Potential intervention target of atherosclerosis: Ferroptosis (Review)

JIA LI, LING XU, YI XUAN ZUO, XUE QIN CHANG and HAI TAO CHI

Department of Neurology, Xinhua Hospital Affiliated to Dalian University, Dalian, Liaoning 116021, P.R. China

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Abstract. Atherosclerosis (AS) is a chronic inflammatory disease of the blood vessels, which is mainly characterized by the form of atherosclerotic plaques and vascular endothelial injury. Its formation involves abnormal lipid metabolism, oxidative stress and inflammation, as well as other processes. AS is the direct cause of various acute cardiovascular and cerebrovascular diseases, such as acute myocardial infarction and acute ischemic stroke. Early intervention in the atherosclerotic inflammatory process and lesion progression is beneficial, and has been associated with the primary prevention of a range of related diseases. Ferroptosis is a non-apoptotic form of cell death different from cell necrosis and autophagy, which has been shown to participate in atherogenesis and atherosclerotic progression through numerous signaling pathways. The main characteristic of ferroptosis is the formation of high levels of cellular iron catalytic free radicals, unsaturated fatty acid accumulation and iron-induced lipid reactive oxygen species accumulation, which can cause oxidative stress, and subsequent DNA, protein and lipid damage. There are numerous hypotheses about the pathogenesis of AS. At present, it has been suggested that ferroptosis can accelerate the progression of AS and that inflammation is associated with the whole process of AS. The mechanisms and signaling pathways related to the involvement of neuroinflammation and ferroptosis in the progression of AS, and therapeutic targets associated with ferroptosis have not yet been elucidated. The present review article evaluated the involvement of ferroptosis in the progression of AS from the perspectives of ferroptotic cell death, the pathogenesis of AS and nervous system inflammation, with the aim of exploring new therapeutic targets for AS.

Correspondence to: Professor Hai Tao Chi, Department of Neurology, Xinhua Hospital Affiliated to Dalian University, 156 Wansui Street, Shahekou, Dalian, Liaoning 116021, P.R. China
E-mail: 723290269@qq.com

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Contents

1. Introduction

Ferroptosis is an iron-dependent lipid reactive oxygen species (ROS)-induced form of non-apoptotic cell death, first proposed by Dixon *et al* in 2012 (1). This previous study revealed that the Ras-selective lethal molecule erastin can affect glutathione (GSH) peroxidase (GPX) activity, leading to redox imbalance, ROS accumulation, membrane lipid peroxidation and ultimately to the destruction of the integrity of the cell membrane (1). Ferroptosis induces morphological changes that manifest as smaller mitochondria, increased mitochondrial membrane density, decreased or lack of mitochondrial cristae, and outer membrane breaks (2). The main characteristic of ferroptosis is redox imbalance and the whole ferroptosis process, which involves iron metabolism, lipid metabolism, oxidative stress mechanisms, and the biosynthesis of nicotinamide adenine dinucleotide phosphate, GSH and coenzyme Q10 (3), participates in the development and progression of atherosclerosis (AS) (4). AS is characterized by the accumulation of lipids in the arteries and the formation of plaques. As the disease progresses, the blood vessels narrow, and resulting in blood flow restriction or even blockage (5). In 1999, Ross (6) proposed that AS is a chronic and progressive inflammatory disease, the mechanism of which involves endothelial cell dysfunction and lipid accumulation, and that AS is an important pathological cause of acute cardiovascular and cerebrovascular diseases.

Epidemiological studies have reported that the incidence rate of cardiovascular and cerebrovascular diseases caused by AS has increased year on year, and that it has become the primary cause of death in China and also worldwide (7). Notably, >75% of disabling and fatal cardiovascular and cerebrovascular diseases in China are caused by AS (8). Intracranial atherosclerotic stenosis (ICAS) is an important

cause of ischemic stroke. A previous study on symptomatic intracranial arterial stenosis and occlusion in China reported that the incidence of intracranial AS in patients with ischemic stroke or transient ischemic attack was 46.6% (9), among which patients with ICAS had more severe symptoms, longer hospital stays and higher stroke recurrence rates; moreover, the recurrence rate increased with stenosis severity. Worldwide, ~2 billion individuals have carotid AS and the incidence of AS is higher among male individuals than female individuals (7). AS is closely related to blood pressure, blood sugar and blood lipid levels, smoking and obesity among other risk factors, which over a period of 20-30 years can gradually lead to vascular stenosis, causing a range of vascular diseases.

AS is an inflammatory lesion caused by numerous risk factors that damage vascular endothelial cells and is mainly characterized by disordered lipid metabolism. It is well known that lipid peroxidation, namely lipid oxidation degradation, generates lipid peroxide radicals and hydrogen peroxide that serve crucial roles in AS by causing inflammation and endothelial dysfunction, whereas lipid peroxidation is also a core feature of ferroptosis (10). Therefore, ferroptosis may be a key factor in the occurrence and development of AS. Ferroptosis is associated with various stages of AS development through numerous physiological mechanisms, such as iron ion metabolism, lipid metabolism and amino acid metabolism (11). Atherosclerotic stenosis is the root cause of vascular-related diseases, such as coronary heart disease, stroke and peripheral vascular disease. In particular, the stability of neck AS plaques has been reported to be closely related to the occurrence of ischemic stroke (12). According to global data (13,14), stroke currently remains the second most common cause of mortality and disability, with ischemic stroke accounting for 62.4% of all new stroke cases in 2019, thus placing a heavy burden on society and the families of patients. A previous meta-analysis on the subtypes of ischemic stroke demonstrated that ischemic stroke due to large artery atheromatous sclerosis accounted for 23% of cases and that the occurrence of cardiogenic stroke was also closely related to major AS in the Asian population (15). Therefore, a more precise understanding of the causes and mechanisms of AS progression may be beneficial for the treatment of patients.

2. Iron and ferroptosis

Regulation of iron homeostasis. Iron is the most abundant transition element in the brain; it participates in the oxidation reaction, myelination, neurotransmitter synthesis and metabolism, and it serves a key role in cell respiration and energy generation (16). The body ingests iron through the diet, and intestinal epithelial cells absorb iron and transport it to the blood (17). In addition, macrophages phagocytose aged red blood cells, which increases the iron content in the blood (18), which has been reported to be an independent predictor of vascular damage (19). Extracellular Fe^{3+} is transported into cells by transferrin receptor 1 (TfR1) and is converted into Fe^{2+}, which participates in the synthesis of heme in mitochondria (20) or is exported from cells by ferroportin; iron is also stored in monocyte macrophages in the form of ferritin, which helps to regulate iron storage and balance in the body (Fig. 1). The intracellular iron content is in a dynamic equilibrium and reduction of the iron content in the body is protective (21). Nuclear receptor coactivator 4 (NCOA4) can recognize ferritin and promote its autophagic degradation, leading to the release of free iron (22). Therefore, knocking out the NCOA4 gene can block ferritin deposition and avoid the accumulation of free iron, thus protecting neurons from ferroptosis. Iron is transported from the cytosol to the mitochondria by mitochondrial ferritin (FTMT) and is used to synthesis iron porphyrin to reduce free iron levels and protect mitochondria from oxidative stress. Moreover, the upregulation of FTMT can increase iron consumption and inhibit ferroptosis (23). It has been reported that cyanidin-3-glucoside can inhibit the occurrence of ferroptosis, affecting ischemia-reperfusion injury in the myocardium by alleviating oxidative stress, reducing the free iron content and downregulating TfR1 expression in cells and tissues (24).

The imbalance of iron metabolism mediates the occurrence of ferroptosis, and promotes the formation and development of AS. Initially, a toxic iron reaction is associated with an excess of stored iron (25), which can accelerate cerebral tissue oxidation by increasing oxygen radical generation (26). This leads to super-oxidation damage in the inner wall of the cerebral artery, further aggravating the iron overload through the oxidized low-density lipoprotein (ox-LDL)-mediated Toll-like receptor 4 (TLR4)/nuclear factor κB (NF-κB) signaling pathway in macrophages (27). Moreover, the occurrence of ferroptosis activates the TLR4/NF-κB signaling pathway and can increase the expression of pro-inflammatory cytokine genes (28). When intracellular free iron levels increase, the intracellular labile iron pool also increases (3). Excess divalent iron may be involved in the Fenton reaction and other lipid peroxidation processes that induce ROS overproduction and may be involved in oxidative stress, with ROS promoting lipid peroxidation and inducing ferroptosis (29). ROS mainly include oxygen molecules, hydroxyl radicals, superoxide anions and hydrogen peroxide radicals. Furthermore, iron is a potent oxidant, which through the Haber-Weiss reaction catalyzed by a large number of ROS, promotes the intracellular lipid peroxidation reaction. The intracellular lipid peroxidation reaction causes damage to proteins and nucleic acids, promotes macrophage apoptosis and leads to the release of numerous components of the cell contents; these substances can further promote the infiltration of macrophages and enhance the lipid peroxidation reaction (30). LDL-C passes through damaged endothelial cells and enters macrophages through oxidation, resulting in the formation of foam cells. The aggregation of foam cells becomes the lipid core of atherosclerotic plaques, which aggravates cell damage and lipid peroxidation, increasing the production of ROS and lipid peroxide products, such as MDA (31). In addition, activated macrophages releases inflammatory factors, such as TNF-α, IL-6 and IL-1β, increase the inflammatory response, mediate the oxidation of lipoprotein, and further accelerate the occurrence of AS.

Lipid metabolism pathway. Lipid peroxidation is the core process of ferroptosis; specifically, the peroxidation of cell membrane phospholipids by free radical-driven arachidonate lipoxygenases (ALOXs) (32). Concurrently, iron is associated with various stages of lipid peroxide generation, including iron-catalyzed lipid oxidation and esterification, the oxidation

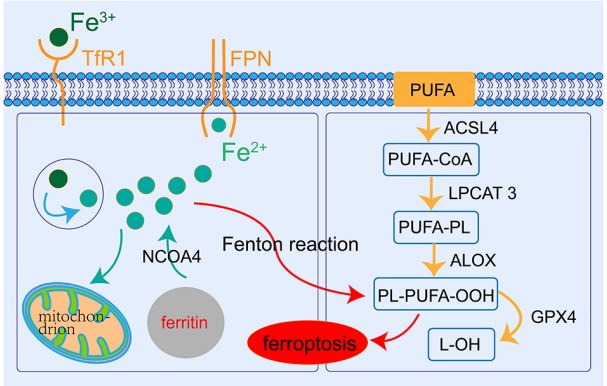

Figure 1. Molecular mechanisms of ferroptosis. Extracellular Fe^{3+} is transported to the cells byTfR1. The Fe^{3+} is reduced to Fe^{2+} to play a role in mitochondria or for storage in the form of ferritin, and the remainder is exported from the cell through ferroportin to maintain iron homeostasis. The reaction of PUFAs and excess reactive oxygen species, which occurs on the cell membrane, is catalyzed by ACSL4 and LPCAT3, and mediated by ALOX, which leads to lipid peroxidation. GPX4 can reduce ferroptosis by inhibiting lipid peroxidation. PUFAs, n-3 polyunsaturated fatty acids; ACSL4, acyl-CoA synthetase long chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; ALOX, arachidonate lipoxygenase; PL, phospholipid; GPX4, glutathione peroxidase 4; NCOA4, nuclear receptor coactivator 4; TfR1, transferrin receptor 1.

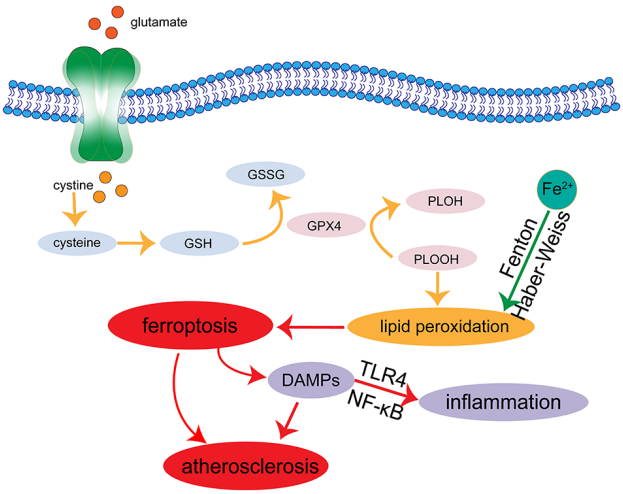

Figure 2. Ferroptosis and atherosclerosis. Intracellular free iron generates hydroxyl radicals through the Fenton reaction and participates in the synthesis of lipoxygenase to generate lipid peroxides. System x_c^- functions as a cystine/glutamate transporter that imports a cystine molecule in exchange for an intracellular glutamate molecule. Cystine is converted to GSH through a series of reactions and GPX4 suppresses ferroptosis by reducing lipid peroxides to their lipid alcohol forms using GSH. When this process is inhibited, ferroptosis occurs, resulting in the release of DAMPs, the expression of pro-inflammatory factors and inflammation, which ultimately lead to the formation and exacerbation of atherosclerosis. GSH, glutathione; GPX4, GSH peroxidase 4; DAMPs, danger-associated molecular patterns; GSSG, oxidized glutathione.

of polyunsaturated fatty acids and lipid ROS generation via the Fenton reaction. Mitochondria are the main organelles where iron utilization occurs in catabolic and anabolic pathways, and they serve an important role in iron metabolism, as well as material and energy metabolism (33). The Fenton reaction and other peroxidation processes that incorporate iron (Fig. 2) can convert the mitochondrial oxidation respiration product, hydrogen peroxide, into hydroxyl radicals through the catalysis of ferrous ions. During the oxidative phosphorylation of the electron transport chain, which takes place on the inner mitochondrial membrane, electrons leak from the complex and oxygen forms ROS through a series of redox processes (34). Excessive ROS generation can cause DNA damage, protein degeneration, lipid peroxidation and can induce ferroptotic cell death. However, ROS derived from mitochondria can also activate NLRP3 inflammatory bodies and lead to the activation of the iron death signaling pathway (35). Therefore, as a mitochondrially derived antioxidant, the free radical scavenger MitoQ can reduce ROS production by inhibiting mitochondrial respiration and enhancing glycolytic function, thus protecting the mitochondria and preventing GPX4-dependent ferroptosis (36). XJB-5-131 has been reported to have a dual antioxidant effect, which can scavenge free radicals by targeting mitochondria and inhibit DNA damage, providing protection from ischemia-reperfusion-induced kidney injury and inflammation in mice (37). Recently published research has focused on the ROS produced by mitochondria, which may promote the occurrence of ferroptosis by enhancing lipid peroxidation, heme degradation and free iron overload, promoting ferroptosis through HMOX-1 in the mitochondria (2). However, this process involves the participation of multiple signaling pathways, and these factors involved may have cross or offset effects, and the clear participating mechanism requires further assessment in animal experiments. Mitochondria-targeting antioxidants have been reported to be effective in animal experiments (38); they can attenuate kidney injury and promote tubular epithelial cells repair after ischemia/reperfusion injury, and the overexpression of mitochondrial ferritin has been shown to inhibit ferroptosis by promoting the storage of mitochondrial iron.

GPX4 is a GPX that catalyzes the reduction of hydrogen peroxide, organic hydroperoxide and lipid hydroperoxides, thereby protecting cells from oxidative damage and ferroptosis (39). As a ferroptosis inducer, Ras-selective lethal small molecule 3 directly binds and inhibits GPX4 activity, and can cause lipid ROS accumulation, mitochondrial damage, disruption of ATP production, lipid peroxide accumulation in cells and the promotion of ferroptosis (40). Acyl-CoA synthetase long chain family member 4 (ACSL4) converts free arachidonic acid into arachidonic arachidonoyl-CoA (41) and promotes unsaturated phospholipid production, the main substrate for lipid peroxidation (42). GPX4 can reduce lipid peroxide, and following GPX4 inhibition (43), ACSL4 is considered to be required for the occurrence of ferroptosis. Furthermore, microRNA-17-92 can protect endothelial cells from ferroptosis in AS by mediating the A20-ACSL4 axis (44). Doll *et al* (45) reported that knockdown of the ACLS4 gene significantly inhibited ferroptotic cell death, thus suggesting that ACLS4 may participate in the ferroptosis cascade via the lipid oxidation pathway. ACSL4 may also aggravate neuronal damage through neuronal ferroptosis and promote the release of pro-inflammatory cytokines from microglia (46). Baicalin has been reported to inhibit ROS production, reduce ACSL4 expression, and mediate iron uptake and autophagic degradation of ferritin to reduce intracellular iron levels, which may participate in the prevention of myocardial ischemia/reperfusion injury through anti-ferroptotic mechanisms (47).

Ebselen (Ebs) is a small molecule organo-selenium compound, which simulates GPX activity. As a lipid-soluble compound, Ebs can easily enter the cell through the cell membrane to exert antioxidant effects (48). Ebs can also inhibit the activity of ALOX5 and ALOX15, and the synthesis of leukotriene; inhibition of lipoxygenase and factors such as leukotriene are important for preventing AS and inhibiting inflammation. It has been reported that promoting the phosphorylation of AKT can increase the expression of endothelial nitric oxide synthase in vascular endothelial cells, increase nitric oxide release, and protect against myocardial ischemia and reperfusion injury, while also reducing oxidative stress and protecting the myocardial mitochondria (49).

Amino acid metabolism pathway. Under normal physiological conditions (50), extracellular cystine is imported into cells in exchange for intracellular glutamate via the cystine/glutamate antiporter system x_c^-, maintaining glutamate balance inside and outside the cell. Intracellular cystine is converted into cysteine by cysteine reductase, and GSH is generated by glutamate-cysteine ligase and GSH synthetase. With antioxidants, GPX4 prevents ferroptosis by reducing lipid peroxides to the alcohol form using GSH (Fig. 2). Furthermore, intracellular cysteine deficiency caused by cysteine uptake disorder can lead to the depletion of the antioxidant peptide, GSH, which is composed of glutamate, cysteine and glycine, thus also leading to GPX4 inactivation and peroxide accumulation at lethal levels (Fig. 2). The depletion of GSH also leads to glutamate-mediated iron and ROS generation, and triggers oxidative cell death; specifically, ferroptosis through the amino acid metabolic pathway (51). GSH levels have been reported to be reduced in a mouse model of middle cerebral artery occlusion where the infarct size was reduced following intervention with the ferroptosis-related inhibitors, liproxstatin-1 and ferrostatin-1 (46). Carvacrol has also been reported to reduce the level of lipid peroxide in the ischemic brain tissue of gerbils by increasing the expression of GPX4, inhibiting ferroptosis, reducing cell death, and conserving the memory and learning ability of gerbils following ischemia-reperfusion (52).

It has been reported that the deposition of iron at atherosclerotic plaques leads to the accumulation of ROS and the death of macrophages; therefore, the loss of the antioxidant capacity of macrophages directly leads to ferroptosis and plaque formation (3). GSH, as a tripeptide antioxidant and a cofactor of GPX4,is a key substrate for GPX4, which can reduce the production of lipid peroxide. The cell defense against lipid peroxidation is decreased due to the depletion of GSH; however, cells do not reduce the amount of ROS produced by Fenton reactions and other iron peroxidation reactions in response to GSH depletion and therefore they are more sensitive to ferroptosis (53). Erastin functions as a ferroptosis inducer (54) that restrains the activity of the x_c^- system, inhibiting cystine uptake, which means that the cysteine in the cells cannot be used for GSH synthesis. Intracellular reduced-GSH and oxidized GSH depletion (55), the accumulation of peroxidized phospholipids, the accumulation of lipid ROS and protein or membrane damage can all trigger ferroptotic cell death. GPX4 is a peroxide inhibitor protein discovered in 1982, which belongs to the seleno-proteins, that produces water or alcohol and protects cells by catalyzing certain reducing reactions of hydrogen peroxide; therefore, a single dose of selenium delivered to the brain can promote antioxidant GPX4 expression, protect neurons and inhibit plaque growth in AS (56). Impaired GPX4 activity caused by GPX4 deficiency or GSH depletion can lead to the inactivation of T cells and promote ferroptosis; however, it can also promote the differentiation of peripheral blood monocytes into B-cells and natural killer cells (51).

Solute carrier family 7 member 11 (SLC7A11, also known as xCT) is a key component of the cystine/glutamate antiporter system x_c^-, which transports extracellular cystine into cells in exchange for intracellular glutamate, maintaining the glutamate balance inside and outside the cell. The inhibition of cysteine-dependent GSH synthesis via SLC7A11 leads to the inactivation of GPX4 and ultimately causes ferroptosis in cells SLC7A11 has been reported as a well-validated target for the prevention of ferroptotic cell death. Furthermore, nuclear factor erythroid 2-related factor 2 (Nrf2) regulates the occurrence of ferroptosis at the transcriptional level (57). Nrf2 regulates phagocytosis following oxidative stress in macrophages by inhibiting cellular iron uptake, reducing ROS production and upregulating SLC7A11 expression, as demonstrated by a high Nrf2 expression in astrocytes, preventing neuronal cell death (58). Therefore, Nrf2 also functions as a key regulator of lipid peroxidation and ferroptosis. Kaempferol has been reported to protect cells from ferroptosis via activation of the Nrf2/SLC7A11/GPX4 signaling pathway (59).

Nrf2 is a transcription factor, whose activation promotes iron storage, reduces cellular iron uptake and limits ROS production (55). Nrf2 is one of the key regulators of the oxidative stress pathway (60), which negatively regulates ferroptosis. Under normal physiological conditions, Kelch-like ECH-associated protein 1 binds to Nrf2 via its C-terminal Kelch domain and Nrf2 expression remains low through the ubiquitination of the proteasome (61). When oxidative stress occurs, Nrf2 dissociates from KEAP1 and undergoes nuclear translocation, where it recognizes antioxidant response sites and activates antioxidant genes, including heme oxygenase-1 (HO-1) and SLC7A11 (62). The expression of SLC7A11, as a target of Nrf2, is upregulated when Nrf2 is activated, protecting neuronal cells against ferroptosis, and high Nrf2 expression in astrocytes protects against neuronal cell death (63). HO-1 is a stress-inducing enzyme encoded by the Hmox1 gene. The activation of Nrf2 can initiate the downstream signal of HO-1, thus preventing oxidative stress and scavenging free radicals (64). The knockout of Nrf2 can significantly reduce the protein expression levels of SLC7A11 and HO-1, and can promote the accumulation of lipid peroxides, trigger ferroptosis through iron overload, excess ROS generation and lipid peroxidation, and aggravate the progression of AS. Furthermore, transglutaminase 2 can lead to neuronal death during stroke by inducing GSH depletion, thereby promoting ROS accumulation and ferroptosis (65).

3. Ferroptosis is involved in the formation and progression of AS

Formation of AS. The pathogenesis of AS involves endothelial cell dysfunction, lipid accumulation, foam cell formation, vascular smooth muscle cell (SMC) migration

and inflammatory factor infiltration. The occurrence of AS begins with abnormal lipid metabolism and a large amount of LDL being deposited on the intima of the vascular wall of endothelial cells where it is oxidized to ox-LDL during the process of oxidative stress (10). Free iron accelerates this modification process through the action of hydroxyl free radicals. Monocytes adhere to the vascular endothelium and are transferred to the subendothelium through chemotaxis to transform into macrophages (66). Macrophages serve a pivotal role in AS progression, recognizing and destroying endothelial cells. Macrophages phagocytize ox-LDL through protease and oxygen free radicals secreted by scavenger receptors and are then converted into foam cells, and excessive lipid deposition forms lipid streaks that progress into lipid-containing plaques; iron deposition is visible in the plaque formed by apolipoprotein E-deficient mice and can be seen by staining analysis or imaging (67). SMCs then migrate to the inner membrane and proliferate, forming a fibrous cap of plaque, which is the fibrous plaque phase of AS. Moreover, ox-LDL triggers the immune response, damaged endothelial cells are activated and express monocyte chemoattractant proteins (MCP)-1 and -8, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin and other inflammatory factors (68). These factors attract lymphocytes and monocytes to bind to endothelial cells and infiltrate the arterial wall, which causes inflammation. For macrophages, high mobility group protein B1 (HMGB1) is considered necessary for the macrophage inflammatory response, as the ferroptosis inducer erastin promotes the release of damage-associated molecular patterns and HMGB1 (69). The plaque formation process promotes the cooperation of iron and lipid accumulation in macrophages, which lead to the development of AS (70). Moreover, AS plaques with macrophage-derived foam cells as the main components are less stable and more prone to rupture (71).

Immune responses are present throughout the development of AS and inflammation relies on mediators, such as immune cells and inflammatory factors, which are involved in AS development through signaling pathways, such as TLR4, NF-κB and JAK/STAT (72). As well as innate immune cells, such as macrophages, adaptive immune cells participate in the formation of AS by exerting pro-inflammatory or anti-inflammatory effects through the secretion of various cytokines or antibodies (73). Th1 cells promote the development of AS by the secretion of IFN-γ, TNF-α and IL-2. Th2 cells regulate the progression of AS inflammation by the secretion of the anti-inflammatory factor IL-13 and the pro-inflammatory factor IL-4. IL-13 stimulates macrophage polarization to the M2 isoform, releases IL-10 and TGF-β, and functions against AS development through the activation of STAT3; however, IL-4 increases CD36 expression, thereby enhancing macrophage phagocytosis and promoting AS progression (74). Th17 accelerates the progression of AS and Th17 cells produce IL-17, IL-22 and IL-23, recruit neutrophils and promote inflammation at the site of infection (75). Tregs inhibit the activity of multiple immune cells, exerting anti-AS effects by secreting TGF-β1 and IL-10 (76).

Effects of ferroptosis on AS. AS is mainly caused by vascular endothelial dysfunction and plaques that form on blood vessel walls. Atherosclerotic plaque progression is characterized by ox-LDL accumulation within macrophages, macrophage death, necrotic core formation, the rupture or shedding of unstable plaques, and entry into the secondary lesion stage of AS. The majority of acute ischemic events, such as acute coronary syndromes and stroke, are attributed to vulnerable plaques (77). Typically, the core characteristics of vulnerable plaques are active inflammation; in addition, vulnerable plaques are more prone to plaque rupture, and they include the following characteristics (78): i) A thin fibrous cap, large lipid core and micro-calcifications; ii) intraplaque hemorrhage; iii) fiber cap rupture or ulcer formation; and iv) numerous infiltrating macrophages and neo-angiogenesis. The stability of atherosclerotic plaques is closely related to the size of the lipid core, the thickness of the fiber cap and the number of inflammatory cells within the plaque (77). Generally, macrophage death is considered to be a major factor in necrotic core formation and plaque instability, and ferroptosis is mainly involved in oxidative stress and the breakdown of endothelial function (79). Macrophages secrete matrix metalloproteases that degrade collagen fibers in the plaque extracellular matrix, causing plaque rupture, hemorrhage and thrombosis, the release of peroxides and nitrogen radicals, and cause the death of surrounding cells (72). The lipid accumulation process produces a large number of oxygen radicals involved in the inflammatory reaction, promoting the formation of AS, while releasing cytokines and proteases to degrade the collagen matrix of the fiber cap, prompting the plaque to become brittle and rupture, affecting its stability (80). A previous study showed that GPX4 was highly expressed in the early stage of plaque formation. With the progression of plaques, the expression levels of NLRP3 and caspase-1 were increased, and in the late stage, the expression of NLRP3 was significantly upregulated and the expression of GPX4 was decreased. These vulnerable plaques are closely related to a large number of acute ischemic events, including acute coronary syndrome and ischemic stroke (77).

As well as the accumulation of LDL, numerous other factors, such as high uric acid and high homocysteine levels are considered independent risk factors for AS progression. High uric acid levels cause vascular endothelial cell dysfunction by inhibiting the protein expression of endothelial nitric oxide synthase; furthermore, high uric acid levels stimulate monocytes to produce IL-1, IL-6 and TNF-α, which is supported by homocysteine (HCY) via JAK2/STAT3 signaling. Reducing HCY-enhanced STAT3 phosphorylation can significantly reduce HCY-induced microglial activation, and IL-6 and TNF-α production (81). Moreover, endothelial cells generate chemokines and adhesion molecules, promote the migration and adhesion of SMCs, aggravate vascular stenosis and plaque instability, promote vascular calcification, promote the precipitation of uric acid crystallization, increase blood viscosity, induce 5-HT release, increase platelet number and induce internal platelet activation; thus promoting thrombosis. High HCY levels damage endothelial cells, further exacerbating oxidative injury and inflammatory processes, increasing fibrinogen production, abnormal coagulation and platelet dysfunction (82). Platelets release arachidonic acid to produce ROS, resulting in calcium and lipid deposits in the endothelium, which reduces the elasticity of the arterial wall and

also causes oxidative stress by affecting cellular respiration, resulting in LDL oxidation. In addition, vascular endothelial cells are damaged, and the normal proportion of endothelin-1 and NO secreted by them to maintain the vasomotor function is broken; the impaired bioavailability of NO, and subsequent vasoconstriction and proliferation of smooth muscle can lead to the progression of AS (83).

Furthermore, gut dysbiosis exacerbates the progression of AS by regulating intestinal structure, intestinal barrier integrity, the inflammatory status and host metabolism (84). It has been reported that the inflammatory response caused by remote infection can aggravate plaque development or cause plaque rupture, and that the metabolism of cholesterol and lipids by intestinal microorganisms can affect the development of atherosclerotic plaques (85). Notably, ferroptosis may also be involved in this process. Chapkin *et al* observed that n-3 polyunsaturated fatty acids (PUFAs) and the short-chain fatty acid, butyrate, induced apoptosis via a mitochondrially-targeted antioxidant, mitoQ, which is a ubiquinone derivative, and overexpression of GPX4. This mirrored the phenomenon that fatty acid metabolism by the gut microbiota occurs by disrupting the energy metabolism balance of mitochondria, eliciting abnormalities in intracellular Ca^{2+} homeostasis systems and releasing ROS, which induce cell ferroptosis (86). Similar reports, such as that by Hayase and Jenq (87) reported that the gut flora metabolite, trimethylamine N-oxide (TMAO), enhanced M1 macrophage polarization through inflammasome formation and the activation of NLRP3 in endothelial cells. Furthermore, TMAO has been reported to cause the overexpression of TNF-α, IL-6 and C-reactive proteins, directly inducing vascular inflammation and endothelial dysfunction (88). This process mainly promotes the occurrence and development of AS through the NF-κB pathway. Concurrently, TMAO can increase platelet hyperreactivity and promote arterial thrombosis through Toll receptor signaling pathways (89). Therefore, it could be concluded that AS risk factors break the intestinal ecological balance and that gut microbial metabolites promote AS progression with the involvement of ferroptosis and inflammation.

Following ischemic stroke, the NLRP3 inflammasome has been reported to promote neuroinflammation, and to trigger the apoptosis of glial cells and neurons. NLRP3 activates caspase-1, triggers the release of inflammatory mediators, such as the cytokines IL-18 and IL-1, exacerbates oxidative stress and endoplasmic reticulum stress, and promotes brain edema and atherosclerotic processes (90).

Ferroptosis is involved in AS progression. The progression of AS is often inevitable and is generally considered to occur due to the accumulation of oxidative lipids in the intima, activation of the inflammatory process and the presence of two types of macrophages in its plaque (91). In the early stages, M2 macrophages are the main type of infiltrated cells, the plaque is relatively stable and the anti-inflammatory M2 macrophages produce IL-10 and IL-13, which inactivate Th1 lymphocytes, reduce inflammation and promote tissue damage repair (92). As the disease progresses, long-term iron overload causes the number of M1 macrophages to increase and dominate, further promoting the development of AS; M1 macrophages have a potent phagocytic activity and secrete proinflammatory

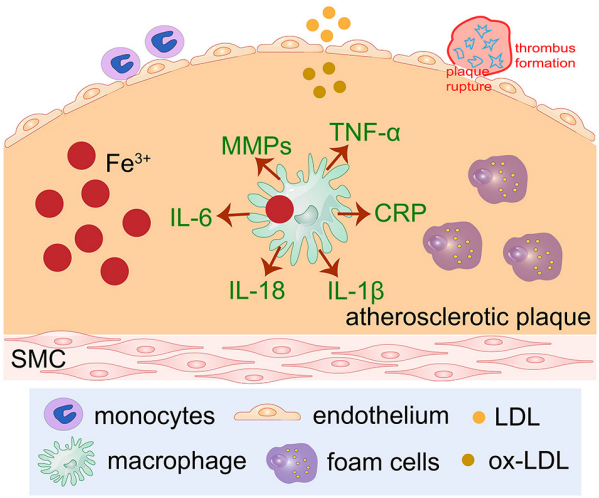

Figure 3. Ferroptosis is triggered by excessive lipid peroxidation. ox-LDL induces iron accumulation. Free iron has strong endothelial cytotoxicity and, at the same time, lipid-laden macrophages accumulate in the arterial subendothelial space, which promotes the inflammatory response of the arterial wall. Iron deposition in plaques causes ox-LDL to activate the TLR4/NF-κB signaling pathway and promotes foam cell formation. Ferric iron retention in atherosclerotic plaques promotes oxidative stress, lipid peroxidation and increases the instability of plaque. The occurrence of ferroptosis is involved in numerous processes of atherosclerosis. Lipid peroxidation, plaque hemorrhage and iron deposition are important characteristics of advanced atherosclerotic plaques. LDL, low-density lipoprotein; ox-LDL, oxidized LDL; MMPs, matrix metalloproteases; CRP, C-reactive protein; SMC, smooth muscle cell.

factors, such as TNF-α (93) (Fig. 3). Blood vessels that are constantly infiltrated by inflammatory factors for a long time are prone to the aggregation and oxidation of LDL, and the deposition of atherosclerotic plaques, promoting AS and the ferroptosis of macrophages in the plaque (94).

In the early stage, AS appears in the form of hemosiderin deposition. In the highly oxidative environment of atherosclerotic lesions, red blood cells rapidly dissolve and release hemoglobin, which is easily oxidized and releases heme. The pro-oxidant and pro-inflammatory effects of heme affect the functions of endothelial cells and macrophages, and promote the oxidation of LDL. Therefore, cholesterol levels are closely related to iron deposition, and activation of the cellular heme oxygenase-1/ferritin system can slow down the progression of this process. Notably, iIncreased iron deposition has been reported to occur in the cerebral cortex of rabbits fed a high-cholesterol diet (95). In MRI T_2-weighted images, intra-plaque hemorrhage and iron deposition have been observed (96). Therefore, macrophage ferroptosis may be considered to be involved in the progression of AS (97) and could serve as a potential target for intervention with AS progression. Vinchi *et al* (98) reported that AS was profoundly aggravated in iron-loaded mice. It has also been reported that iron can exacerbate AS by lipid profile alterations, vascular permeabilization, sustained endothelial activation, elevation of pro-atherogenic inflammatory mediators and reduced nitric oxide availability (99). Treatment with iron chelators in mice has been reported to restore vascular endothelial function, reduce the levels of IL-6, TNF-α and MCP-1, and suppress AS (100). Iron overload leads to endothelial cell dysfunction

Table I. Ferroptosis-related factors and possible targets.

First author (year)	Ferroptotic factor	Regulatory mechanism	Possible intervention target to inhibit ferroptosis	(Refs.)
Lu *et al* (2020)	TFR1	lncRNA PVT1 regulates miR-214-mediated TFR1 expression	Silencing of lncRNA PVT1 and miR-214 overexpression markedly decrease PVT1 levels to suppress ferroptosis *in vivo*	(125)
Li *et al* (2021)	NCOA4	Degradation of ferritin leads to free iron release	Knockout of NCOA4 notably abrogates ferritinophagy and thus inhibits ferroptosis	(126)
Chen *et al* (2021)	ACSL4	Enhancing lipid peroxidation	ROSI inhibits ACSL4 and blocks the lipid peroxidation process	(127)
Lu *et al* (2018)	ROS	Nrf2/NADPH/ROS pathway	Artesunate suppresses oxidative toxicity and inflammatory by activating Nrf2 and downregulating ROS	(128)
Liu *et al* (2020)	GPX4	x_c^-/GSH/GPX4	Sulforaphane alleviates the cytotoxicity of erastin by promoting the expression of genes related to GSH synthesis	(129)
Dong *et al* (2020)	Nrf2	Nrf2/SLC7A11/HO-1	Nrf2 alleviates OGD/R-induced ferroptosis by upregulating SLC7A11 and HO-1	(64)
Wang *et al* (2020)	HMOX1	Nrf2/HO-1	The upregulation of Nrf2 iron-related target gene HMOX-1 exerts antioxidant and anti-inflammatory effects	(2)
Ratan (2020)	HIF-1α	HIF-1α/HO-1	Adaptaquin selectively inhibits HIF prolyl hydroxylases and stabilizes HIF-1 to protect neurons from ferroptosis	(57)

TFR1, transferrin receptor 1; NCOA4, nuclear receptor coactivator 4; ACSL4, acyl-CoA synthetase long-chain family member 4; ROS, reactive oxygen species; GSH, glutathione; GPX4, GSH peroxidase 4; Nrf2, nuclear factor erythroid 2-related factor 2; HMOX1, heme oxygenase 1; HIF-1α, hypoxia inducible factor-1α; lncRNA, long non-coding RNA; miR, microRNAs; x_c^-, system x_c^-cystine/glutamate antiporter; SLC7A11, solute carrier family 7 member 11; HO-1, heme oxygenase-1; ROSI, rosiglitazone; OGD/R, oxygen-glucose deprivation and reoxygenation.

through pro-oxidative and proinflammatory effects, directly causing AS progression, whereas the occurrence of ferroptosis affects AS progression through increased ROS production and cytokine secretion (101). Consistent with this, it has been confirmed in human coronary artery endothelial cells *in vitro* that Tanshinone IIA can inhibit ferroptosis-induced endothelial lipid peroxidation and dysfunction by activating the Nrf2 pathway, thus ultimately alleviating AS (102). Furthermore, it has also been reported that ferroptosis is involved in the progression of AS mainly through oxidative stress and the exacerbated breakdown of endothelial function (67).

In a previous study, iron content was directly quantified by nuclear magnetic resonance spectroscopy and it was reported that the level of redox active iron was increased in carotid atherosclerotic lesions compared with in normal healthy human endothelium, and it was further increased in late AS, inducing oxidative stress and inflammatory responses (103). Notably, Bai *et al* (104) assessed $ApoE^{-/-}$ mice with high-fat diet-induced AS and evaluated the expression of ferroptosis-related factors. This previous study reported significant reductions in the mRNA and protein expression levels of SLC7A11, a decrease in endothelial cell and angiogenic markers, such as CD31, and mitochondrial damage in endothelial cells. Moreover, the opposite results were reported in the same model treated with an iron chelator; the expression levels of the pro-angiogenic factor VEGF, and the adhesion molecules ICAM-1 and VCAM-1 were decreased, and the lesion area in AS was reduced, which indicated that ferroptosis may be involved in the progression of AS (104).

Processes, such as lipid oxidation, inflammatory reactions and iron accumulation, can occur during the pathogenesis of AS. Currently, the applied targets of ferroptosis inhibitors focus on the imbalance of lipid peroxidation and ferrous ions. Iron chelators, antioxidants and free radical scavengers reduce the ferroptosis of endothelial cells by reducing the lipid peroxidation of the plaques in the AS. *In vitro,* vascular endothelial cells treated with ox-LDL have ferroptotic properties and the use of ferroptotic inhibitors can interfere with this process (Table I).

4. Ferroptosis accelerates the progression of AS, leading to ischemic stroke

Ferroptosis accelerates AS progression mainly through vascular endothelial disorder and the lipid peroxidation of vascular endothelial cells, endothelial function impairment, platelet adhesion aggregation and eventually, thrombosis (105). The causal association between iron and AS is as follows. Firstly, iron overload leads to the activation of lipoxygenase (106), the upregulation of ACSL4 and

ALOX, and catalyzes the lipid peroxidation of phospholipids containing PUFAs in the lipid bilayer of the cell membrane, which are degraded by oxidation, resulting in damage to the cell membrane. Secondly, the interaction between iron and oxygen radicals leads to lipid peroxidation and neuronal death, promotes thrombosis by accelerating the progression of AS and intravascular platelet activation, and eventually, ischemic stroke (98). It has previously been reported that ALOX15 knockout can reduce iron deposition in the cerebral cortex, the level of ROS and the level of 4-hydroxynonenol, which is the final product of lipid peroxidation, and reduces nerve injury through the spermidine/spermine N1-acetyltransferase 1/ALOX15 axis (107).

During the process of an ischemic stroke, iron accumulation can exacerbate neuronal injury in patients or in animal models; iron accumulation is associated with AS and the occurrence of ferroptosis accelerates plaque formation (3). However, this process can be prevented by iron chelation therapy (108). A previous study reported a reduction in iron accumulation and attenuated neuronal degeneration in mice that had been treated with Fer1 (109). Clinical trials have reported that reducing the systemic iron content within 1-3 days of ischemic stroke may provide benefits for patients with acute ischemic stroke (110). Hypoxia-inducible factor (HIF)-1 prolyl hydroxylases (PHDs) may be a target of iron chelators to inhibit ferroptosis. In the brains of hypobaric hypoxic rats pre-treated with deferoxamine, hypoxia inactivated PHDs, causing the accumulation of HIF-1α and the level of HIF-1α protein to be significantly upregulated (111). Cells were more able to tolerate the hypoxic environment and hypoxic cells could recover faster, eventually reducing the volume of cerebral infarction. Moreover, HIF-1 may downregulate ACSL4 expression by binding the ACSL4 promoter to inhibit its transcription and alleviate ischemic brain injury (112).

Neuronal death and secondary inflammation due to cerebral ischemia are directly related to poor functional outcomes, with inflammation occurring throughout the entire phase of ischemia-reperfusion injury and ferroptosis interlinking with inflammation. Following cerebral ischemia, the release of inflammatory cytokines and neurotoxic mediators can be induced through multiple signaling pathways, such as NF-κB and STAT3, leading to neuronal damage and death (113). NF-κB activation following ischemia, and the expression of TNF-α, IL-1 and IL-6, is upregulated in cells to promote the inflammatory response (11). The high expression of IL-6 promotes the continuous phosphorylation of signal transducers and STAT3, and the transcription factor NF-κB enters the nucleus from the cytoplasm, regulating the expression of inflammatory cytokines, causing endothelial cell dysfunction and macrophage polarization, and promoting inflammation (114). IL-6/STAT3 is an important pathway for mediating intracellular inflammatory signaling, which can mediate the production of the proinflammatory cytokines, TNF-α and IL-1, and the anti-inflammatory cytokines, IL-4 and IL-10. Furthermore, the NF-κB/IL-6/STAT3 signaling pathway participates in the regulation of ferroptosis through the inflammatory response after cerebral ischemia and increases the expression of hepcidin (115). Notably, red wine polyphenol extract has been shown to efficiently suppress the inflammation of intestinal epithelial cells by inhibiting JAK/STAT and promoting Nrf2 pathways (116). Therefore, it may be hypothesized that enhancing the activity of Nrf2 and inhibiting the JAK/STAT signaling pathway, may reduce inflammation and monocyte differentiation to macrophages, and regulate SLC7A11 to inhibit ferroptosis. Artesunate is an antimalarial drug, and research has indicated that it also has antitumor and anti-inflammatory effects; it can inactivate the generation of pro-inflammatory mediators in microglia by affecting the NF-κB, p38/MAPK and Nrf2/ARE-dependent signaling pathways, thus inhibiting activation of the immune response following cerebral ischemia (117).

The presence of ferroptosis will continuously damage neural tissue for days to weeks; following ferroptosis, danger-associated molecular patterns (DAMPs) trigger neutrophil recruitment, neutrophil infiltration, proinflammatory cytokine expression (118), leukocyte death, and changes the immune status of the body (119). DAMPs associated with ferroptosis include HMGB1 and IL-33 (120). HMGB1 is released by ferroptotic cells and acts as an adjuvant to activate the recognition receptor of the NF-κB pathway (20); moreover, it triggers an inflammatory response in peripheral macrophages and exacerbates the poor prognosis of ischemic stroke, including cerebral edema and the risk of ischemia-reperfusion (121,122). Furthermore, it has been reported that ferroptosis is the main cause of neuronal death after ischemic stroke. Abnormal tau phosphorylation aggregation leads to neuronal winding, which is involved in the mechanism of ischemic and hemorrhagic stroke. Therefore, inhibiting tau protein expression can inhibit the excitatory cytotoxicity of cells, promote iron outflow to prevent the occurrence of ferroptosis and reduce damage to nerve cells (123). Moreover, the main role of tau protein in neurons is to promote the formation of neuronal microtubule structures and play a key role in axonal transport and cognitive function, but as mice grow older, tau has double effects (124); the tau protein is hyperphosphorylated and amyloid protein is formed, causing nerve fiber damage and nerve fiber degeneration, thus aggravating the neurotoxic iron accumulation.

5. Conclusion and future perspectives

The mechanisms of AS are complex and involve multiple processes in numerous cell types. Crucially, with the participation of foam cells and macrophages, ferroptosis drives the progression of AS through oxidative stress and inflammatory responses. The present review summarized that ferroptosis is involved in the entire period of atherogenesis and progression through numerous signaling pathways, including lipid pattern, atherosclerotic plaque, fiber plaque and plaque rupture, while interlinking with inflammation to exacerbate the poor prognosis of AS-related diseases. During the development of AS, lipids are deposited under the vascular endothelium forming fatty streak plaques. With the progression of the disease, vascular endothelial cells are damaged, ferroptosis and inflammation participate in the atherosclerotic plaque stage, smooth muscle cells gradually migrate, and the formation of fibrous caps on the surface of the plate indicates the progression of the fibrous plaque stage. The presence of ferroptosis and inflammation further damages endothelial cells, rupture the plaque and forms a series of ischemic events. Undoubtedly, advances in the study of ferroptosis-associated mechanisms will change the traditional concept of AS, and may improve the ability to

manage AS risk and address the inevitable risks that remain following current interventions. In conclusion, ferroptosis serves a crucial role in the pathogenesis of AS, ischemic stroke and coronary heart disease, and with the exploration of clinical feasibility, the targeting of ferroptosis may provide novel insights into the treatment of vascular-related diseases.

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Authors' contributions

JL, LX contributed to the conception and design of this study. JL prepared the tables and figures, and wrote the manuscript. YXZ, XQC revised the manuscript critically and and added relevant relevant literature. HTC was responsible for revising the manuscript and given final approval of the version to be published. All authors read and approved the final version of the manuscript. Data authentication is not applicable..

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Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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