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Review

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Novel perspective in transplantation therapy of mesenchymal stem cells: targeting the ferroptosis pathway

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Abstract: Ex vivo culture-amplified mesenchymal stem cells (MSCs) have been studied because of their capacity for healing tissue injury. MSC transplantation is a valid approach for promoting the repair of damaged tissues and replacement of lost cells or to safeguard surviving cells, but currently the efficiency of MSC transplantation is constrained by the extensive loss of MSCs during the short post-transplantation period. Hence, strategies to increase the efficacy of MSC treatment are urgently needed. Iron overload, reactive oxygen species deposition, and decreased antioxidant capacity suppress the proliferation and regeneration of MSCs, thereby hastening cell death. Notably, oxidative stress (OS) and deficient antioxidant defense induced by iron overload can result in ferroptosis. Ferroptosis may inhibit cell survival after MSC transplantation, thereby reducing clinical efficacy. In this review, we explore the role of ferroptosis in MSC performance. Given that little research has focused on ferroptosis in transplanted MSCs, further study is urgently needed to enhance the in vivo implantation, function, and duration of MSCs.

Key words: Mesenchymal stem cells (MSCs); Ferroptosis; Oxidative stress (OS); Iron metabolism; Lipid peroxidation; Glutathione peroxidase 4 (GPX4)

1 Introduction

Mesenchymal stem cells (MSCs) are multipotent cells that can be differentiated into osteoblasts, adipocytes, and chondrocytes. In recent years, MSCs have been investigated for curing a diverse range of diseases, and much has been learned from in vivo and in vitro models (Wang S et al., 2022; Zhang L et al., 2022a). Considerable evidence has demonstrated that MSC transplantation can promote the recovery of cell viability and regeneration of cells to heal tissue injury and degeneration (Ma T et al., 2022; Shao et al., 2022; Zhang L et al., 2022b). However, the perturbation microenvironment in grafted areas, especially in hypoxia- or ischemia-injured tissues, imposes major obstacles to the clinical application of MSCs (Hamid et al., 2022). For example, in the hypoxic, acidic, and hypertonic environments of an intervertebral disc center, transplanted MSCs are in a state of oxidative stress (OS), thus affecting their biological functions and therapeutic efficacy (Zhou ZM et al., 2022). Under OS, excess reactive oxygen species (ROS) weaken the cellular antioxidant capacity and contribute to cell death (Shi et al., 2022). This process also involves iron deposition and lipid peroxide accumulation, which are reminiscent of ferroptosis (Noubari et al., 2022; Teli et al., 2022). Recent studies have shown that ferroptosis affects the efficacy of MSC transplantation (Xu et al., 2021; Tan et al., 2022). Previous mechanisms could not completely explain the inferior survival rate of transplanted MSCs. Hence, we investigated the mechanisms of ferroptosis in MSCs to improve the potential for future applications of MSC transplantation.

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2 Basic properties of ferroptosis

Ferroptosis is a novel category of regulated cell death that is morphologically and biochemically different from pyroptosis, necroptosis, autophagy, and apoptosis (Dixon et al., 2012; Conrad and Pratt, 2019; Lei et al., 2019; Su et al., 2019; Lin et al., 2020; Liu et al., 2020; Macías-Rodríguez et al., 2020; Minagawa et al., 2020; Su et al., 2020; Tang et al., 2020; Christidi and Brunham, 2021; Koren and Fuchs, 2021) (Table 1). From the perspective of morphology, ferroptotic cells are characterized by shrunken mitochondria, reduced or absent mitochondrial ridges, and condensed mitochondrial membranes. These abnormalities are attributed to OS, which leads to the absence of selective permeability of the plasma membrane (Su et al., 2019). The key biochemical features of ferroptosis are the depletion of unsaturated fatty acids in the plasma membrane and an increased ferrous ion concentration, thus accelerating the accumulation of iron-dependent lipid peroxides (Conrad and Pratt, 2019). Lipid peroxidation damages the cellular membrane structure, leading to cell death (Minagawa et al., 2020). Another central regulator of ferroptosis is glutathione peroxidase 4 (GPX4), which specifically catalyses the reduction of lipid peroxides to the corresponding alcohol (Tang et al., 2020). Therefore, the absence or inactivation of GPX4 results in the accumulation of lipid peroxides, which represents the lethal signal of ferroptosis. The level of GPX4 is strictly controlled by the Xc^- system, which sustains the synthesis of glutathione (GSH) and provides oxidative protection (Macías-Rodríguez et al., 2020). GSH serves as a cofactor of GPX4. In addition, ferroptosis can be suppressed by radical-trapping antioxidants (e.g., vitamin E (α-tocopherol) and ferrostatin-1 (Fer-1)) or iron chelators (e.g., deferoxamine (DFO)) (Liu et al., 2020). Initially, a unique set of genes, e.g., iron response element-binding protein 2, heme oxygenase-1 (HO-1), ribosomal protein L8, adenosine triphosphate (ATP) synthase F0 complex subunit C3 and acetyl-CoA synthetase family member, was thought to be involved in the ferroptotic mechanism

Table 1 Comparison of characteristics of ferroptosis, necroptosis, apoptosis, autophagy, and pyroptosis

Category	Morphological features	Biochemical features
Ferroptosis	Shrunken mitochondria (Su et al., 2020); Condensed mitochondrial membrane (Dixon et al., 2012); Reduced or absent mitochondrial ridges (Lei et al., 2019); Rupture of the outer membrane (Dixon et al., 2012; Conrad and Pratt, 2019)	ROS and iron deposition (Lei et al., 2019); Inhibition of Xc^- system (Dixon et al., 2012); GSH exhaustion (Lin et al., 2020); Suppression of GPX4 (Conrad and Pratt, 2019); Accumulation of iron-dependent lipid peroxidation (Lin et al., 2020; Su et al., 2020)
Necroptosis	Plasma membrane breakdown (Minagawa et al., 2020); Swelling of organelles and cytoplasm (Koren and Fuchs, 2021); Moderately condensed chromatin (Tang et al., 2020); Liberated cell contents (Minagawa et al., 2020)	Caspase inhibition (Tang et al., 2020); Drop in ATP level (Conrad and Pratt, 2019)
Apoptosis	Cell and nuclear volume reduction (Su et al., 2019); Condensation of nuclear chromatin (Christidi and Brunham, 2021); Nuclear disruption (Su et al., 2019); Blebbing of plasma membrane (Lei et al., 2019); Rounding up of the cell (Koren and Fuchs, 2021); Generation of apoptotic bodies (Macías-Rodríguez et al., 2020)	Caspase activation (Macías-Rodríguez et al., 2020); DNA fragmentation (Koren and Fuchs, 2021); Phosphatidylserine exposure (Macías-Rodríguez et al., 2020)
Autophagy	Formation of double-membraned autolysosomes (Koren and Fuchs, 2021); Lysosome degradation of the substrate (Liu et al., 2020)	Conversion from LC3-I to LC3-II (Liu et al., 2020); Substrate degradation (Christidi and Brunham, 2021); Elevated lysosomal vitality (Koren and Fuchs, 2021)
Pyroptosis	Karyopyknosis (Christidi and Brunham, 2021); Cytoplasmic swelling (Koren and Fuchs, 2021); Plasma membrane rupture (Tang et al., 2020); Osmotic lysis of the cell (Koren and Fuchs, 2021)	Liberation relied on caspase-1 and proinflammatory cytokine (Tang et al., 2020; Christidi and Brunham, 2021)

ROS: reactive oxygen species; GSH: glutathione; GPX4: glutathione peroxidase 4; ATP: adenosine triphosphate; LC3: microtubule-associated protein light chain 3.

(Chen Z et al., 2022; Liu and Gu, 2022). Subsequent studies showed that this specific oxidative and nonapoptotic cell death process involves more proteins and genes, including tumor repressor p53, mitogen-activated protein kinase (MAPK), cyclooxygenase-2, nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidases (NOXs), nuclear factor erythroid 2-related factor 2 (Nrf2), and transferrin (Tf) receptor 1 (TfR1) (Forcina et al., 2022; Miao et al., 2022; Yuan et al., 2022). Even though the exact mechanisms are unclear, ferroptosis is tightly connected with iron, ROS, lipid peroxidation, and antioxidant metabolism.

3 Ferroptosis in MSCs

MSCs have recently been studied because of their proliferative and regenerative properties. They can repair degenerative and injured tissues by directly differentiating into various cells and secreting diverse paracrine factors (Deng et al., 2022; Ma JJ et al., 2022). However, when grafted into a target area, MSCs are affected by several ambient factors. One of the most important factors is OS, which results in the loss of grafted MSCs at the transplant site (Ma JJ et al., 2022). Therefore, methods to handle MSCs should be established to reduce ROS in the deleterious oxidising microenvironment to improve MSC engraftment and enhance tissue repair. Iron is essential for the proliferation and differentiation of cells, including MSCs (Buschhaus et al., 2022), but iron accumulation has been clearly proven to induce OS and hinder the self-renewal of MSCs (di Paola et al., 2022). Ferroptosis is considered as an iron overload that triggers OS, thereby contributing to subsequent cell death. Thus, inhibiting ferroptosis is expected to increase the efficacy of MSC therapy.

3.1 Iron metabolism in MSCs

The human body carefully controls cellular iron homeostasis. Irreversible damage may occur once iron levels exceed the storage capacity of a cell. Ferroptosis induces damage typically by iron overload. Ferric iron (Fe^{3+}) can be recognized by Tf and is transported into the cell through TfR1 (Brown et al., 2019). Fe^{3+} is then deposited in the endosome and converted into ferrous iron (Fe^{2+}) by the six-transmembrane epithelial antigen of prostate 3 (STEAP3) (Battaglia et al., 2020). Divalent metal transporter 1 (DMT1) transports the unstable Fe^{2+} from the endosome to the cellular labile iron pool (LIP) in the cytoplasm (Pandrangi et al., 2022). Excess irons can either be stored as Fe^{3+} in ferritin or released extracellularly by ferroportin to sustain the LIP at an appropriate level to avoid cell toxicity (Zhang and Li, 2022) (Fig. 1). As the chief iron reservoir protein in the cell, ferritin comprises the ferritin light chain (FTL) and ferritin heavy chain 1 (FTH1). Liu J et al. (2022) found that the overexpression of NOP2/Sun RNA methyltransferase 5 (NSUN5) elevates the expression levels of the FTL and FTH1 proteins to resist ferroptosis in bone marrow MSCs (BMSCs) (Fig. 2).

The disruption of iron metabolism homeostasis in MSCs reduces their capabilities for pluripotent differentiation and self-renewal. In the in vitro model of BMSCs, iron overload induced by ferric ammonium citrate suppresses osteogenic differentiation and cell multiplication (Yao et al., 2019). Furthermore, iron accumulation upregulates phosphorylated extracellular signal-regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK) in rat BMSCs (Yao et al., 2019) and increases the protein expression of ERK in mouse BMSCs (Yang et al., 2017), indicating mitogen-activated protein kinase (MAPK)/ERK and MAPK/JNK pathway activation. In mouse BMSCs, iron overload also elevates the expression levels of phospho-p38 and p38 (Yang et al., 2017; Shen et al., 2018). Other studies showed that the activation of MAPK signalling pathways triggers ferroptosis (Park et al., 2019; Song QX et al., 2022). Moreover, inhibiting MAPK pathways prevents the occurrence of ferroptosis in lung cancer cells (Poursaitidis et al., 2017). Therefore, we speculated that iron accumulation may motivate ferroptosis in MSCs through the activation of MAPK pathways (Fig. 2). Interestingly, in the iron-overload model of umbilical cord-derived MSCs, the expression of p53 is elevated in conditions of ferric ammonium citrate (FAC)-induced oxidative damage, which may be attributed to the activation of the p38-MAPK signalling pathway (Lu et al., 2013). However, Xie et al. (2017) suggested that upregulated p53 expression represses ferroptosis induced by erastin in human colorectal cancer (CRC) cells. p53 exhaustion blocks the nuclear deposition of dipeptidyl peptidase 4 (DPP4), thereby increasing the interaction between DPP4 and nicotinamide adenine dinucleotide phosphate oxidases 1

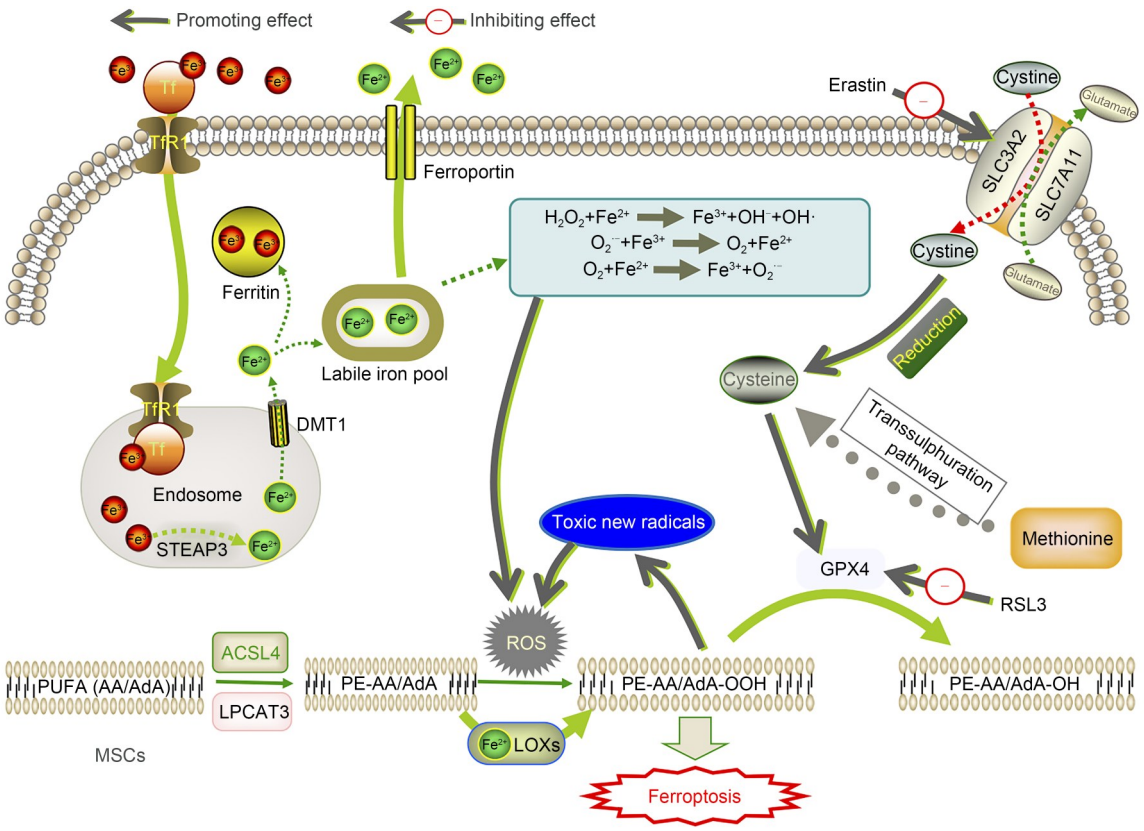

Fig. 1 Molecular mechanisms of ferroptosis in MSCs. MSCs: mesenchymal stem cells; Tf: transferrin; TfR1: transferrin receptor 1; STEAP3: six-transmembrane epithelial antigen of prostate 3; DMT1: divalent metal transporter 1; PUFA: polyunsaturated fatty acid; AA/AdA: arachidonic acid/adrenic acid; PE: phosphatidylethanolamine; ACSL4: acyl-CoA synthetase long-chain family membrane 4; LPCAT3: lysophosphatidylcholine acyltransferase 3; LOXs: lipoxygenases; GPX4: glutathione peroxidases 4; ROS: reactive oxygen species; RSL3: RAS-selective lethal 3; SLC7A11: solute carrier family 7 member 11; SLC3A2: solute carrier family 3 member 2.

(NOX1). This process is essential for lipid peroxidation and results in ferroptosis in CRC cells (Xie et al., 2017) (Fig. 3). Differences between CRCs and MSCs might explain these conflicting results. Nevertheless, the role of p53 in iron-induced oxidative damage in MSCs needs further study. Khoshlahni et al. (2020) demonstrated that DFO effectively restored the viability of BMSCs to normal levels in iron-overload circumstances. Lactoferrin participates in the transport of ferritin and has as an important role in iron homeostasis. Park et al. (2017) found that the pretreatment of human MSCs with lactoferrin effectively retrieved cell vitality and decreased cell death in hydrogen peroxide (H_2O_2)-induced conditions, which may involve the phosphorylation of serine/threonine kinase (AKT). The activation of AKT can mediate the regulation of sirtuin 3 (SIRT3) on acyl-CoA synthetase long-chain family 4 (ACSL4), which inhibits ferroptosis (Liu et al., 2021) (Fig. 3).

3.2 Oxidative stress in MSCs

ROS are highly reactive oxygen-containing molecules, including hydroxyl radicals (OH·), superoxide anion radicals ($O_2^{\cdot-}$), H_2O_2, peroxyl radicals (ROO·), lipid hydroperoxides (L-OOH), and alkoxyl radicals (LO·) (Chen GH et al., 2022). The cytosolic Fe^{2+} iron constituting LIP has high chemical reactivity and can participate in the Fenton reaction directly, thereby generating highly reactive ROS (Hu et al., 2022) (Fig. 1). Excess OH· contributes to protein oxidation, resulting in protein structural change and dysfunction, oxidation of DNA, and disruption of the cell membrane (Yu et al., 2022). Surplus lipid ROS also leads to the generation of OS, which is defined as an imbalance between ROS production and elimination. With decreased antioxidant capacity, MSCs are more vulnerable to OS than differentiated cells (Orciani et al., 2010; Ko et al., 2012). Furthermore, MSCs from individuals

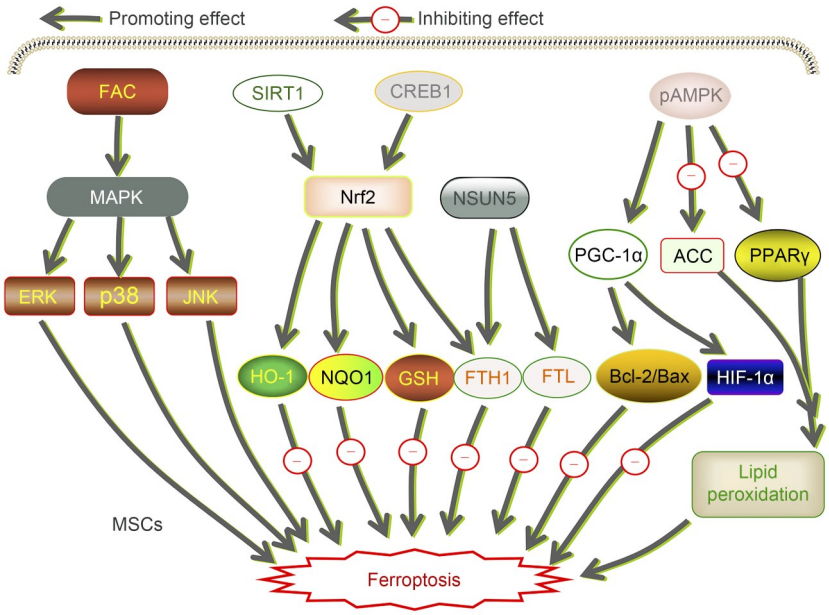

Fig. 2 Pathways promoting or inhibiting ferroptosis in MSCs. FAC: ferric ammonium citrate; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; SIRT1: sirtuin 1; CREB1: cyclic adenosine monophosphate (cAMP)-responsive element-binding protein 1; Nrf2: nuclear factor erythroid 2-related factor 2; NSUN5: NOP2/Sun RNA methyltransferase 5; HO-1: heme oxygenase-1; NQO1: nicotinamide adenine dinucleotide phosphate (NAD(P)H) quinone oxidoreductase 1; GSH: glutathione; FTH1: ferritin heavy chain 1; FTL: ferritin light chain; pAMPK: phosphorylated adenosine monophosphate (AMP)-activated protein kinase; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator-1α; ACC: acetyl-CoA carboxylase; PPARγ: peroxisome proliferator-activated receptor γ; Bcl-2/Bax: B-cell lymphoma 2/Bcl-2-associated X protein; HIF-1α: hypoxia-inducible factor-1α; MSCs: mesenchymal stem cells.

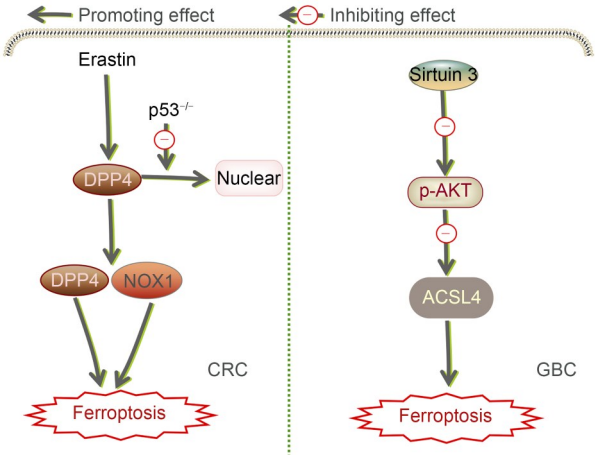

Fig. 3 Pathways promoting or inhibiting ferroptosis in CRC and GBC. CRC: colorectal cancer; GBC: gall bladder cancer; DPP4: dipeptidyl peptidase 4; NOX1: nicotinamide adenine dinucleotide phosphate hydride oxidases 1; p-AKT: phospho-serine/threonine kinase; ACSL4: acyl-CoA synthetase long-chain family 4.

with coronary artery disease display high levels of intracellular ROS (Kizilay Mancini et al., 2018). Therefore, transplanting MSCs into a pre-existing OS microenvironment could disrupt the intracellular metabolism of MSCs, leading to iron overload and toxic ROS accumulation. OS caused by ROS affects the survival rate of transplanted MSCs. In the in vitro model of erastin-treated BMSCs, Li et al. (2020) reported that quercetin (a flavonol antioxidant) inhibits ROS deposition and restores cell proliferation to normal levels, thereby protecting cells from ferroptosis. Mohammadi et al. (2021) found that astaxanthin (ATX, an antioxidant) can weaken functional disturbance caused by H_2O_2 stimulation by upregulating Nrf2 expression to combat OS. Nrf2 blocks the occurrence of ferroptosis by enhancing the expression of HO-1 and NAD(P)H quinone oxidoreductase 1 (NQO1), both of which are involved in iron and ROS metabolisms (Fig. 2). These results support the opinion that transplanted MSCs may undergo ferroptosis as a result of OS.

Evidence supporting a direct role of OS in causing ferroptosis in MSCs is lacking. Regulating SIRT is a potential method to reduce OS in MSCs (Denu and Hematti, 2016). As nicotinamide adenine dinucleotide

(NAD(+))-dependent deacetylases, SIRTs can protect against age-associated pathologies, including neurodegenerative diseases, cancers, and metabolic diseases. The knockdown of *SIRT1* suppresses the differentiation and proliferation of MSCs, whereas the overexpression of SIRT1 shows the opposite results (Yuan et al., 2012). SIRT1 deacetylates Nrf2. Activated Nrf2 translocates into the nucleus and enhances HO-1 function to eliminate ROS (Patel et al., 2022) (Fig. 2). In mice and cells treated with acetaminophen, the downregulation of the SIRT1/Nrf2/HO-1 pathway greatly increases ROS and 4-hydroxynonenal (4-HNE) levels, ultimately aggravating ferroptosis (Wang C et al., 2021). The downregulation of SIRT1 is restored by ulinastatin to ameliorate ferroptosis (Wang C et al., 2021). In addition, many enzymes and proteins that avoid lipid peroxidation and inhibit ferroptosis are target genes of Nrf2 (Dodson et al., 2019). In the doxorubicin-induced cardiotoxicity model of rats, increased SIRT1 and Nrf2 expression levels also activate the expression of FTH1, thereby restricting the iron level and attenuating ferroptosis (Li DL et al., 2022a) (Fig. 2). SIRT3 is localised in the mitochondria, which are major sites of ROS generation and have a vital role in ferroptosis (Wang CF et al., 2021). SIRT3 improves the efficiency of the electron transport chain by reprogramming the metabolism of mitochondria, which is thought to decrease ROS production (Wang LJ et al., 2022). Moreover, SIRT3 deacetylates and activates manganese superoxide dismutase, which can counteract ROS (Hayashi et al., 2022). Brown et al. (2013) revealed that SIRT3 is indispensable for maintaining haematopoietic stem cell homeostasis under OS or old-aged conditions. SIRT6 regulates the repair, transcription, and metabolism of DNA in response to OS (Munnur and Ahel, 2017; Tatone et al., 2018). For example, SIRT6 stimulates the activity of poly(ADP-ribose) polymerase 1 (PARP1) and elevates the repair efficiency of double-stranded DNA in OS conditions (Mao et al., 2011). *SIRT6* knockdown inhibits the osteogenesis of MSCs derived from rats (Sun et al., 2014). The roles of SIRT3 and SIRT6 in ferroptosis have not been clarified in detail and indicate a significant direction for future research.

3.3 Lipid peroxidation in MSCs

Polyunsaturated fatty acids (PUFAs) are vital for physiological functions and membrane fluidity. PUFAs are esterified into membrane phospholipids (PLs; e.g., phosphatidylethanolamines (PEs)) and can be oxidised by ROS in a nonenzymatic process and generate lethal lipid peroxides, which are crucial in promoting ferroptosis (Fan et al., 2022; Qu et al., 2022). OH· react with PUFAs in membrane PLs particularly arachidonic acid (AA) and adrenic acid (AdA), thereby promoting the formation of lipid peroxyl radicals and lipid radicals (Liu MY et al., 2022) (Fig. 1). Subsequently, these lipid ROS undergo chain reactions with PUFAs to generate lipid peroxides, thus initiating or propagating oxidative fragmentation of PUFAs (Jiang et al., 2022). In the presence of iron, LO· are converted from lipid peroxides and react with adjacent PUFAs, thus triggering lipid autoxidation via a chain of autocatalytic radical reactions, producing ROS and damaging cellular membranes (Zhang YC et al., 2022). L-OOH are usually believed to be the crucial mediators of ferroptosis due to their capacity to modify the structure and function of the membrane. In addition, L-OOH are unstable and easily decompose into secondary products that generate additional toxicity (Li DL et al., 2022b). As degradation products, malondialdehyde (MDA) and 4-HNE can amplify cellular impairment by attacking proteins or DNA (Khan et al., 2022). The formation of lipid peroxides catalysed in an iron-enzymatic manner also plays an important role. Three enzymes are involved in the conversion of AA/AdA to PE-AA/AdA-OOH, including lysophosphatidylcholine acyltransferase 3 (LPCAT3), ACSL4, and lipoxygenases (LOXs) (Wang Y et al., 2022). The functions of ACSL4 and LPCAT3 are important for the biosynthesis and remodelling of PEs, whereas LOXs catalyse free PUFAs to generate lipid peroxides (Zhu et al., 2022) (Fig. 1).

Adenosine monophosphate (AMP)-activated protein kinase (AMPK), which functions as a redox and energy sensor, plays a vital role in preserving the normal function of MSCs. The activated AMPK signalling pathway increases peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) expression (Lee et al., 2006) and subsequently elevates the hypoxia-inducible factor-1α (HIF-1α) expression level, B-cell lymphoma 2/Bcl-2-associated X protein (Bcl-2/Bax) ratio, proangiogenic-related factor expression level, and survival rate of MSCs (Lu et al., 2012) (Fig. 2). AMPK has also been reported to regulate lipid peroxidation, thus engaging in ferroptosis. Acetyl-CoA

carboxylase (ACC) converts acetyl-CoA into malonyl-CoA and expedites fatty acid synthesis (Vancura et al., 2018; Lee et al., 2020). Lee et al. (2020) discovered that cotreatment with erastin or RAS-selective lethal 3 (RSL3) and 5-(tetradecyloxy)-2-furoic acid (an inhibitor of ACC) alleviates lipid peroxide accumulation and inhibits ferroptosis in immortalised mouse embryonic fibroblasts (MEFs). Phosphorylated AMPK inhibits the activity of ACC, thereby reducing fatty acid synthesis (Fig. 2). Lipidomic analyses showed that AMPK deletion increases the biosynthesis of PUFAs, thus elevating the sensitivity to ferroptosis (Lee et al., 2020). Consistent with this, *ACSL4* knockdown notably blocks ferroptosis induced by erastin in AMPK deletion MEFs (Lee et al., 2020). Peroxisome proliferator-activated receptor γ (PPARγ) is thought to be an additional signal that strengthens the synthesis of fatty acids (Chyau et al., 2020). Han et al. (2021) proved that PPARγ participates in lipid metabolism and drives ferroptosis induced by RSL3. Phospho-AMPK inhibits the expression level of PPARγ, thereby blocking lipid synthesis and alleviating lipid peroxide accumulation (Fig. 2). Several studies also detected the vital role of PPARγ in the differentiation and commitment of MSCs (Li Y et al., 2018; Ko et al., 2022; Svensson et al., 2022). Therefore, understanding the function of the AMPK/PPARγ signalling pathway underlying ferroptosis in MSCs will help improve transplantation and regeneration therapies in degenerative diseases. Although lacking direct evidence, the hypothesis that AMPK signalling regulates ferroptosis in MSCs is worthy of attention.

3.4 Xc^- system/GSH/GPX4 axis in MSCs

A complex network of antioxidant defence systems is established in cells to combat excessive OS and maintain redox homeostasis. The Xc^- system is a sodium- and chloride-dependent antiporter composed of solute carrier family 3 member 2 (SLC3A2) and solute carrier family 7 member 11 (SLC7A11) (Chen Y et al., 2022). The Xc^- system can input cystine and output glutamate at a ratio of 1:1. Cystine is changed into cysteine via the reduction reaction and synthesises GSH, which maintains the homeostasis of cellular redox and serves as a cofactor of GPX4 (Feng et al., 2022). GPX4 can degrade lipid peroxides directly and maintain oxidation–reduction balance (Yang et al., 2022) (Fig. 1). Cysteine starvation can lead to the depletion of GSH, which affects the efficiency of GPX4 synthesis and subsequently results in lipid peroxide accumulation and ferroptotic death. As a ferroptotic-inducing molecule, erastin binds the Xc^- system directly and inhibits its function, resulting in the inactivation of GPX4 (Liang et al., 2022). Thus, erastin has a pro-ferroptotic effect by indirectly restraining the activity of GPX4. Notably, a complementary mechanism is enhanced by inhibition of Xc^- system. This result is the intracellular upregulation of SLC7A11, which can oppose erastin-induced ferroptosis (Fu et al., 2022). Despite the predominance of extracellular uptake of cystine, the trans-sulfuration pathway (TSP) also performs a compensatory role for sourcing cysteine. Under cysteine-insufficient conditions, methionine can be converted into cysteine to counter the OS response via the TSP (Rodríguez-Graciani et al., 2022). RSL3 acts as a straight inhibitor of GPX4 by binding and inactivating it, impeding the process of lipid peroxide degradation (Liu M et al., 2022).

High levels of ROS and resultant OS induce ferroptosis. OS can be aggravated by a weakened defence ability of antioxidants, including GPX4 and GSH. GSH is proven to be crucial for maintaining the migration and stemness abilities of MSCs, which determine their efficiency of therapy in chronic musculoskeletal diseases (Lim et al., 2020). Ferroptosis is attributed not only to the excessive accumulation of iron, but also to the limitation of antioxidant defense pathways with iron levels unchanged. Hence, the disruption of GSH homeostasis in MSCs also leads to the initiation of ferroptosis. Recognised as regulating redox homeostasis, Nrf2 transcribes GSH metabolic genes to preserve GSH stability. Lim et al. (2020) found that cyclic adenosine monophosphate (cAMP)-responsive element-binding protein 1 (CREB1) upregulates Nrf2 expression to stabilise the redox homeostasis of MSCs, thereby promoting the enhancement of the therapeutic functions of MSCs (Fig. 2). Moreover, human umbilical cord MSCs with a cascade of CREB1-Nrf2 signalling show increased resistance to cell death induced by OS. To further verify the role of GSH in MSCs under OS, Bonilla-Porras et al. (2019) established a molecular model involving deletion of GSH. Interestingly, the cotreatment of buthionine sulfoximine (BSO, inhibitor of GSH) with 6-hydroxydopamine (6-OHDA, inducer of OS) induces cell death in MSCs by generating H_2O_2. The degree of OS and ratio of cell death are decreased

by treating MSCs with *N*-acetylcysteine (as a GSH synthesis precursor) and BSO+6-OHDA (Bonilla-Porras et al., 2019). These studies emphasised the significance of maintaining the stability of the GSH-dependent antioxidant system in protecting MSCs from oxidative injury, thereby increasing the proportion of grafted MSCs that survive transplantation. In an in vitro experiment of BMSCs, bleomycin (pulmonary fibrosis inducer) reduced cell vitality and caused MSCs to suffer OS (Li XH et al., 2018). The aggravated OS further strengthened the activity of Xc^- system, expediting the synthesis of GPX4 to counter oxidative injury. In the disease modeling of cerebral ischemic reperfusion injury (Zhai et al., 2022) and cardiac arrest (Xu et al., 2021), MSC treatment has been shown to upregulate GPX4 expression and suppress ferroptosis in host cells. We speculate that the increased defence capacity of host cells may be attributed to the antioxidants secreted by MSCs. However, owing to their susceptibility to OS, the functions of MSCs, including paracrine migration and proliferation, remain inhibited, perhaps because of ferroptosis of the grafted MSCs in the transferred region.

3.5 Other ferroptosis-related pathways in MSCs

In addition to the classical ferroptosis-related pathways mentioned above, BTB and CNC homology 1 (BACH1) also affect susceptibility to ferroptosis (Nishizawa et al., 2022). As an OS-responsive transcription factor, BACH1 can promote ferroptosis by suppressing the expression of FTL, FTH1, and SLC7A11 (Nishizawa et al., 2022). By modifying the expression of these genes, BACH1 may affect the regulation of LIP, generation of GSH, and degradation of lipid peroxides by GPX4. The expression of BACH1 is stable under the condition of homeostasis and inhibits several target genes of Nrf2 (Hushpulian et al., 2021). When intracellular ROS levels are increased, BACH1 is inactivated and thus elevates the expression of genes induced by Nrf2, which are involved in combatting ROS (Nishizawa et al., 2022). However, when cells are under OS, the activity of Nrf2 is decreased while the activity of BACH1 is increased, leading to ferroptosis (Lignitto et al., 2019). Wang D et al. (2022) found that lipopolysaccharide-pretreated MSCs significantly improved hypoxia/reoxygenation-induced injury in cardiomyocytes through the inactivation of BACH1. Further studies revealed that the knockdown of *BACH1* could increase the protective benefits of MSCs on injured cardiomyocytes, as evidenced by decreased levels of cardiac troponin and lactic dehydrogenase and elevated expression of hepatocyte growth factor and vascular endothelial growth factor (Wang D et al., 2022).

HIFs are indispensable factors that maintain the homeostasis of oxygen at the gene transcription level and participate in modulating multiple proteins related to iron metabolism (Song YH et al., 2022). In combination with hypoxia response elements, HIF-1 can activate TfR transcription (Song YH et al., 2022). Additionally, upregulated expression of HIF-1α and HIF-2α can elevate ferroportin and DMT1 expression in astrocytes (Yang et al., 2012). The autophagic degradation of ferritin, called ferritinophagy, can facilitate ferroptosis by increasing LIP (Zhou H et al., 2022). Ni et al. (2021) showed that HIF-1α could restrain ferritinophagy by inhibiting the formation of autophagosomes, thereby avoiding ferroptosis in osteoclasts. HIF-1α also has the ability to repress ferroptosis by upregulating HO-1 and Nrf2 expression and expediting the synthesis of GSH (Li DL et al., 2022a). HIF-1α is degraded by prolyl hydroxylases (PHDs), which requires the participation of iron ions (Song QX et al., 2022). Yang et al. (2018) showed that knockdown or inhibition of PHDs significantly ameliorated OS-induced kidney tubular injury. But when intracellular GSH is depleted, increased iron levels are bound to activate PHDs, promoting the occurrence of ferroptosis (Haase, 2021). MSCs have powerful stabilizing effects for treating hypoxic-ischemic injury. However, the insufficient amount of engrafted MSCs in injured regions has restricted their therapeutic potential. Therefore, great attention has been devoted to solving the low survival and efficiency in MSCs homing to damaged tissues. In the rat model of hypoxic-ischemic brain injury, overexpression of HIF-1α in MSCs greatly enhanced their vitality and migration ability. Their therapeutic efficiency was also improved, as shown by the greater number of MSCs migrating to the injured region and alleviating histological and behavioral changes (Lin et al., 2017). Zhou et al. (2020) also showed that HIF-1α could drive biological effects in MSCs by increasing their viability and migration ability. Interestingly, in in vitro experiments, DFO upregulated the expression of genes associated with angiogenic and osteogenic differentiation in

MSCs via activation of HIF-1α, thus promoting tissue regeneration and repair (Ran et al., 2018). Given the important role of ferroptosis in the transplantation therapy of MSCs and that HIF-1α has been proven to resist ferroptosis, we propose that HIF-1α inhibitors may have a pro-ferroptosis effect in MSCs. As expected, Meng et al. (2018) reported that the application of the HIF-1α inhibitor KC7F2 restrains the migration and survival capacity of MSCs. But more studies need to be undertaken to support this opinion.

4 Inhibition of ferroptosis in MSCs

In the field of transplantation engineering, MSCs are viewed as crucial seed cells for treating degenerative diseases such as degenerative joint and bone diseases and neurodegenerative diseases (Staff et al., 2019; Herger et al., 2022). However, studies have shown that MSC transplantation associated with OS increases the risk of failure. The perturbations of the extracellular microenvironment increase lipid ROS and weaken antioxidant capacity, inducing cellular death through ferroptosis; therefore, inhibiting ferroptosis in MSCs is a promising new therapeutic strategy. Shatrova et al. (2021) reported that the application of DFO prevented the deposition of lipid ROS in human endometrial stem cells. Pretreatment with DFO before transplantation of MSCs obviously enhanced stem cell homing in the target region, resulting in enhanced efficiency of cell therapy (Peyvandi et al., 2018). OS is considered a harmful factor for osteogenesis. Polycystin-1 (PC1, also known as PKD1, encoded by *PKD1*) is associated with skeletogenesis and bone remodeling (Chen et al., 2021). Zhang et al. (2021) observed that cells lacking PKD1 exhibited typical features of ferroptosis, including reduced expression of Xc^- system, GPX4, and ferroportin, and increased expression of TfR1 and DMT1, which in turn caused high ROS levels and the generation of OS. In rat BMSCs, researchers found that cell vitality and PKD1 expression were reduced in a time- and dose-dependent manner by H_2O_2, but their further studies showed that PKD1 could appropriately extenuate OS-suppressed rat BMSC osteogenesis by activating transcriptional coactivator with PDZ-binding motif (TAZ) (Chen et al., 2021). Vitamin E is an effective lipid antioxidant and thus could restrain lipid peroxidation, thereby inhibiting ferroptosis (Villalón-García et al., 2022). Bhatti et al. (2017) showed that vitamin E pretreatment could upregulate proliferative markers and the cell viability of grafted MSCs. Pretreated MSCs resisted H_2O_2-induced OS in vitro, and this phenomenon was recapitulated in a rat model of osteoarthritis. Xc^- system, GSH, and GPX4 are involved in establishing the antioxidant defence system and can be regulated to resist ferroptosis. Studies have shown that all-trans retinoic acid (ATRA) has a meaningful level of antioxidant activity in vitro (Yucel et al., 2019). Also, pretreatment of Wharton's jelly MSCs with ATRA extenuated OS, as evidenced by an obvious elevation in GSH concentrations (Barakat et al., 2022).

5 Research prospects

Recently, MSCs research has attracted broad attention in the domain of tissue engineering and regeneration. The potential therapeutic benefits of MSCs can be divided into direct differentiation and the secretion of various cytokines and growth factors, which enhance protective efficiency in injured tissues, limit further inflammation, promote proliferation, and motivate angiogenesis (Barrachina et al., 2020). These functions revealed the possible value of MSCs in future clinical applications, but therapeutic effectiveness is highly dependent on the transplantation, migration, and vitality of MSCs in the degenerated and damaged region. OS is one of the major factors facilitating cell death in vivo and in vitro. The reduction of OS, by chelating iron ions or adding radical-trapping antioxidants such as DFO or vitamin E, has been proven to prolong the lifespan of human MSCs while preserving their differentiation potential (Jin et al., 2010; Lang et al., 2022). After the transplantation of MSCs into a targeted region, a range of molecular processes are involved in the interaction between OS and the process of ferroptosis, including iron deposition, lipid peroxidation, and GSH exhaustion. In this review, we have described the damaging role of ferroptosis in MSCs in detail. However, experimental research on ferroptosis in MSCs is still lacking, and the protective role of ferroptosis inhibitors in transplanted MSCs engrafted into target areas has not been fully clarified. But the long-established effects of targeting ferroptosis in other cell lines imply the potential value of this

approach. Given that little research has focused on ferroptosis in transplanted MSCs, more studies are required to identify and validate relevant findings. Such studies could also explore new ways of improving the therapeutic efficiency of MSC treatments.

6 Conclusions

Although MSCs have achieved some therapeutic effects in the early stages of clinical application, there is great potential to expand their use. This potential has not been fully exploited for several reasons. One of the reasons is that oxidative injury is induced by OS, which affects the engraftment and function of MSCs in vivo. As an OS-induced regulated cell death, ferroptosis is characterised by the iron-dependent accumulation of lethal ROS and lipid peroxides and plasma membrane rupture, ultimately leading to cell death. Therefore, methods to inhibit ferroptosis in MSCs should be established to enhance their proliferative and regenerative capacities to achieve their therapeutic potential. Iron chelators and radical-trapping antioxidants perhaps represent a novel avenue to accomplish this and are worthy of further investigation in MSCs.

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Author contributions

Yuzhu XU and Yuntao WANG designed the manuscript. Yuzhu XU, Pan FAN, and Yuntao WANG wrote the manuscript. Yuzhu XU, Lei LIU, Xuanfei XU, Lele ZHANG, and Xiaolong LI provided opinions. Yuzhu XU, Jiadong WANG, Yuao TAO, and Xi LI were involved in the discussion. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Yuzhu XU, Pan FAN, Lei LIU, Xuanfei XU, Lele ZHANG, Jiadong WANG, Yuao TAO, Xiaolong LI, Xi LI, and Yuntao WANG declare that they have no conflict of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

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