Ferroptosis and renal fibrosis: A new target for the future (Review)

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Abstract. Ferroptosis is a type of non-apoptotic controlled cell death triggered by oxidative stress and iron-dependent lipid peroxidation. Ferroptosis is regulated by signalling pathways that are associated with metabolism, including glutathione peroxidase 4 dysfunction, the cystine/glutamate antiporter system, lipid peroxidation and inadequate iron metabolism. Ferroptosis is associated with renal fibrosis; however, further research is required to understand the specific molecular mechanisms involved. The present review aimed to discuss the known molecular mechanisms of ferroptosis and outline the biological reactions that occur during renal fibrosis that may be associated with ferroptosis. Further investigation into the association between ferroptosis and renal fibrosis may lead to the development of novel treatment methods.

Contents

1. Introduction

Regulated cell death (RCD) is a key biological mechanism in the body that is required for healthy development, homeostasis maintenance and disease prevention. RCD primarily includes apoptosis, necroptosis, autophagy and ferroptosis (1). Ferroptosis is fuelled by oxidative stress and iron-dependent lipid peroxidation, which differs from apoptosis, necroptosis and autophagy morphologically, biochemically and genetically (2). It is characterized by increased intracellular free iron and accumulation of toxic lipid peroxides, leading to cell death (3,4). Previous research has demonstrated that renal fibrosis, which is defined by the breakdown of healthy kidney architecture, fibroblast proliferation and excessive extracellular matrix deposition, is a common pathological state of almost all types of chronic and progressive kidney disorder (5,6). Ferroptosis is closely associated with the pathological process of numerous renal diseases and plays a key role in numerous fibrotic diseases; however, the specific mechanisms underlying development of renal fibrosis remain to be fully elucidated. Ferroptosis serves a key role in the development of renal fibrosis, and a comprehensive understanding of this involvement may identify novel targets and approaches for the development of disease prevention and therapy.

2. Ferroptosis

Ferroptosis is an iron-dependent and lipotoxic RCD. Erastin is a cell-permeable substance that was discovered by Dolma *et al* (7) in 2003 using a high-content screening assay. In that study, it was demonstrated that erastin selectively inhibits genetically engineered cells with oncogenic RAS mutations without harming healthy cells. In 2012, Dixon *et al* (2) named erastin-induced iron-dependent non-apoptotic RCD as 'ferroptosis'. Ferroptosis is characterized by high levels of lipid peroxidation at the cytoplasmic membrane and/or intracellular locations, such as the mitochondria, endoplasmic reticulum or lysosome, and is a caspase-independent type of cell death (2,8-10). Morphologically, ferroptosis is characterized by decreased mitochondrial density, decreased or absent mitochondrial cristae and rupture of the outer mitochondrial membrane. Moreover, these features are accompanied by intact membranes, normal nuclear size and non-condensed chromatin (11). Biochemically, ferroptosis is characterized by accumulation of reactive oxygen species (ROS), increase of lipid peroxides and the deposition of intracellular iron ions (2). Ferroptosis is regulated by factors associated with metabolism, including glutathione peroxidase 4 (GPX4) dysfunction, the cystine (Cys)/glutamate (Glu) antiporter system (system Xc^{-1}), lipid peroxidation and iron metabolism dysfunction (12). It is also associated with signalling pathways, such as p53 (13), ferroptosis suppressor protein 1 (FSP1)/coenzyme Q_{10} (COQ10)/NAD(P)H (14), PI3K/Akt/mTOR (15), sequestosome 1 (p62)/Kelch-like ECH-associated protein 1

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(Keap1)/nuclear factor erythroid 2-related factor 2 (NRF2) (16) and autophagy-related (ATG) 5/ATG7/nuclear receptor coactivator (NCOA) 1 (17).

GPX4. The antioxidant enzyme GPX4 is a member of the GPXs family and a key target in the regulation of ferroptosis (18). GPX4 interrupts the lipid peroxidation chain reaction by reducing complex hydroperoxides (including phospholipid hydroperoxides and cholesterol hydroperoxides) to their corresponding Sub-units (19). GPX4 is a selenoprotein with selenocysteine (Sec) in its active site, and its active state requires the catalysis of glutathione (GSH). GPX4 activity is reduced or inactivated when GSH is depleted, and GPX4 also converts GSH to glutathione disulfide (GSSG), thereby reducing esterified oxidized fatty acids and cholesterol hydroperoxide, and reducing lipid hydroperoxide (L-OOH) to a nontoxic lipid hydroxy derivative (L-OH), thus resisting oxidative damage (13). During GPX4 maturation, Sec-tRNA is mediated by mevalonate, one of the key regulatory factors in positive regulation of the pathway product, isopentenyl pyrophosphate (13). Both erastin and RAS-selective lethal 3 (RSL3) compounds induce ferroptosis via inactivation of GPX4, according to Dixon *et al* (20). A recent study also demonstrated that erastin upregulates the expression of ATF3, which inhibits expression of GPX4, in addition to inactivating GPX4 and inhibiting its expression (21).

System Xc^-. System Xc^- is an antioxidant system located on the cell membrane and an amino acid antiporter that is broadly distributed in the phospholipid bilayer. It is a heterodimer consisting of two subunits and primarily consists of solute carrier family 7 member 11 (SLC7A11) and solute carrier family 3 member 2 (22). System Xc^- regulates the 1:1 ratio of Cys and Glu entering and leaving cells (23). The primary antioxidant in cells is the reducing agent GSH and GSH biosynthesis is limited by cysteine (2,24). Cys is reduced to cysteine in the cell and then synthesized into GSH. GSH impacts intracellular redox homeostasis and activates GPX4. Inhibiting activity of system Xc^- reduces the uptake of Cys, therefore impacting the synthesis of GSH. In turn, this decreases activity of GPX4 and the antioxidant capacity of cells and accumulates ROS, ultimately leading to oxidative damage and ferroptosis (25,26). Erastin impairs cellular antioxidant defenses by inhibiting system Xc^- mediated cystine uptake, thereby promoting the accumulation of ROS and thus ferroptosis (7). Embryonic fibroblasts derived from SLC7A11 knockout mice have ferroptotic cell death due to SLC7A11 gene deletion (27). And deletion of SLC7A11 gene in mice can lead to ferroptotic-like impairment (28). Chang *et al* (29) found that the increase of SLC7A11 significantly inhibited the occurrence of ferroptosis. This shows that system Xc^- plays an important role in the occurrence of ferroptosis.

Lipid peroxidation. Lipid metabolism is key in the process of ferroptosis and is a typical free radical chain reaction. Polyunsaturated fatty acids (PUFAs) are involved in almost all pathways of ferroptosis because they are susceptible to lipid peroxidation due to the presence of easily extractable hydrogen atoms at bis-allylic carbon positions (30). Any free radical that can extract hydrogen atoms from oxidizable substrates can initiate the lipid peroxidation process, and the abundance and location of intracellular lipid peroxidizable substrates determines the extent of lipid peroxidation and ferroptosis (8). Free PUFAs are substrates for synthesis of lipid signal transduction mediators, and these are esterified into membrane phospholipids during lipid metabolism and oxidized into ferroptosis signals (13). Lipidomic analysis has revealed that phosphatidylethanolamines (PEs) are key membrane phospholipids that drive ferroptosis by oxidizing phospholipid hydroperoxides [arachidonic acid (AA) and adrenic acid (AdA)-hydroperoxides-PE] via a non-enzymatic process (13,16). Acyl-CoA Synthetase is a long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyl-transferase 3 (LPCAT3) are two enzymes involved in the biosynthesis and remodeling of PE, which activate PUFA and affect its transmembrane properties (12). Therefore, blocking expression of ACSL4 and LPCAT3 inhibits the esterification of AA or AdA to PE and decreases accumulation of intracellular lipid peroxidative substrates, thereby inhibiting ferroptosis (13). As enzymatic effectors, lipoxygenases, of which free PUFAs are the preferred substrates, mediate the peroxidative reaction of ferroptosis (31). SLC38A1 is a regulator of glutamate uptake and metabolism in lipid peroxidation (32). Yang *et al* (33) found that lncRNA ZFAS1 could regulate the expression of SLC38A1 through miR-150-5p and activate the conversion of fibroblasts into myofibroblasts in lung tissue with the development of cytosolic iron death.

Iron metabolism dysfunction. Iron can form iron (Fe^{3+}) ions and ferrous (Fe^{2+}) ions, which are one of the essential trace elements for the human body and are involved in a variety of biological processes (like the formation of hemoglobin from proteins, the transport of oxygen, and the formation of enzymes necessary for the human body) (34). One of the primary characteristics of ferroptosis is accumulation of iron ions (2). Under normal conditions, intracellular iron is balanced by the iron transport system, while extracellular iron is taken into the cell by transferrin (TF) and transferrin receptor (TFR) and stored and transported as ferritin complexes (mainly ferritin) (35). Ferritin is mainly composed of ferritin light chain (FTL) and ferritin heavy chain (FTH1), which has iron oxidase activity and can catalyze the conversion of Fe^{2+} to Fe^{3+}, promote iron binding to ferritin and reduce free iron levels (36). Ferroportin 1 (FPN1) is the only protein known to control iron export in mammalian cells and serves an important role in iron metabolism (FPN1 is a target molecule of hepcidin. FPN1, in the action of hepcidin, controls the amount of dietary iron, circulating iron and stored iron released into the plasma by altering its distribution across the cell membrane to maintain iron homeostasis in the body.) (2,3). Fe^{2+} is the form involved in the reaction during iron death, and when the body's iron metabolism is upset, intracellular iron stores are reduced and excess Fe^{2+} is involved in the Fenton reaction catalyzing the production of large amounts of ROS and hydroxyl radicals, which leads to the occurrence of ferroptosis (24). Alvarez *et al* (37) demonstrated that iron-sulphur cluster biosynthetic enzyme could resist the onset of iron death by inhibiting the elevation of intracellular iron levels. Fang *et al* (38) and Chang *et al* (29) observed that heme oxygenase 1 (HO-1) induces ferroptosis via promotion of heme decomposition to release Fe^{2+} ions. Serine/threonine

protein kinase ataxia-telangiectasia mutated protein, a crucial DNA damage response regulator, promotes ferroptosis by preventing nuclear translocation of metal regulatory transcription factor 1, a transcription factor that triggers production of FTH1 and FTL to decrease iron toxicity (39).

Other molecular mechanisms. Results of a recent study demonstrated that p53 is essential for inducing ferroptosis (13). p53 responds to different stress signals via the coordination of specific cellular responses and the corresponding cell cycle arrest and apoptosis play important roles in inhibiting development of cancer (13). It was found that p53 could inhibit the uptake of System Xc- to Cys by suppressing the expression of SLC7A11, which resulted in a large decrease in GSH production and affected the activity of GPX4, leading to reduced antioxidant capacity and ROS accumulation, thus promoting cellular iron death (40). The communication between mitochondria and other organelles is aided by voltage-dependent anion channels (VDACs), channel proteins located in the outer mitochondrial membranous layer (41). Yagoda *et al* (42) demonstrated that erastin alters the permeability of the mitochondrial outer membrane via direct binding to VDAC2/3, thereby decreasing the oxidation rate of NADH and inducing ferroptosis. FSP1 is a potent ferroptosis-resistance factor (43). Doll *et al* (14) demonstrated that myristoylation of FSP1 inhibits lipid peroxidation via NAD(P)H reduction of COQ10, thereby inhibiting ferroptosis. Moreover, methionine is converted to Cys under oxidative stress via the sulphur transfer pathway to create GSH, which exerts its antioxidant action (44). In addition, the PI3K/Akt/mTOR (15), p62/Keap1/NRF2 (16) and ATG5/ATG7/NCOA4 (17) signalling pathways serve regulatory roles in the occurrence of ferroptosis.

3. Ferroptosis and renal fibrosis

Oxidative stress. When the redox system is damaged, ROS and reactive nitrogen species are excessively produced and oxidative stress occurs (45). Well-established mechanisms of oxidative stress-mediated renal injury include production of ROS and the ensuing disruption of the antioxidant system, which result in apoptosis, ferroptosis and necrosis (46,47). There are numerous causes of renal fibrosis, including oxidative stress (48). Specific inhibitors, including ferrostatin-1 (Fer-1), which is characterized by lipid-dependent peroxidation, prevent ferroptosis (2). Roxadustat is an emerging therapeutic option for treatment of anaemia in patients with chronic kidney disease (CKD). It is an oral inhibitor of hypoxia-inducible factor (HIF) prolyl hydroxylase, which stimulates erythropoiesis and regulates iron metabolism. Using a folic acid-induced kidney injury model, Li *et al* (49) demonstrated that roxadustat pre-treatment decreases ferroptosis and inhibits inflammation by stabilizing HIF-1α and activating the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway, thereby inhibiting renal fibrosis. Ide *et al* (50) demonstrated that lipid peroxidation induces ferroptotic stress and ferroptosis. Following injury, renal proximal tubule (PT) cells may exhibit a pro-inflammatory state and genes involved in high ferroptosis stress may trigger accumulation of inflammatory PT cells, thereby enhancing inflammation and fibrosis. Feng *et al* (51) demonstrated that diabetes increases HIF-1α and HO-1 in the kidney of mice, resulting in increased lipid peroxidation due to increased ROS production and tubular iron deposition. This leads to renal tubular damage and fibrosis in mice. In a diabetic mouse model, inhibition of ferroptosis prevents lipid peroxidation by decreasing ROS production in the kidneys and reducing iron deposition in the renal tubules (51). This alleviates diabetes mellitus, renal tubular injury and renal fibrosis progression in mice (51). Nobiletin (Nob), an important active flavonoid found in citrus fruits, exhibits antioxidant, anti-inflammatory, antifibrotic and antiapoptotic properties (52). Lo *et al* (53) demonstrated that Nob partially decreases oxidative stress and ferroptosis or apoptosis in unilateral ureteral obstruction (UUO) mice, which decreases inflammatory responses and subsequently inhibits development of renal fibrosis. In conclusion, the inhibition of ferroptosis via regulation of oxidative stress may represent a novel method for treatment of renal fibrosis.

Inflammation. Inflammation is an immune response to exogenous or endogenous injury and contributes to the maintenance of tissue homeostasis under stressful conditions (54). A high inflammatory burden is associated with kidney damage. Results of a clinical study on type 2 diabetes demonstrated that the ratio of C-reactive protein expression to serum albumin is increased in patients with diabetic nephropathy compared with those without diabetic nephropathy (55). Using an adenine-induced mouse model of aging, the model group demonstrated increased levels of extensive tubular damage and fibrosis, as well as increased inflammatory responses, compared with groups of control (56). Inflammation is the main pathogenesis of diabetic kidney injury (DKI), and the monocyte to lymphocyte ratio (MLR) is considered a marker of inflammatory disease. microalbuminuria (MA) is the Microalbuminuria (MA) is the last reversible stage of DKI treatment, and in type 2 diabetic patients, MLR expression levels are significantly higher in the MA group compared to the normoalbuminuria (NA) group (57). Monocyte chemoattractant protein-1, macrophage colony-stimulating factor and neopterin levels are markedly increased in patients with chronic renal disease compared with controls (58). Moreover, numerous inflammatory cytokines, such as neuregulin (59), kidney injury molecule-1 (KIM-1) (60) and omentin (61) are associated with degree of kidney damage. In addition, interleukin (IL)-10 exerts anti-inflammatory effects. In a renal ischemia-reperfusion injury model, IL-10 knockout mice demonstrated decreased levels of renal function, upregulation of renal injury biomarkers, such as KIM-1, and increased expression of certain pro-inflammatory cytokines, compared with the control group (62). Therefore, renal damage is associated with inflammation.

Damage to renal tissue induces the inflammatory and fibrotic processes that aid in regeneration and repair (63). Results of previous studies demonstrated that macrophages are a potential therapeutic target for renal injury and fibrosis and play a significant role in the pathophysiology of kidney disease (64-66). Notably, renal fibrosis may be reversed as different subpopulations of macrophages in the kidney can either promote or inhibit deposition of extracellular matrix in the kidney (64). Inflammatory cell infiltration is a key characteristic of renal fibrosis (67). Renal tubular injury is considered

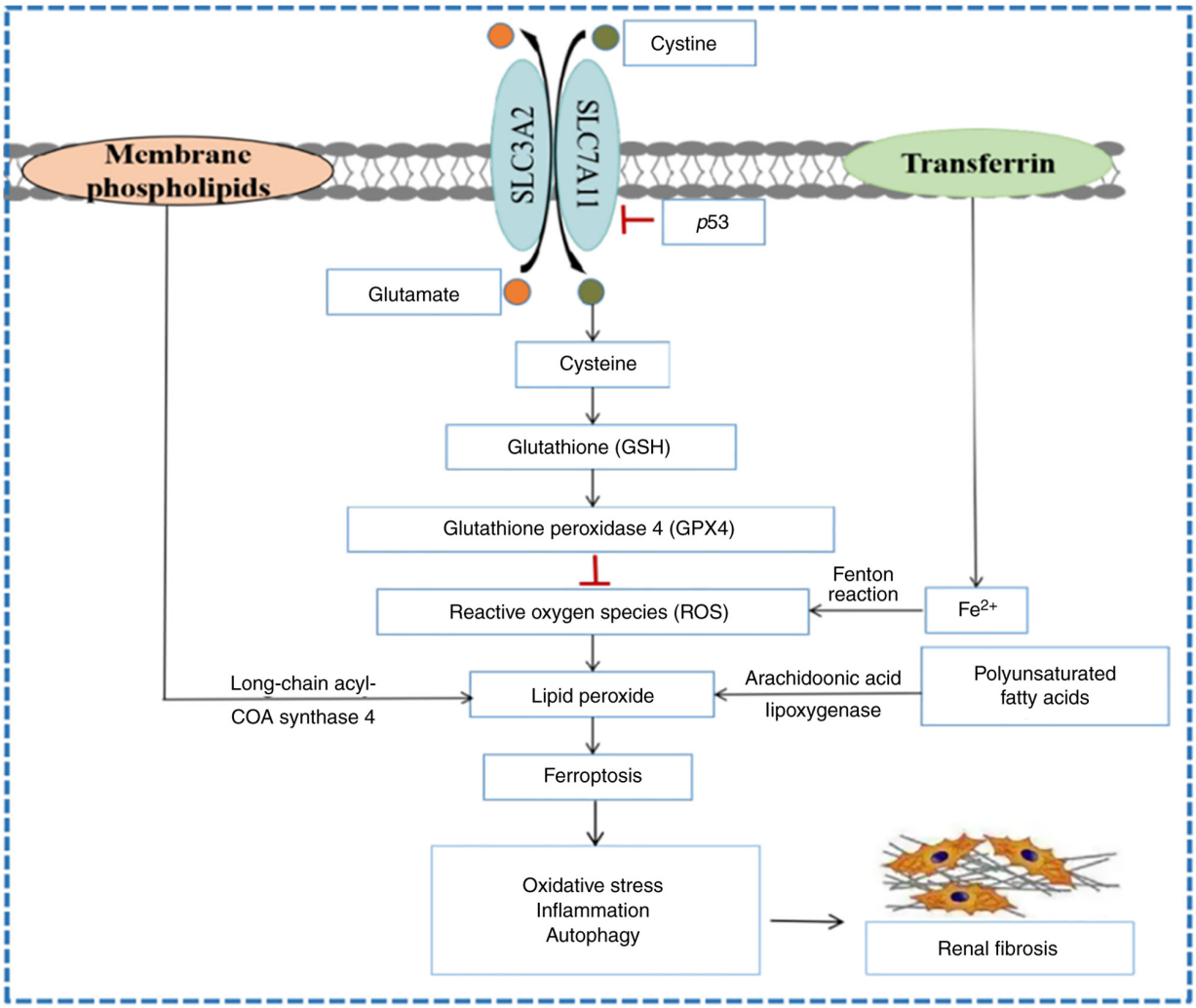

Figure 1. Principal mechanism of ferroptosis-mediated renal fibrosis. Ferroptosis occurs through various pathways including GPX4, System Xc-, lipid peroxidation, and iron metabolism dysfunction. Ferroptosis may be involved in the process of renal fibrosis through multiple pathways such as oxidative stress, inflammation and autophagy.

a proinflammatory driving force in fibrosis. Following renal tubular injury, renal tubular epithelial cells (TECs) produce immune responses and release inflammatory mediators. The aggravation of inflammation leads to cell death, while cell death also has a strong pro-inflammatory effect, further worsening tubular injury, and continued inflammation and injury can lead to tubulointerstitial fibrosis (68). In conclusion, renal fibrosis and inflammation are associated with renal damage.

Due to increased permeability and rupture of the cell membrane during ferroptosis, associated contents, including damage-associated molecular pattern (DAMP) may be released, causing an inflammatory response and activation of the innate immune response. However, the specific mechanism requires further investigation (69). Necroinflammation associated with ferroptosis is observed acute kidney injury model in mice, as well as in GPX4-deficient knockout mouse models (70,71). In the kidneys of GPX4 knockout mice induced by tamoxifen, a large number of renal tubular cells died, and the release of cellular debris, mitochondria and even nuclei from ruptured cells into the tubular lumen could be observed at the histological level; this may be associated with DAMP (72). In ferroptotic tissues, F4/80 immunofluorescent staining has demonstrated that macrophages are markedly activated (73), releasing pro-inflammatory substances, thus triggering inflammatory responses.

However, to the best of our knowledge, the interaction between ferroptosis and inflammation in renal fibrosis remains unclear. Tectorigenin, a compound derived from the iris plant *Belamcanda chinensis*, is an active ingredient used in Traditional Chinese Medicine (74). Tectorigenin exhibits numerous pharmacological activities, such as anti-inflammatory and antioxidant properties, liver protection and diabetes control (75,76). Li *et al* (77) demonstrated that tectorigenin inhibits ferroptosis and fibrosis induced by external stimuli in primary TECs. Moreover, Fer-1, a ferroptosis inhibitor, inhibits the pro-fibrotic effect of TGF-β1-stimulated TECs, suggesting that tectorigenin may alleviate renal fibrosis via inhibition of ferroptosis in TECs (77). Results of a recent study also demonstrated that Fer-1 attenuates oxalate-induced TEC damage and renal fibrosis via inhibition of ferroptosis (78). Zhang *et al* (79) demonstrated that ferroptosis of TECs may be induced following UUO in mice, while liproxstatin-1 (Lip-1), a ferroptosis inhibitor, inhibits downregulation of GPX4 expression and ferroptosis in TECs and attenuates expression of

pro-fibrotic factors in UUO mice. These results suggested that Lip-1 alleviates renal fibrosis in UUO mice via inhibition of ferroptosis in TECs. Moreover, Luo *et al* (80) demonstrated that obesity induces ferroptosis in the kidney while Fer-1 inhibits the development of high-fat diet-induced inflammation and fibrosis in renal tissue. Tocilizumab is an emerging interleukin-6 (IL6) receptor-targeting drug Yang *et al* (81) demonstrated that tocilizumab attenuates renal fibrosis in mice via inhibition of ferroptosis. Renal fibrosis is a common pathological process in diabetic nephropathy (82). Results of previous studies have demonstrated that expression levels of ACSL4 are increased in diabetic nephropathy mice and expression levels of GPX4 are decreased (83). Using the ACSL4 inhibitor rosiglitazone, both ferroptosis and production of pro-inflammatory cytokines are inhibited in TECs, preventing development of diabetic nephropathy (83). Zhou *et al* (84) confirmed that inhibiting ferroptosis in TECs reduces interstitial inflammation and renal fibrosis. Collectively, these results suggested that attenuating cellular inflammation development via inhibition of ferroptosis may be a novel approach for the treatment of renal fibrosis.

Autophagy. Autophagy refers to self-phagocytosis of cells, which removes misfolded proteins and damaged organelles in cells awaiting degradation, thereby maintaining cell homeostasis (85). Autophagy is a self-protection mechanism of eukaryotic cells (85). A previous study demonstrated that changes in autophagy activity are associated with renal fibrosis (86). The regulatory function of autophagy in fibrosis is associated with coordinated regulation of tubular cell death, interstitial inflammation and, in particular, production of pro-fibrotic secretory proteins (87). Results of a previous study demonstrated that, as a relatively recently discovered regulatory mode of cell death, ferroptosis differs from other regulatory modes such as autophagy, apoptosis, necrosis (2). Nonetheless, a more recent study demonstrated that ferroptosis and autophagy exhibit common regulators such as SLC7A11, GPX4, Nrf2 and heat shock protein β-1 (88). The autophagy-related protein beclin 1 (BECN1) inhibits the function of system Xc^- via formation of the BECN1/SLC7A11 complex and induces ferroptosis under the action of erastin and RSL3 (84). Ferritinophagy is a type of cell-selective autophagy mediated by NCOA4. To facilitate the movement of intracellular ferritin to autophagy lysosomes and liberate free iron, NCOA4 functions as a selective autophagy receptor and binds to FTH1 of ferritin (89). Overexpression of NCOA4 increases ferritin degradation in cancer cells and fibroblasts, thereby promoting ferroptosis (90). Wang *et al* (91) demonstrated that expression of NCOA4 is increased in a 5/6 nephrectomy-induced CKD rat model Following the addition of ferroptosis inducer cisplatin or the ferroptosis inhibitor desferrioxamine mesylate, expression of NCOA4 is enhanced or attenuated, respectively. This treatment alters the progression of renal fibrosis. Therefore, ferritinophagy may induce ferroptosis in CKD and promote development of renal fibrosis. Consequently, inhibition of ferroptosis via regulation of autophagy may act as a novel therapeutic method in treatment of renal fibrosis.

4. Conclusion

Renal fibrosis is a common pathological state in almost all chronic and progressive kidney diseases, but effective measures for its clinical prevention and treatment are still not available. Ferroptosis is a novel regulatory cell death modality, and by summarizing the association between renal fibrosis and ferroptosis, we found that ferroptosis is involved in various biological processes such as oxidative stress, inflammation, and autophagy during renal fibrosis (Fig. 1). However, the specific molecular mechanism is still unclear, and further research is needed to investigate the role of ferroptosis in the development of renal fibrosis and to explore effective and highly targeted therapeutic measures against ferroptosis to provide new targets and more valuable therapeutic approaches for renal fibrosis research.

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Authors' contributions

HYZ, MC, LZ and YPW were responsible for the conceptualization of the present review. HYZ and MC were responsible for the original draft preparation. LZ and YPW were responsible for reviewing and editing the manuscript. LZ and YPW were responsible for funding acquisition. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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