Mitophagy and ferroptosis in cerebral ischemia-reperfusion: regulatory mechanisms and therapeutic potential

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Abstract: Nutrient deficiency, excitotoxic injury, and oxidative stress caused by cerebral ischemia/reperfusion injury are important inducing factors of mitophagy and ferroptosis in neurons. Ferroptosis is an iron-dependent mode of cell death usually accompanied by a large accumulation of iron ions and lipid peroxides. Mitophagy is one of the forms of selective autophagy, which can maintain mitochondrial and cellular homeostasis by eliminating dysfunctional mitochondria. Mitophagy and ferroptosis are closely related to the pathological mechanism of ischemia/reperfusion injury. However, the function and mechanism of mitophagy in regulating ferroptosis are only beginning to be understood, and the relationship between mitophagy and ferroptosis after cerebral ischemia/reperfusion has not been elucidated. This article reviews the mechanism pathways of mitophagy and ferroptosis after cerebral ischemia/reperfusion, especially discusses the common regulatory factors of mitophagy and ferroptosis in cerebral reperfusion injury, and focuses on the therapeutic potential of mitophagy in regulating ferroptosis, in order to provide ideas for targeted treatment of cerebral ischemia/reperfusion injury.

Keywords: Ischemia/reperfusion, Ferroptosis, Mitophagy.

1. Introduction

Acute ischemic stroke (AIS), also known as acute cerebral infarction, is the most important type of stroke. It is a disease caused by local cerebral tissue ischemia and hypoxia caused by cerebrovascular occlusion caused by various reasons and is characterized by high incidence, high disability rate, and high mortality rate, bringing a heavy burden to society and families[1]. At present, intravenous thrombolytic therapy and intravascular therapy within the time window are the most effective treatment measures, aiming at reducing tissue damage and cell necrosis caused by ischemia by restoring blood flow and saving injured neurons. However, reperfusion of blood flow in brain tissue often leads to reperfusion injury by inducing oxidative stress, inflammation, and other damage to nerve cells [2,3]. Cerebral ischemia/reperfusion (I/R) involves reoxygenation-induced reactive oxygen species (ROS) production, calcium overload, inflammatory response, ER stress, and cell death [3]. The underlying mechanism of I/R impairment is not fully understood. Therefore, it is very important to study the pathological mechanism of cerebral ischemia/reperfusion injury for treatment and improvement of patient prognosis.

Ferroptosis is a new type of cell death mode, which is regulated by iron-dependent cell

death caused by the accumulation of reactive oxygen species and lipid peroxides [4]. Studies have shown that ferroptosis plays a key role in Cerebral ischemia/reperfusion injury, and ferroptosis inhibitors have successfully prevented or reduced Cerebral ischemia/reperfusion injury. Inhibition of ferroptosis is becoming an effective therapeutic strategy for the treatment of cerebral ischemia/reperfusion injury and helps to reduce cell death during reperfusion injury [5-8].

Mitochondria are important organelles present in eukaryotes, known as the "energy factories" of cells, which can produce the energy required by the body and are also the main producers of ROS. Cerebral ischemia/reperfusion injury can induce mitochondrial dynamic imbalance and further mitochondrial dysfunction, resulting in decreased mitochondrial membrane potential, increased ROS production, and impaired ATP synthesis, affecting the whole function of neuron cells and even causing apoptosis or necrosis [9]. Mitophagy can maintain the stability of cellular functions by removing damaged mitochondria [10]. Studies have shown that various signaling pathways and mitophagy receptor proteins play an important role in cerebral ischemia/reperfusion by mediating mitophagy and regulating mitophagy may be a promising therapeutic strategy to alleviate cerebral ischemia/reperfusion injury and protect brain nerve cells [11-15].

Recent data show that there is a close relationship between mitophagy and ferroptosis [16-18], both of which seriously affect oxidative metabolism and participate in the pathological process of cerebral ischemia/reperfusion injury. In view of the close relationship between mitophagy and ferroptosis after cerebral ischemia/reperfusion and their importance in the development and treatment of diseases, this paper discusses the possible mechanisms of mitophagy and ferroptosis in cerebral ischemia/reperfusion, especially the contradictory relationship between mitophagy and ferroptosis and the possible common regulators of mitophagy and ferroptosis after cerebral ischemia/reperfusion.

2. Ferroptosis and cerebral ischemia/reperfusion
2.1 Overview of ferroptosis

The concept of ferroptosis was first proposed by Dixon et al. in 2012, which is a type of programmed cell death based on iron-dependent lipid peroxidation [4]. The main feature is the excessive accumulation of intracellular lipid peroxides and ROS [19], resulting in excessive oxygen free radicals that seriously damage the membrane, indirectly allowing more oxidation [20], and ultimately leading to cell membrane damage and cell death. In recent years, the role of ferroptosis in I/R in acute ischemic stroke has been extensively studied. Studies have found that the disorders of brain iron metabolism [21], lipid metabolism [22], and amino acid metabolism [23] after ischemia and hypoxia can lead to ferroptosis of nerve cells, and the ischemic injury has been successfully reversed by inhibiting the occurrence of ferroptosis [5-8] and the prognosis of stroke has been improved.

2.2 Mechanism of ferroptosis in cerebral ischemia/reperfusion
2.2.1 Disorders of Iron Metabolism

Intracellular iron overload is an important factor in ferroptosis. Iron, a redox-active metal, is required for ferroptosis [24]. Under normal circumstances, Fe^{3+} binds to

transferrin (TF) and enters cells under the mediation of transferrin receptor 1 (TFR1) [25]. Subsequently, Fe^{3+} is converted to Fe^{2+} by six-transmembrane epithelial antigen of prostate 3 (STEAP3), and Fe^{2+} is transported from endosomes via divalent metal transporter 1 (DMT1) and exported via ferroportin (FPN) [25,26]. Intracellular Fe^{2+} can be stored in the labile iron pool (LIP) and ferritin [27].

During cerebral ischemia/reperfusion, iron metabolism disorders can occur, including iron uptake, storage, utilization, and outflow disorders. Iron metabolism disorder involves the regulation of HIF-1α, BACH1, NCOA4, Hepcidin, and other factors. HIF-1α can promote Hmox1 transcription and the expression of TFR1 and DMT-1 [28,29]. Hmox1 encodes the stress-inducing enzyme HO-1, which can degrade heme and produce Fe^{2+} [30,31], while HIF-1α-mediated increased expression of TFR1 and DMT-1 further promotes the increase of intracellular Fe^{2+}. BACH1 promotes ferroptosis by inhibiting the transcription of ferritin genes (Fth1 and Ftl) and ferroportin genes (Slc40a1) [32], that is, reducing iron storage and efflux. NCOA4 has been identified as a selective cargo receptor for ferritin autophagy [33], which binds to ferritin and delivers it to lysosomes for degradation, releasing large amounts of iron [34]. Hepcidin is emerging as a new important factor in brain iron homeostasis [35]. The increased expression of inflammatory factors after acute cerebral ischemia can increase the expression of hepcidin [36]. Hepcidin inhibits the activity of FPN and promotes its degradation, reducing iron efflux by binding to FPN [37]. Hepcidin has been shown to act not only on FPN but also on DMT1 [38]. Downregulation of FPN and up-regulation of DMT1 were observed in the cerebral cortex of rats after intracerebral injection of hepcidin [39].The above disturbance of iron metabolism during cerebral ischemia/reperfusion eventually leads to abnormal accumulation of Fe^{2+} in the cell, and then Fe^{2+} generates a large number of ROS through the Fenton reaction, which can cause oxidative damage [40]. In addition, iron is a pro-oxidant in ferroptosis, which is crucial for the function of enzymes such as lipoxygenases (LOXs) [41] and helps induce lipid peroxidation. Eventually, it causes cell dysfunction and ferroptosis.

2.2.2 Disorder of Lipid Metabolism

Another important factor causing ferroptosis after cerebral ischemia/reperfusion is the accumulation of intracellular lipid peroxides. Polyunsaturated fatty acids (PUFAs) are the main components of phospholipids in cell and organelle membranes, and also the main substrate of lipid peroxidation during ferroptosis, which can lead to membrane structural and functional damage [42]. After cerebral ischemia/reperfusion, polyunsaturated fatty acids on the cell membrane are easily oxidized to form lipid peroxides. First, long-chain acyl-CoA synthetase 4 (ACSL4) catalyzes polyunsaturated fatty acids, For example, arachidonic acid (AA) or adrenic acid (AdA) form AA/AdA-CoA [43]. Secondly, AA/AdA-CoA can be esterified to AA/AdA-PE by recombinant lysophosphatidylcholine acyltransferase 3 (LPCAT3) [44].AA/AdA-PE is converted to harmful PE-AA-OOH or PE-ADA-OOH by non-enzymatic lipid peroxidation mediated by LOXs through enzymatic reactions or iron-dependent Fenton chemical reactions [45,46], which eventually induces ferroptosis.

ACSL4 is widely expressed in brain tissue, and as a potential target of miR-347 after cerebral ischemia, it is upregulated with the overexpression of miR-347 after cerebral

ischemia in MCAO mice, inducing neuronal death [47]. ACSL4 can also promote the production of pro-inflammatory cytokines by microglia [22], which can further aggravate brain injury by promoting the inflammatory response after AIS. In a mouse model of ischemic stroke, ACSL4 expression is increased, and inhibition of ACSL4 with rosiglitazone can significantly improve nerve function 72 h after stroke and reduce the volume of cerebral infarction [6]. These studies suggest that ACSL4 may be a novel regulator of neuronal death and neuroinflammation, and interference with ACSL4 expression may be a potential therapeutic target for ischemic stroke. LOXs are the key enzymes that induce ferroptosis [48]. Several subtypes of LOXs have been identified, of which 12/15-LOX is a special subtype. It was found that 12/15-LOX was elevated in neurons and endothelial cells after focal ischemia. Overexpression of 12/15-LOX can lead to the death of brain neurons and the destruction of the blood-cerebrospinal fluid barrier, and the use of 12/15-LOX inhibitors can improve nerve function and alleviate brain edema [49]. Other studies have found that after global cerebral ischemia, 12/15-LOX is widely increased and leads to neuronal damage, and gene knockout or use of LOXBlock-1, an inhibitor of 12/15-LOX, can reduce neuronal damage and improve neurological outcomes [50]. 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 to alleviate cerebral ischemic injury.

2.2.3 Disorder of Amino Acid Metabolism

System Xc- is a heterodimer amino acid reverse transporter consisting of two subunits (light chain subunit SLC7A11 and a heavy chain subunit SLC3A2) and is also an important antioxidant [51]. System Xc- introduces extracellular cystine in a 1:1 ratio in exchange for intracellular glutamate [52]. Cystine is first reduced to cysteine, and then GSH is synthesized with glycine and glutamate. GPX4 is a unique intracellular antioxidant enzyme. Under physiological conditions, reduced glutathione (GSH) accounts for the majority of cells. Under the catalytic effect of GPX4, GSH can be converted into oxidized glutathione (GSSG), which plays an antioxidant role, reduces the occurrence of lipid peroxidation and ferroptosis, and protects cells from damage [53]. Systems Xc- and GPX4 are both key regulators of ferroptosis. Cellular uptake of cystine is an important step in the production of GSH. 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, resulting in a decrease in GSH synthesis [54-56]. Other studies have found that GPX4 protein expression and activity decreased after acute ischemic stroke [6]. Decreased GSH synthesis and decreased GPX4 protein expression and activity can lead to the accumulation of lipid peroxides in cells and eventually ferroptosis. It can be seen that the ferroptosis caused by the disorder of amino acid metabolism is manifested in the following three aspects: (1) The inhibition of System Xc-; (2) Reduced glutathione synthesis; (3) The expression and activity of GPX4 were decreased.

3. Mitophagy and cerebral ischemia-reperfusion

3.1 Overview of mitophagy

Mitophagy is one of the forms of selective autophagy, which was proposed by LEMASTERS et al. [57] in 2005. It refers to the depolarized damage of mitochondria in

cells under stress such as excessive accumulation of ROS, insufficient nutrition, cell aging or infection with viruses, and the damaged mitochondria are specifically wrapped into autophagosomes and fused with lysosomes. Complete the degradation of damaged mitochondria, thus limiting the production of mtROS, and controlling the quality and quantity of mitochondria to maintain cell homeostasis. Mitochondria are the main sites of oxidative phosphorylation, energy production, and reactive oxygen species (ROS) production in mammalian cells. Cerebral ischemia and hypoxia lead to the lack of important energy substances such as oxygen and glucose in neuron cells. In this environment of hypoxia and insufficient energy supply, mitochondria cannot properly oxidative phosphorylation to produce energy, and may produce more reactive oxide ROS. Excessive ROS can cause oxidative damage to mitochondrial proteins, lipids, and DNA, and the mitochondrial function is gradually damaged or even abnormal, thus initiating mitophagy. At present, the activation of mitophagy to rescue cerebral ischemia/reperfusion injury has been widely reported in ischemic stroke [14,58-60]. The pathways that induce mitophagy in cerebral ischemia/reperfusion include PTEN-induced putative kinase 1 (PINK1) /Parkin ubiquitin-dependent pathway and receptor-mediated ubiquitin-independent pathway.

3.2 Mechanism of mitophagy pathway in cerebral ischemia/reperfusion
3.2.1 Ubiquitin-dependent pathway mediated by PINK1/Parkin
Currently, the most widely studied and common mechanistic pathway of mitophagy is the ubiquitin-dependent pathway mediated by PINK1/Parkin. This pathway is primarily dependent on PTEN-induced putative kinase 1 (PINK1) and cytoplasmic E3 ubiquitin ligase Parkin, both of which are involved in various physiological and pathological processes. In normal mitochondria, the content of PINK1 is extremely low, mainly because PINK1 is continuously transported from the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM), processed by mitochondrial processing peptidase (MPP) and presenilin-associated rhomboid-like protease (PARL) [61,62], and subsequently broken down and removed. PINK1 is the upstream protein of Parkin. After cerebral ischemia/reperfusion, mitochondrial function is impaired, membrane depolarization is observed, membrane potential is decreased, and input of PINK1 is blocked, resulting in accumulation of PINK1 on the outer membrane of mitochondria [63]. PINK1 recruits and phosphorylates Parkin to translocate it from the cytoplasm to the mitochondrial surface [64,65], and ubiquitinates a variety of outer mitochondrial membrane protein substrates, leading to the recruitment and recognition of autophagosomes, phagocytosis of damaged mitochondria, and subsequent mitochondrial degradation by lysosomes [66-69]. Activation of PINK1 / Parkin pathway can promote the formation of autophagy vesicles, degrade damaged mitochondria, maintain cell homeostasis, improve mitochondrial function, and protect neurons from cerebral ischemia/reperfusion injury [60]. Recent studies have shown that when the PINK1 / Parkin pathway is activated, it promotes mitophagy and rescues brain damage [70,71]. However, knocking out PINK1 or Parkin inhibits the activation of PINK1/Parkin pathways and prevents mitochondrial clearance [70,72]. Notably, the silent information regulator factor 2-related enzyme 1 (SIRT1) is an important regulator of mitophagy. It plays a crucial role in neuroprotection against cerebral ischemia [73].SIRT1 can induce

mitophagy and reduce cerebral ischemia/reperfusion injury by activating PINK1/Parkin signaling pathway [74].

The protective effect of activated PINK1/Parkin mediated mitophagy on neurons has been highlighted in current studies of cerebral ischemia/reperfusion. However, it is not fully understood how neurons PINK1 and Parkin sense ischemic stress to trigger mitophagy in ischemic neurons.

3.2.2 Receptor-mediated ubiquitin independent pathway

Another important pathway of mitophagy is the receptor-mediated ubiquitin-independent pathway. BNIP3L/NIX, BNIP3, and FUNDC1 are the mitophagy receptors that have been studied in cerebral ischemia/reperfusion injury. After cerebral ischemia/reperfusion, mitochondrial function is impaired and membrane potential is reduced. These proteins directly interact with LC3 to induce mitophagy [75]. At present, studies have confirmed that BNIP3L/NIX is involved in mitophagy induced by cerebral ischemia/reperfusion, and knockout of Bnip3l reduces mitophagy and aggravates cerebral ischemia/reperfusion injury in mice, which can be rescued by overexpression of BNIP3L. This study proposed the concept that BNIP3L may be a potential therapeutic target for ischemic stroke [76]. Recent studies have further confirmed that promoting NIX-mediated mitophagy can alleviate ischemia/reperfusion injury in ischemic stroke [77]. Similarly, activation of BNIP3-mediated mitophagy can also protect neurons from ischemia/reperfusion injury [78]. tPA has long been considered the mainstay of ischemic stroke treatment. tPA can induce FUNDC1-dependent mitophagy to protect neurons from cerebral ischemia/reperfusion injury [79]. A recent study showed that FUNDC1 was inactivated in the later stage of neuronal ischemia/reperfusion injury, but FUNDC1 deletion did not destroy neuronal mitophagy, and induction of FUNDC1-mediated mitophagy may be a potential therapeutic strategy for the treatment of ischemic stroke [58]. Interestingly, inhibition of p-ULK1 / FUNDC1 / LC3-II-mediated mitophagy pathway protected neurons from ischemia/reperfusion injury. This is in contradiction with the statement that FUNDC1-dependent mitophagy is induced to protect neurons from cerebral ischemia/reperfusion injury, so it is necessary to further study the specific role of FUNDC1-mediated mitophagy on ischemic neurons in cerebral ischemia/reperfusion injury [80].

Although PINK1/Parkin, BNIP3L, BNIP3, and FUNDC1 mediated mitophagy have been identified in studies of cerebral ischemia/reperfusion, related studies are limited, and how mitophagy is induced after cerebral ischemia/reperfusion has not been clearly elucidated. In addition, Other mitophagy receptors have been found in mammalian cells and various biological models, such as NLRX1, FKBP8, Bcl2L13, PHB2, and CL[75,81]. However, the role of mitophagy mediated by these receptors in cerebral ischemia/reperfusion is still lacking, and it is expected to be further studied in the future. The diversity of mitophagy pathways may enable neurons to sense different environmental stresses to ensure the removal of damaged mitochondria, and although some studies suggest that there may be potential links between these mitophagy pathways, it is unclear whether or how they regulate mitochondrial quality control in ischemic neurons.

4. Possible relationship between mitophagy and ferroptosis in cerebral

ischemia/reperfusion

Mitophagy and ferroptosis are closely related to cerebral ischemia/reperfusion injury. The main characteristic of ferroptosis is the accumulation of lipid peroxides and ROS in cells. It is well known that mitochondria are the main source of intracellular ROS, and damaged mitochondria that are not cleaned in time will produce a large amount of ROS, which further aggravates cell damage and is closely related to ferroptosis [82]. The accumulation of reactive oxygen species in neuronal cells can trigger mitochondrial depolarization, thereby initiating mitophagy. A large number of studies have found that ferroptosis can occur in neurons after cerebral ischemia/reperfusion to further aggravate brain injury and cerebral infarct volume, and inducing mitophagy can alleviate cerebral ischemia/reperfusion injury and reduce neuronal damage. Based on recent findings in other diseases, mitophagy is closely related to ferroptosis, and mitophagy can either induce ferroptosis or inhibit ferroptosis. Despite these conclusions, there are few studies on the mechanism of mitophagy regulating ferroptosis. It is even unclear how mitophagy regulates ferroptosis after cerebral ischemia/reperfusion injury, although both are involved in the regulation of cerebral ischemia/reperfusion injury. Therefore, more research is needed to further explore the relationship between them. Considering the different pathological mechanisms of different diseases, when studying the relationship between mitophagy and ferroptosis in cerebral ischemia/reperfusion, it is of great help to find out the factors regulating the two mechanisms at the same time to improve the condition and treatment. Studies have found that HIF-1, p53, SIRT1, Nrf2, and NLRP3 play an important role in cerebral ischemia/reperfusion injury and can regulate mitophagy and ferroptosis. Therefore, it is an interesting question whether these factors can regulate mitophagy to play an anti-ferroptosis role and thus play a role in reducing brain tissue damage after cerebral ischemia/reperfusion.

4.1 HIF-1α

HIF-1α expression was up-regulated after cerebral ischemia/reperfusion injury.HIF-1α can regulate many target genes closely related to ferroptosis. It can up-regulate the expression of TFR1 and DMT-1 [28,29], promote neuronal ferroptosis, and negatively regulate the expression of ACSL4 to inhibit neuronal ferroptosis [83]. HIF-1α can increase the expression of BNIP3 and BNIP3L [84]. Upregulation of BNIP3 and BNIP3L expression can activate mitophagy and improve brain injury caused by ischemia/reperfusion[76,78]. HIF-1α has also been implicated in PINK1/Parkin mediated mitophagy. Studies in other diseases have shown that HIF-1α can promote PINK1 and Parkin activation, and then promote mitophagy in granulosa cells and restore hypoxia-induced apoptosis [85]. At present, although PINK1/Parkin, FUNDC1, BNIP3, and BNIP3L have been observed to mediate mitophagy to play a neuroprotective role in cerebral ischemia/reperfusion [60,77,78,86], it is not clear whether HIF-1α is involved in the regulation of this process.HIF-1α may play different roles in different periods of ischemic stroke and is an important regulator of potential new treatment methods for ischemic stroke [87]. Therefore, it is worth studying that HIF-1α can induce mitophagy to regulate ferroptosis after cerebral ischemia/reperfusion injury.

4.2 p53

p53 activation is induced by various forms of cellular stress, including DNA damage,

oncogene activation, ribosomal stress, or hypoxia. After cerebral ischemia and hypoxia, p53 can be activated to promote its expression. With the increase of p53 expression, it can increase the sensitivity of cells to ferroptosis by inhibiting the expression of SLC7A11 and GPX4 or promoting the expression of SAT1, PTGS2, and GLS2 [88-94]. Cytosolic p53 was found to bind to Parkin and inhibit mitophagy by preventing the translocation of Parkin from cytoplasm to mitochondria, thereby reducing mitophagy activation and leading to hepatocyte apoptosis in heat troke acute liver injury (HS-ALI). The pharmacology of mitochondrial autophagy induced by inhibition of p53 may be a promising treatment for HS-ALI [95]. SIRT3 deficiency impairs Parkin-mediated mitophagy by increasing p53-Parkin binding and blocking Parkin mitochondrial translocation in cardiomyocytes, which may increase the susceptibility of the aged heart to cardiac dysfunction [96]. In a study of alcoholic liver disease, it was found that the promotion of p53 activation promoted dynamin-related protein 1 (Drp1) related mitochondrial fission and inhibited FUNDC1-mediated mitophagy [97]. However, the use of inhibitors of p53 can regulate and increase mitophagy to exert its neuroprotective activity [98]. There are still studies on p53 and ferroptosis after cerebral ischemia/reperfusion, but there is a lack of research on the regulation of p53 mitophagy. It is possible that inhibiting p53 at the appropriate time may play an anti-ferroptosis role by regulating mitophagy, thereby reducing brain damage.

4.3 SIRT1

SIRT1 is a nicotine-adenine dinucleotide (NAD) dependent enzyme, which plays an important role in ischemic stroke. Regulation of Sirt1 can reduce inflammation in the acute phase, inhibit oxidative stress, enhance blood vessels and neurogenesis, and promote the recovery of nerve function and other neuroprotective effects [73]. SIRT1 is related to ferroptosis, and activation of SIRT1/Nrf2/GPx4 signaling pathway can inhibit ferroptosis in hippocampal neurons [99]. The expression of PUM2 is increased after cerebral ischemia/reperfusion. PUM2 can inhibit SIRT1 and inhibit SLC7A11-mediated ferroptosis in neurons. Further study found that the down-regulation of PUM2 could increase the protein expression levels of SIRT1 and SLC7A11, and the inhibition of SIRT1 reversed the increase of SLC7A11 protein level mediated by the down-regulation of PUM2, indicating that SIRT1 may positively regulate SLC7A11 to inhibit ferroptosis [23]. SIRT1 not only plays a role in neuronal ferroptosis but also participates in the regulation of mitophagy to play a neuroprotective role. Apelin-36 is a neuropeptide that plays a protective role in cerebral ischemia/reperfusion injury. SIRT1 can mediate PINK1/Parkin dependent mitophagy to participate in the neuroprotective effect of Apelin-36 on OGD/R-induced oxidative stress and mitochondrial dysfunction [100]. A recent study pointed out that melatonin can promote mitophagy through SIRT1-mediated signaling pathway to alleviate oxidative stress-induced apoptosis and mitochondrial damage in bovine ovarian granulosa cells [101]. Although SIRT1 activation has been shown to be a potential treatment for ischemic stroke, there is no study linking SIRT1 with mitophagy and ferroptosis. Therefore, further research is needed to identify precise targets for SIRT1, which will help develop new treatment strategies for ischemic stroke.

4.4 NLRP3

NLRP3 is a major contributor to the inflammatory response that exacerbates cerebral ischemia/reperfusion injury. Cerebral ischemia/reperfusion can induce activation of NLRP3 inflammasome and aggravate brain injury. NLRP3 inflammasome activation is associated with cerebral ischemia/reperfusion neuronal ferroptosis. Knockdown of NLRP3 inflammasome can alleviate cerebral ischemia/reperfusion injury by inhibiting ferroptosis and inflammation, and NLRP3 inflammasome inhibitor can reduce cerebral ischemia/reperfusion induced neuronal ferroptosis [102]. Mitophagy plays an important role in regulating the activation of NLRP3 inflammasome. During cerebral ischemia/reperfusion injury, PINK1/Parkin and FUNDC1 can mediate mitophagy to inhibit the activation of NLRP3 inflammasome, thereby alleviating cerebral ischemia/reperfusion injury [86,103]. Therefore, it is hypothesized that the activation of NLRP3 inflammasome can be inhibited by inducing mitophagy in cerebral ischemia/reperfusion injury, thereby reducing neuronal ferroptosis and saving brain injury.

4.5 Nrf2

The nuclear factor erythroid 2-related factor 2 (Nrf2), as one of the most important antioxidant transcription factors in cells, can coordinate various cell protective factors to inhibit oxidative stress. Targeting Nrf2 is considered a potential strategy for the prevention and treatment of ischemic stroke [104]. Nrf2 is involved in regulating ferroptosis. Numerous studies have shown that drugs such as β-caryophyllene[105], dexmedetomidine[106], edaravone[107], and icariside II[108] can activate the Nrf2-regulated pathway to inhibit neuronal ferroptosis and reduce cerebral ischemia/reperfusion injury. Nrf2 can also regulate mitophagy. The combination of xuesaitong (XST) and dexmedetomidine (Dex) can activate Keap1 / Nrf2 signaling and mitophagy to protect rats from cerebral ischemia/reperfusion injury [59]. Mitoquinone inhibits oxidative stress-related neuronal death by activating mitophagy through Keap1/Nrf2/PHB2 pathway after SAH [109]. Based on other diseases, it has been demonstrated that some drugs can activate NRf2-related pathways to promote mitophagy and inhibit ferroptosis. VO-OHpic protects endplate chondrocytes from apoptosis and degeneration by activating Nrf-2 / HO-1 mediated mitophagy process and inhibiting ferroptosis[110]. Icariin promotes Parkin mediated mitophagy and inhibits ferroptosis by activating Nrf-2 / HO-1 pathway, thereby alleviating redox imbalance and mitochondrial dysfunction, and ultimately improving cell survival [111]. Astaxanthin enhances the activation of Nrf-2/HO-1 signaling pathway, thereby promoting the process of mitophagy, inhibiting oxidative stress and ferroptosis of cartilage endplate (CEP) chondrocytes, and ultimately improving extracellular matrix degradation, CEP calcification, and endplate chondrocytes apoptosis [112]. The above studies only show that the activation of Nrf2-mediated signaling pathway can promote mitophagy and inhibit ferroptosis, but it has not been proved that the activation of Nrf2-mediated signaling pathway can promote mitophagy and play an anti-ferroptosis role. Therefore, it is worthy of further study whether the drugs mentioned above that can activate the Nrf2-regulated pathway to inhibit neuronal ferroptosis can inhibit ferroptosis by promoting mitophagy and thereby alleviate cerebral ischemia/reperfusion injury.

5 Conclusion and prospect

Ischemic stroke is a common and serious neurological disease, and its pathogenesis involves many complex biological processes. In recent years, a large number of studies have confirmed that ferroptosis and mitophagy play important roles in cerebral ischemia/reperfusion. This article mainly introduces the mechanism of ferroptosis and mitophagy after cerebral ischemia/reperfusion. After cerebral ischemia/reperfusion, cell ischemia, and hypoxia lead to ferroptosis of nerve cells by affecting iron metabolism, lipid metabolism, and amino acid metabolism. Inhibition of ferroptosis has been shown to effectively reduce cerebral ischemia/reperfusion injury. However, after cerebral ischemia and hypoxia, the accumulation of reactive oxygen species in neuronal cells can not only lead to ferroptosis but also lead to the impairment of mitochondrial function, triggering mitochondrial depolarization and initiating mitophagy. Similarly, induction of mitophagy has also been shown to be effective in attenuating cerebral ischemia/reperfusion injury to help reduce cell death during reperfusion injury. This indicates that inducing mitophagy and inhibiting ferroptosis are of great significance in reducing ischemia/reperfusion injury and can provide guidance for the treatment of ischemic stroke.

Meanwhile, recent studies suggest that regulating mitophagy can inhibit ferroptosis. For example, in the study of acute kidney injury, Lin et al. [113]found that BNIP3 mediated and PINK1-PARK2-mediated mitophagy could prevent cisplatin-induced ferroptosis of renal tubular epithelial cells through ROS/HO-1 / GPX4 axis. Xue et al. [16] found that activated PPARγ could improve mitochondrial function by promoting PINK1/Parkin dependent mitophagy, inhibit ferroptosis of chondrocytes, and delay the progression of osteoarthritis. In their study on the effects of melatonin on mitophagy and cell damage during kidney stone formation, Zhou et al. [114]found that melatonin could enhance PINK1-Parkin-regulated mitophagy through AMPK phosphorylation, reduce excessive ROS release and inhibit oxidative stress, inflammation and ferroptosis. Du et al.[115] demonstrated that silibinin attenuated ferroptosis in rat islet β cells INS-1 induced by palmitic acid and high glucose treatment by enhancing PINK1/Parkin mediated mitophagy. Surprisingly, the commonality of these results is that regulation of mitophagy can inhibit ferroptosis. Despite these conclusions, there are few studies on the mechanism of mitophagy regulating ferroptosis. It is even unclear how mitophagy regulates ferroptosis after cerebral ischemia/reperfusion injury, although both are involved in the regulation of cerebral ischemia/reperfusion injury. Therefore, this paper introduces HIF-1α, p53, SIRT1, NLRP3, and Nrf2 which are important regulatory factors that may regulate mitophagy and ferroptosis in cerebral ischemia/reperfusion injury. These factors may be important targets for cerebral ischemia/reperfusion to regulate mitophagy and play an important role in anti-ferroptosis. The introduction of the mechanism and important regulatory factors of mitophagy and ferroptosis in this paper also leads to the following thoughts: (1) Although there are many studies on the mechanism of mitophagy and ferroptosis after cerebral ischemia/reperfusion, how to induce mitophagy and ferroptosis is not clear, and whether there is a common regulatory mechanism between the two is still unknown, and further exploration and research are needed in this field. (2) Although this paper introduces the important regulatory factors that may regulate mitophagy and ferroptosis

after cerebral ischemia/reperfusion, there is no research on the induction of mitophagy and ferroptosis by these factors in cerebral ischemia/reperfusion injury, and it has great potential to study the specific regulatory mechanism and then target the application of drugs in clinical therapy. With the deepening of research, finding appropriate drugs and targeting specific factors to regulate ferroptosis by inducing mitophagy is likely to become an effective strategy for the treatment of ischemic stroke. (3) Up to now, studies on ferroptosis and mitophagy after cerebral ischemia/reperfusion injury have mostly focused on animal and cell experiments, and clinical trials are still lacking. Therefore, more population-based data are needed to determine whether inducing mitophagy and inhibiting ferroptosis can improve the prognosis of AIS patients. (4) At present, there are many studies on the mechanism of ferroptosis and mitophagy, but the drug research on cerebral ischemia/reperfusion injury treatment mainly focuses on inhibiting ferroptosis or regulating mitophagy alone. Therefore, future studies should focus not only on the therapeutic effect of inhibiting ferroptosis or regulating mitophagy in brain cells but also on the comprehensive treatment of all aspects of ischemic stroke.

Author Contributions

Haoxiu Li wrote the main manuscript text. Zhongqiang Cheng, Meng Bi, Jing Shi and Huiling Xu conducted the literature search. Weirong Li supervised the implementation and revised the manuscript. All authors reviewed the manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Approval

not applicable.

Data Availability Statement

not applicable.

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