also promotes the production of pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-6(IL-6), and interleukin-1β (IL-1β) by microglia, which further exacerbates brain injury by promoting the inflammatory response after AIS[53]. It was found that ACSL4 expression was increased in a mouse model of ischemic stroke, and inhibition of ACSL4 with rosiglitazone significantly improved neurological function and reduced infarct volume 72 h after stroke[39]. These studies suggest that ACSL4 may be a novel regulator of neuronal death and neuroinflammation, and intervention of ACSL4 expression may be a potential therapeutic target for ischemic stroke. However, there are still few related studies in this field, so more studies should be conducted in the future to reveal the specific mechanism of action of ACSL4 in the occurrence of ferroptosis in patients with ischemic stroke.

LOXs are key enzymes in the induction of ferroptosis[54]. There are several subtypes of LOXs, of which 12/15-LOX is a particular subtype. The study found that after focal ischemia, 12/15-LOX was increased in neurons and endothelial cells. Overexpression of 12/15-LOX leads to neuronal death in the brain and disruption of the blood-cerebrospinal fluid barrier, and the use of 12/15-LOX inhibitors can improve neurological function and reduce cerebral edema[55]. And research has found that 12/15-LOX is up-regulated following global cerebral ischemia, and contributes to neuronal injury. Either gene knockout or treatment with the 12/15-LOX inhibitor LOXBlock-1 reduce injury and improve neurological outcome[56]. These findings suggest that increased expression of 12/15-LOX is involved in neuronal cell death after cerebral ischemia, and its inhibitor may be a novel therapeutic approach for alleviating cerebral ischemia injury. However, whether 12/15-LOX is involved in post-AIS neuronal cell death mainly by mediating ferroptosis remains unclear and deserves further study.

**(Figure 1)**

## 3. Risk factors associated with ferroptosis and acute ischemic stroke

### 3.1 Ferroptosis and atherosclerosis

Atherosclerosis (AS) is the most common cause of acute ischemic stroke, which is related to the TOAST classification of large artery atherosclerosis. Therefore, a more precise understanding of the pathological mechanism of the occurrence and progression of AS may benefit patients in preventing AIS. Studies have found that ferroptosis is closely related to the occurrence of AS and participates in various pathophysiological processes of AS[57].

#### 3.1.1 Ferroptosis is involved in endothelial dysfunction in AS

An important mechanism by which ferroptosis occurs is iron overload. Studies have found that chronic iron

overload can exacerbate the atherosclerotic process, which is associated with oxidative stress and vascular endothelial dysfunction [58,59]. A clinical trial has reported that iron levels increase in carotid atherosclerotic lesions compared to normal healthy human endothelium and are further elevated in advanced AS, inducing oxidative stress and inflammatory responses[60]. Induced oxidative stress is mainly manifested by excessively stored iron leading to peroxidative damage in the inner wall of the artery by increasing the production of oxygen free radicals, which further aggravates AS. Inflammatory response induction mainly shows that with the progression of the disease, long-term iron overload leads to the increase and dominance of M1 macrophages, which can secrete pro-inflammatory factor TNF-α, etc[61]. Blood vessels infiltrated by long-term inflammatory factors are prone to the aggregation and oxidation of LDL, promoting the formation of foam cells and the deposition of atherosclerotic plaques. In turn, it promotes the development of AS and ferroptosis of macrophages in plaques[62]. Administration of ferroptosis inhibitor ferrostatin-1 significantly ameliorated ROS-induced lipid peroxidation and endothelial dysfunction, thereby attenuating atherosclerotic lesions[11]. A recent study demonstrated for the first time that ionizing radiation can induce ferroptosis in endothelial cells through NCOA4-mediated ferritin autophagy to promote plaque progression in AS. At the same time, this study also proposed that the knockdown of NCOA4 has a therapeutic effect in alleviating ionizing radiation-induced ferroptosis in endothelial cells[63]. The above studies suggest that ferroptosis is involved in endothelial dysfunction and plays an important role in the occurrence and development of AS.

### 3.1.2 Ferroptosis is involved in AS foam cell formation and death and promotes plaque formation

HIF-1α, an upregulated DEG between atherosclerosis and normal, co-regulates autophagy and ferroptosis. HIF-1α inhibitor PX-478 attenuates foam cell formation and lessens atherosclerosis by enhancing autophagy and depressing ferroptosis in macrophages[64]. Nuclear factor NF-E2-related factor (Nrf2) has been considered as a major regulator of antioxidant in previous studies, which can mediate the transcriptional regulation of multiple target genes and play a key role in regulating ferroptosis. It was found that by inducing a macrophage autophagy defect model, the Nrf2-mediated antioxidant defense was turned off, while the negative effects of Nrf2 manipulation were initiated, leading to iron deposition and lipid peroxidation, and finally ferroptosis of foam cells[65]. It has also been shown that a high level of uric acid-induced iron death in macrophages is involved in the formation of atherosclerotic plaques. More importantly, inhibition of ferroptosis by activation of Nrf2 may attenuate atherosclerosis induced by high levels of uric acid[5]. In conclusion, ferroptosis is not only involved in inducing the formation and death of foam cells in AS but also in promoting the formation of AS plaques. At the same time, the regulation of the Nrf2 pathway may be an important mechanism of ferroptosis resistance in AS.

### 3.1.3 Ferroptosis is involved in inducing plaque progression in AS

Plaque rupture, intra-plaque bleeding, and thrombosis are also important causes of AS progression. In the process of plaque formation in the late stage of human carotid artery atherosclerosis, oxygen consumption in the blood vessel wall increases and plaque lesions undergo hypoxia, which stimulates angiogenesis and the resulting blood vessels are highly permeable and brittle[66]. Moreover, advanced plaques contain a large number of macrophages with pro-inflammatory phenotypes that secrete stroma-degrading enzymes, leading to plaque instability, plaque rupture, intra-plaque bleeding, and thrombotic events[67].

GPX4 is an important antioxidant and plays an anti-ferroptosis role in the body. The severity of atherosclerosis was negatively correlated with GPX4 expression[68]. Overexpression of GPX4 inhibits the development of atherosclerosis by decreasing lipid peroxidation and inhibiting the sensitivity of vascular cells to oxidized lipids[69]. By up-regulating GPX4 expression and enhancing GPX4 catalytic activity, vascular smooth muscle cell proliferation can be inhibited and arterial protection can be enhanced[70]. A single dose of selenium delivered to the brain promotes the expression of the antioxidant GPX4, protects neurons, and inhibits plaque growth in AS[71]. It was found that Qing-Xin-Jie-Yu Granule partially inhibited ferroptosis in vulnerable AS plaques through GPX4/ Xc- signaling pathway and weakened AS progression[72]. Thus, upregulation of GPX4 expression can slow plaque progression in AS by inhibiting ferroptosis.

Long non-coding RNAs (lncRNAs) have been associated with atherosclerosis (AS), and lncRNA PVT1 was found to be significantly upregulated in serum of patients with AS. In vitro experiments using human vascular endothelial cells (HUVECs) showed that knockdown of PVT1 and miR-106b-5p overexpression inhibited increases in iron content, MDA levels, lipid ROS, ACSL4, and PTGS2, as well as decreases in GSH and GPX4 in oxygen-LDL-induced HUVECs. The study also found that PVT1 knockdown decreased lipid deposition, atherosclerotic plaque number and size in ApoE mice. These results suggest that PVT1 plays a key role in the progression of AS by regulating the miR-106b-5p/ACSL4 axis in HUVECs, and therefore may be a potential therapeutic target for AS[73].

#### 3.1.4 Possible ferroptosis biomarkers for AS

Prostaglandin peroxidase synthase 2 (PTGS2) gene encodes cyclic oxygenase-2 (COX-2)[74]. PTGS2 has been proposed as a potential ferroptosis-related biomarker for ischemic stroke[75]. The pathological analysis of human coronary arteries showed that the late ferroptosis-related proteins PTGS2 and ACSL4 were up-regulated, while GPX4 was down-regulated. The severity of atherosclerosis was positively correlated with the expression of PTGS2 and ACSL4, and negatively correlated with the expression of GPX4. This study further confirmed that cellular ferroptosis can regulate the development and progression of atherosclerosis. The study also suggests that *PTGS2* may be a central gene in atherosclerosis[68]. Then, by constructing a protein-protein interaction (PPI) network and histological verification of related genes, five AS-related ferroptosis hub genes ( *TP53* ,*MAPK1* , *STAT3* , *HMOX1* , and *PTGS2* ) were finally identified. This study further demonstrated that *PTGS2* may be a key hub gene for AS and its expressed protein may serve as a biomarker of atherosclerosis severity[76]. These findings may provide new ideas and potential targets for the prevention and treatment of atherosclerosis. Atherosclerosis eventually leads to clot deposits and narrowing of blood vessels, which reduces blood flow to the brain and restricts oxygen supply, eventually leading to hypoxia-ischemia and AIS. Therefore, it is necessary to understand the specific mechanism of ferroptosis in AS, so AS to provide thinking for the treatment of AS and prevention of AIS by targeting ferroptosis. However, research on ferroptosis and AS is still in its infancy. More studies will reveal the specific molecular mechanism of ferroptosis and thus provide more evidence for ferroptosis prevention and treatment of AS. **(Figure 2)**

### 3.2 Ferroptosis and atrial fibrillation

Atrial fibrillation (AF) is an important risk factor for AIS, resulting in a five-fold increased risk of stroke and two-fold increased mortality[77]. Atrial fibrillation (AF) is also one of the most common preventable causes of AIS and is often associated with cardiogenic stroke. Compared with other ischemic stroke subtypes, cardiogenic stroke is associated with more severe severity, poorer prognosis, and a relatively higher recurrence rate[78]. Therefore, understanding the pathogenesis of atrial fibrillation and selecting appropriate drugs based on the pathological mechanism may greatly help the treatment and prognosis of patients with cardiogenic stroke.

#### 3.2.1 Ferroptosis is involved in the occurrence of AF

Iron overload and oxidative stress are essential pathological processes in ferroptosis. Studies have confirmed that iron overload and oxidative stress affect cardiac pathophysiology and can lead to AF[79,80]. Nomani et al. found that AF was more common in beta-thalassemia patients than in the normal population, for whom iron overload of the heart and iron-dependent oxidative stress is one of the most important causes of atrial fibrillation[81].

Iron overload is related to AF occurrence. It was found that AF was induced by chronic iron overload[79]. FPN mediates iron efflux, an important protein that regulates iron and calcium homeostasis in cells. After knocking out FPN, rats' intracellular iron concentration and oxidative stress were further increased, and AF was more likely to be induced[12].

Oxidative stress is associated with AF occurrence. In myocardial tissue, elevated ROS levels are associated with AF. Elevated ROS levels lead to protein, lipid, and DNA damage, and are also involved in cardiac

structural and electrical remodeling, increasing susceptibility to AF[82,83]. Connexin distribution and protein level changes were associated with enhanced AF susceptibility[84]. Studies have found that ROS may induce AF by decreasing the expression of connexin 40 and connexin 43[85].

Given the above basis, it has been confirmed that ferroptosis occurs in AF. This study pointed out that cardiac fibroblasts promote the ferroptosis of cardiomyocytes by secreting exosomes, and reduce the loss of cardiomyocytes and oxidative stress damage by interfering with exosome miRNA, which can prevent the continuous development of AF[6].

#### 3.2.2 Different AF risk factors affect AF susceptibility by regulating ferroptosis

Alcohol consumption is one of the common risk factors for AF. A study on the effect of ferroptosis on the susceptibility to AF at different drinking frequencies indicated that frequent excessive drinking would activate ferroptosis and increase the induction rate of AF. Inhibition of ferroptosis could balance the iron overload disorder and reduce the production of reactive oxygen species (ROS), ultimately reducing the susceptibility to AF[86]. Another study to explore the pharmacological effects of icariin on ethanol-induced atrial remodeling found that excessive ethanol use resulted in severe atrial damage, as indicated by increased susceptibility to AF, altered atrial conduction patterns, enhanced atrial enlargement and fibrosis markers, and up-regulation of ferroptosis related proteins (PTGS2, ACSL4, P53). The expression of anti-ferroptosis-related molecules (GPX4, FTH1) was down-regulated, and these deleterious effects were reversed by treatment with ferrostatin-1 or icariin[87].

Obesity is an important risk factor for AF and intestinal microbiota imbalance plays an important role in the pathogenesis of obesity-related AF, which can increase LPS and cause atrial pathological remodeling by activating ferroptosis and inflammasome signaling pathways. Inhibition of ferroptosis or inflammasome signaling significantly ameliorated atrial fibrosis and reduced susceptibility to obesity-related intestinal dysbiosis-induced AF[88].

Sepsis is a risk factor for new-onset AF, and ferroptosis is involved in the development of sepsis-induced organ damage. In a recent study, researchers generated an LPS-induced endotoxemia model to understand the mechanism of the link between sepsis-induced ferroptosis and atrial fibrillation. Selective knockdown of Fpn using gene transfection technology was found to increase the intracellular iron concentration and oxidative stress and increase the susceptibility of LPS-induced endotoxemia rats to AF[12]. **(Figure 3)**

The above studies have shown that ferroptosis plays an important role in the pathogenesis of atrial fibrillation susceptibility, and intervention of ferroptosis can reduce the susceptibility and progression of AF. However, there is still a lack of relevant research on the specific mechanism of AF and ferroptosis. Clinically, whether the use of ferroptosis inhibitors can reduce the incidence of cardiac stroke and improve the prognosis of some patients who are susceptible to AF or who have already suffered from AF requires further exploration and research by more scholars.

### 3.3 Ferroptosis and hypertension

Hypertension is one of the risk factors for cardiovascular and cerebrovascular diseases such as atherosclerosis and ischemic stroke. Its pathophysiological mechanism is mainly related to increased vascular resistance, manifested by endothelial dysfunction, increased vasoconstriction, and arterial remodeling. In recent years, more and more studies have found that ferroptosis is closely related to the occurrence and development of hypertension[7,89]. Therefore, understanding the specific relationship between ferroptosis and hypertension will be of great benefit to the treatment of hypertension and the prevention of cerebrovascular disease complications caused by hypertension.

#### 3.3.1 Ferroptosis is involved in the pathophysiology of hypertension

Existing studies have found that ferroptosis is involved in endothelial cell dysfunction, which can lead to changes in vasomotor function and cause hypertension[11]. Vascular smooth muscle cells (VSMCs), as one of the main cellular components of the vascular wall, can maintain the tension of the vascular wall through a slow

and mild contraction. The phenotypic transformation of VSMCs is closely related to the pathophysiological process of hypertension. Recent studies have found that ferroptosis can induce the transition of VSMCs from contractile phenotype to synthetic phenotype[90]. This suggests that ferroptosis is related to the pathogenesis of hypertension.

Many studies have shown that ROS plays an important role in vascular remodeling and endothelial dysfunction associated with hypertension. Increased ROS can promote endothelial dysfunction, and accelerate VSMCs proliferation and vascular remodeling, leading to vascular injury, increased peripheral resistance, and elevated blood pressure[91,92]. An important feature of ferroptosis is an increase in intracellular ROS levels. A recent in vitro study found that VSMC undergoes ferroptosis under high hydrostatic pressure, accompanied by iron accumulation, increased ROS production, and lipid peroxidation[7].In addition, it has been proposed that lenvatinib can induce ferroptosis in endothelial cells, which subsequently leads to vascular dysfunction and hypertension[13]. The above studies have proved that ferroptosis is involved in the pathophysiology of hypertension.

### 3.3.2 Possible ferroptosis biomarkers in Hypertension

It has been mentioned above that PTGS2 may be a potential biomarker of iron death in AIS and AS, and PTGS2 encodes COX-2. COX-2 has been implicated in increased vascular hardness and extracellular matrix deposition, increased vasoconstriction response, endothelial dysfunction, and vascular inflammation. Reduction of COX-2 can reduce vasoconstriction response and endothelial dysfunction, and reduce the increase in vascular hardness in hypertensive animals[93]. It was found that high hydrostatic pressure induced iron death in VSMC, accompanied by up-regulation of COX-2[7]. Reducing COX-2 expression can improve endothelial function and slow the progression of hypertension[94]. In conclusion, it is suggested that COX-2 may be a biomarker of ferroptosis in hypertension, which can be proved by more studies in the future.

### 3.3.3 Antiferroptosis may be a new target for hypertension treatment in the future

Nrf2 can activate the transcription of target antioxidant genes and play a key role in the oxidation reduction reaction regulation of hypertension[95]. Studies have found that inhibiting Nrf2 can promote oxidative stress and inflammation, and further aggravate hypertension in mice[96]. On the contrary, it promoted Nrf2 signaling pathway transduction and alleviated renal ferroptosis and lipid peroxidation in hypertensive state[97]. It was also found that activation of Nrf2 inhibited the progression of hypertension in hypertensive mice 12-14 days after infusion of Ang II[98] . At present, studies on Nrf2 in the development and progression of hypertension are limited. Whether Nrf2 can protect hypertension by regulating related mechanisms to inhibit ferroptosis needs more research to determine.

It was also found that ferrostatin-1 administration could reverse the ferroptosis of VSMCs induced by high hydrostatic pressure[7], while also significantly ameliorating hypertension and endothelial ferroptosis induced by lenvatinib in mice[13] . This suggests that ferrostatin-1 may alleviate hypertension by inhibiting ferroptosis of VSMCs and endothelial cells, but it has not been applied in the clinic so far, and more in-depth and comprehensive research exploration of ferrostatin-1 is needed to ensure its safety and efficacy.

Elabela (ELA) is a novel endogenous ligand of the Apelin receptor (APJ) that regulates oxidative stress and plays a protective role in cardiovascular and cerebrovascular diseases. Zhang et al[89]. found that ELA could significantly inhibit the up-regulation of iron levels and lipid peroxidation in Ang II-induced hypertensive mice. Further studies found that ELA inhibited cardiac microvascular endothelial cell ferroptosis by regulating the IL-6/STAT3/GPX4 signaling pathway. It has also been shown that ELA binds to APJ and alleviates neuronal ferroptosis after ischemic stroke by activating antioxidant signaling pathways[99]. The above studies suggest that ELA inhibition of the ferroptosis pathway has a certain potential in the treatment of hypertension and cardiovascular and cerebrovascular diseases, which needs further clinical and basic experiments to verify.

### 3.3.4 Iron overload in the brain caused by hypertension leads to ferroptosis of neuronal cells

Increased iron reserves in the body are associated with adverse outcomes and symptomatic hypertension,

and iron overload may offset the beneficial effects of thrombolytic therapy[100]. Yang et al[101]. found that elevated iron content, increased lipid peroxide content, and changes in indicators related to ferroptosis were found in the brain tissue of hypertensive rats compared with normotensive rats, suggesting that hypertension may lead to brain iron overload, and iron overload increases lipid peroxidation, thereby inducing ferroptosis in neurons. This suggests that iron homeostasis plays an important role in the development of hypertension. It has been established that hypertension is the most common risk factor associated with stroke because it puts pressure on the blood vessels of the brain, resulting in hemodynamic changes that worsen clinical stroke outcomes[102,103]. However, how hypertension leads to iron overload in the brain and what is the specific mechanism of ferroptosis in hypertensive brain injury need further study and discussion. As mentioned above, ferroptosis may play a key role in the pathogenesis of hypertension and related brain damage, and inhibition of ferroptosis may be a potential therapeutic target for the prevention and treatment of hypertension and hypertensive heart and brain damage. However, whether ferroptosis plays a key role in the impact of hypertension on stroke severity and clinical outcome still needs further study.

### 3.4 Ferroptosis and diabetes mellitus

Diabetes mellitus(DM) is a disease caused by insulin secretion deficiency or insulin dysfunction, which leads to metabolic abnormalities with chronic hyperglycemia as the prominent manifestation. Diabetes mellitus is also another important risk factor for AIS and increases the mortality and stroke recurrence rate associated with it[104,105].

#### 3.4.1 Φερροπτοσις μαψ μεδιατε ισλετ β-ςελλ δψσφυνςτιον

Islet beta cell dysfunction plays an important role in the occurrence and development of diabetes. It has been proposed that pancreatic β-cells have low expression levels of the antioxidant enzymes superoxide dismutase (SOD), glutathione (GSH) peroxidase, and catalase[106], which suggests that they are susceptible to oxidative stress and thus ferroptosis. Killion et al[107]. observed small atrophy of mitochondria, increased membrane density, and disappearance of mitochondrial crista in pancreatic islet β cells of T2DM mice, and iron deposition in or near the center of the islet, which was consistent with typical changes of ferroptosis, further suggesting that ferroptosis may mediate the dysfunction of pancreatic islet β cells.When the iron homeostasis in pancreatic β cells is destroyed, a large amount of iron accumulates in the cells. Excessive free reactive Fe2+ can produce lipid peroxides through catalyzing lipid peroxidation, and form ROS through the Fenton reaction. Due to the lack of antioxidant enzymes expressed by pancreatic β cells themselves, ROS and lipid peroxides cannot be eliminated in time, and a large accumulation may cause ferroptosis. Make the islet cell differentiation disorder and even death, resulting in reduced insulin secretion. In vitro studies, erastin, an iron death inducer, was associated with significantly reduced insulin secretion and impaired islet function. Pretreatment with ferroptosis inhibitor ferrostatin-1 or DFO rescued the above injury[8]. These findings suggest that ferroptosis may mediate islet β-cell dysfunction.

#### 3.4.2 Ferroptosis may mediate insulin resistance

ACSL4 is another important factor in the occurrence of ferroptosis, and its increase will enhance lipid peroxidation and promote the production of more lipid peroxides, finally leading to the ferroptosis of cells[108]. Up-regulation of ACSL4 expression was observed in mice fed a high-fat diet(HD). In the same study, adipocyte-specific ACSL4 knockout mice were found to be protected from HD-induced cell death and insulin resistance[109]. Another experiment showed that ACSL4 protein is present in human and rat islet β cells, concentrated around insulin secretory granules and mitochondria, and participates in insulin secretion by modifying fatty acids in insulin secretory granules and mitochondria[110]. These findings suggest that ACSL4 may promote insulin secretion in beta cells, but it may exacerbate peripheral insulin resistance. Therefore, more experiments on the function of ACSL4 are needed to explore whether ACSL4 mediates ferroptosis and plays an important role in the pathogenesis of DM and whether targeting ACSL4 can prevent and treat DM.

#### 3.4.3 Ferroptosis induced by hyperglycemia plays an important role in the progression of diabetes and its complications

Pancreatic β-cell dysfunction and insulin resistance can lead to hyperglycemia(HG), which can increase MDA levels in cells, reduce GPX4 activity[111], promote ferroptosis, and form a vicious cycle. This vicious cycle accelerates the process of diabetes and its complications. Previous studies have identified endothelial dysfunction as one of the most important factors in vascular complications of diabetes[112]. Hyperglycemia-induced oxidative stress and increased ROS production play an important role in the development of endothelial dysfunction. It has been proposed that HG induces ferroptosis in human umbilical vein endothelial cells (HUVECs), accompanied by a significant increase in p53 in HUVECs, and p53 siRNA and the use of ferroptosis inhibitors can attenuate HG-induced ferroptosis in HUVECs[113]. And studies show that in the condition of diabetes, endothelial p53 expression raised obviously, and endothelial-dependent vasodilation significant damage[114]. In an in vitro experiment mimicking diabetic brain ischemic injury, Meg3 expression was found to be increased, which could mediate p53 to cause ferroptosis by regulating GPX4 transcription and expression. Knockdown of p53 protected rat brain microvascular endothelial cells from ferroptosis induced by OGD + hyperglycemic reperfusion, whereas overexpression of p53 produced the opposite effect[115]. A recent study showed that HG intervention after MCAO aggravated neurological deficits, infarct size, oxidative stress, iron accumulation, and BBB damage in a hemorrhagic transformation model. Inhibition of P53 signaling attenuated ferroptosis in the endothelium and reduced HG-induced hemorrhagic transformation after MCAO[116]. In conclusion, p53 may mediate ferroptosis and play an important role in the progression of diabetes and its complications, and inhibition of the p53 signaling pathway may be a new therapeutic target for diabetes and its complications. Together, these findings suggest that ferroptosis plays an important role in the onset and progression of diabetes and its complications, and therefore, the treatment and prevention of ferroptosis is a very promising target for diabetes and its complications.

### 3.5 Ferroptosis and obstructive sleep apnea

Obstructive sleep apnea (OSA) has been associated with acute ischemic stroke, hypertension, and atrial fibrillation[117] . OSA is considered an independent risk factor for recurrent stroke[118] . Its main pathophysiological feature is chronic intermittent hypoxia (CIH)[9].

Intermittent hypoxia(IH) can induce ROS increase, lead to GSH depletion through endoplasmic reticulum stress and affect GPX4 activity[119]. Other studies have found that the levels of Nrf2-ARE path-related antioxidant enzyme GPX4 in the peripheral blood of patients with moderate to severe OSA are significantly reduced, affecting the antioxidant capacity of the central nervous system, resulting in an imbalance between oxidation and antioxidant, and ultimately leading to neuron damage and signal transduction abnormalities[120]. Other studies have confirmed that Nrf2 plays a protective role in the process of ferroptosis induced by IH[9,121]. This suggests that IH may affect the expression of the antioxidant protein in the Nrf2-ARE pathway, resulting in decreased expression of GPX4 and GSH in brain tissue, decreased antioxidant capacity, increased expression of ROS and MDA, and lipid peroxidation. In addition, elevated extracellular glutamate levels were found in the brains of OSA patients[122,123]. The abnormal increase of extracellular glutamate level may lead to the dysfunction of System Xc- transport, further inhibit the synthesis of GSH, affect the ability to resist lipid peroxidation and promote ferroptosis. Therefore, it can be inferred that ferroptosis may play an important role in OSA-induced brain injury, and the mechanism may be related to lipid peroxidation, abnormal increase of glutamate level, and Nrf2 regulation disorder. However, there is still a lack of research on OSA-mediated ferroptosis to promote the occurrence and progression of AIS, which still has great potential.

## 4. Conclusion and prospect

The occurrence of cerebrovascular events is the result of many factors. It is closely associated with various diseases, such as atherosclerosis, atrial fibrillation, diabetes mellitus, hypertension, obstructive sleep apnea, and so on. Comorbidities are the hallmark of stroke, which can both increase the incidence of stroke and worsen the outcome. More and more studies have shown that ferroptosis is not only involved in the occurrence and progression of AIS but also involved in the chronic pathogenesis of AIS risk factors.

This article mainly reviews the mechanism of ferroptosis in the occurrence and development of acute ischemic stroke. By introducing the relationship between ferroptosis and AS, AF, HF, DM, OSA, and the specific

mechanism of ferroptosis after acute ischemic stroke, it is further explained that ferroptosis plays a very important role in the occurrence and development of AIS. At the same time, the following thoughts are also triggered: (1) Although this paper describes the correlation between ferroptosis and AIS risk factors, the research on the mechanism of ferroptosis and AIS risk factors is limited, and the specific mechanism of ferroptosis is not clear, which is worthy of further research by scientists. (2) Smoking, alcohol consumption, and hyperlipidemia are also common risk factors for AIS. In future studies, we can establish an acute ischemic stroke model in mice with AIS risk factors, study the specific mechanism of neuronal ferroptosis, and find targeted drugs and inhibitors to observe the impact on the prognosis and treatment of AIS. (3) At least two or more types of programmed cell death may occur in the occurrence and development of AIS. Therefore, only targeting ferroptosis may not achieve the desired effect, and in the future, multiple targets combined with cell death methods such as apoptosis, autophagy, and necrosis can effectively treat the disease. (4) So far, there is no clinical trial of inhibitors of ferroptosis for the treatment of ischemic stroke. This field needs further exploration and more population-based data are needed to determine whether the prognosis of AIS patients can be improved by inhibiting ferroptosis. With the deepening of research, inhibition of ferroptosis is likely to become an effective strategy for the treatment of ischemic stroke.

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**Figure 1** Mechanism and regulation of ferroptosis in acute ischemic stroke

Related regulatory mechanisms of iron metabolism: damage of the blood-brain barrier, imbalance of iron transport, and NCOA4-mediated ferritin autophagy can lead to iron overload and induce the Fenton reaction to produce a large number of ROS. Lipid metabolism related regulatory mechanisms: The increased expression of acyl-CoA synthetase long-chain family member 4 (ACSL4) and lipoxygenases (LOXs) induced lipid peroxidation. Amino acid metabolism related regulatory mechanisms: The expression of solute carrier family 7 member 11(SLC7A11) was inhibited and the synthesis of glutathione (GSH) was restricted. The decreased content of glutathione peroxidase 4 (GPX4) eventually led to excessive accumulation of lipid peroxide. Polyunsaturated fatty acids (PUFAs); prostaglandin-endoperoxide synthase 2 (PTGS2); cyclooxygenase-2 (COX2); arachidonic acid (AA); adrenic acid (AdA); lyso-phosphatidylcholine acyltransferase-3 (LPCAT3); lipid peroxide (L-OOH); Phospholipids-H (L-OH); solute carrier family 3 member A2 (SLC3A2); glutathione-cysteine ligase (GCL); glutathione synthase (GSS); oxidized glutathione (GSSH); transferrin (TF); transferrin receptor1 (TFR1); Divalent metal transporter 1 (DMT1); Six-transmembrane epithelial antigen of prostate 3 (STEAP3); labile iron pool (LIP); ferroportin (FPN)

**Figure 2** Atherosclerosis associated ferroptosis hub gene

*TP53* , *MAPK1* , *STAT3* , *HMOX1* and *PTGS2*may be the key hub genes of AS-associated ferroptosis.

**Figure 3** Atrial fibrillation risk factors regulate ferroptosis and affect atrial fibrillation susceptibility

Alcohol consumption, obesity, and sepsis are risk factors for atrial fibrillation, which can participate in the regulation of ferroptosis and affect the susceptibility to atrial fibrillation.