Therapeutic Implications of Ferroptosis in Renal Fibrosis

Yao Zhang¹, Yanhua Mou², Jianjian Zhang¹, Chuanjian Suo¹, Hai Zhou³, Min Gu³, Zengjun Wang¹ and Ruoyun Tan¹*

¹Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China, ²Department of Oncology, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, Xiangyang, China, ³Department of Urology, The Second Affiliated Hospital of Nanjing Medical University, Nanjing, China

Renal fibrosis is a common feature of chronic kidney disease (CKD), and can lead to the destruction of normal renal structure and loss of kidney function. Little progress has been made in reversing fibrosis in recent years. Ferroptosis is more immunogenic than apoptosis due to the release and activation of damage-related molecular patterns (DAMPs) signals. In this paper, the relationship between renal fibrosis and ferroptosis was reviewed from the perspective of iron metabolism and lipid peroxidation, and some pharmaceuticals or chemicals associated with both ferroptosis and renal fibrosis were summarized. Other programmed cell death and ferroptosis in renal fibrosis were also firstly reviewed for comparison and further investigation.

Keywords: renal fibrosis, ferroptosis, iron homeostasis, lipid peroxidation, programmed cell death

1 INTRODUCTION

Chronic kidney disease (CKD) is an important public health concern causing a high economic cost to the health systems, with an overall prevalence of more than 10% (Hill et al., 2016). Progressive CKD often results in end-stage renal failure and the only options are dialysis or kidney transplantation. Renal fibrosis is a common characteristic of CKD independent of the underlying etiology, leading to the destruction of normal kidney structure and loss of renal function. Renal fibrosis, one of the common characteristics of CKD, includes tubulointerstitial fibrosis and glomerulosclerosis, regardless of the underlying etiology, is synthesized from an imbalance between excessive synthesis and reduced breakdown of the extracellular matrix, and it may be due to normal wound healing response become uncontrolled, with an uncontrolled inflammatory response and myofibroblast proliferation, eventually leading to destruction of normal kidney structure and loss of renal function (el Nahas et al., 1997). Currently, therapies against renal fibrosis exhibit limited effectiveness, and little progress has been made in reversing fibrosis in recent years.

In 2003, Sonam Dolma et al. discovered a new form of cell death induced by erastin without nuclear morphological changes, DNA fragmentation, and caspase-3 activation, which could not be reversed by caspase inhibitors (Dolma et al., 2003) but could be inhibited by iron chelator (Yang and Stockwell, 2008). Subsequently, Dixon named this cell death “ferroptosis” in 2012 (Dixon et al., 2012), which is characterized by lipid peroxidation-mediated membrane damage, leading to iron-dependent regulated necrosis (Stockwell et al., 2017). Different from autophagy, necrosis, pyroptosis, and apoptosis, ferroptosis morphologically exhibits normal nuclei and shrinking mitochondria with condensed mitochondrial membrane, diminished or abolished mitochondria cristae, and ruptured outer mitochondrial membrane (Yagoda et al., 2007; Cao and Dixon, 2016; Latunde-Dada, 2017). The connection between necrotic cell death and long-term renal fibrosis may be dominated by the inflammatory infiltration, which could be defined by the type of necrotic death (Tang et al., 2020).
Although our knowledge about the role of ferroptosis in renal fibrosis is still in its infancy, the cytotoxicity of iron and lipid peroxidation has been known since the 1900s and 1950s, respectively (Moore and Hawkes, 1908; Bieri, 1959). Before the proposing of ferroptosis, many studies confirmed that the ferroptosis-related indicators, including GPXs, iron, and lipid peroxides are participated in renal fibrosis (Sponsel et al., 1996; Ueda et al., 1996; Zachara et al., 2004; Himmelfarb, 2005; Dominguez et al., 2006; Seiler et al., 2008). This review is to summarize the relationship between renal fibrosis and ferroptosis from the perspective of iron metabolism and lipid peroxidation.

2 MECHANISM OF FERROPTOSIS

2.1 Oxidative Stress and Lipid Peroxidation in Ferroptosis

Ferroptosis is a regulated form of cell death characterized by iron-dependent oxidative stress induced lipid peroxidation (Stockwell et al., 2017). In general, ferroptosis is mainly caused by the inactivation of cellular antioxidant system; it is caspase- and necrosmine-independent, which is precisely regulated at epigenetic, transcriptional, post-transcriptional and post-translational levels (Galluzzi et al., 2018).

2.1.1 Oxidative Stress

Oxidative stress is a disruption of redox signaling and control (Jones, 2006), resulting from either excessive free radicals or insufficient degradation of these free radicals. Oxidative stress is a causative factor in the pathogenesis and pathophysiology of numerous degenerative disorders.

Firstly, ROS and reactive nitrogen species (RNS) are the most common oxidative compounds, which can mediate oxidative stress under various pathophysiological conditions, and are removed by antioxidant defense mechanisms. Secondly, reduced glutathione, is an essential intracellular antioxidant synthesized by the ATP-dependent cytosolic enzymes glutamate-cysteine ligase (GCL) and glutathione synthetase (GSS) in a two-step synthesis of cysteine, glutamate and glutathione disulfide (GSSG) in a two-step synthesis of cysteine, glutamate and glycine (Stockwell et al., 2017). Thirdly, extracellular cystine and intracellular cysteine are essential for the maintenance of glutathione biosynthesis and for the inhibition of a type of cell death, which can also be prevented by iron chelators or lipophilic antioxidants (α-tocopherol) (Bannai et al., 1977; Murphy et al., 1989). Cysteine is imported into cells through the oxidized form-cystine of system Xc− where cystine is immediately reduced to cysteine within the cells. Finally, GSH is a major soluble non-enzymatic antioxidant, which relieves the oxidative damage and protects cellular macromolecules, including membrane lipids, against ROS by donating electrons to free radicals. This donation leads to its own oxidation, which converts GSH into glutathione disulfide (GSSG). In this process, glutathione peroxidases (GPXs) play an antioxidant role and converts potentially toxic lipid hydroperoxides (L-OOH) to non-toxic lipid alcohols (L-OH) by buffering H2O2 with GSH (Battin and Brumaghim, 2009).

2.1.2 Lipid Peroxidation

Oxidative stress might impact various macromolecules including lipids, proteins and nucleic acid, leading to the formation of oxidative damage products. Lipid peroxidation, broadly defined as the insertion of hydroperoxyl group into a lipid, has recently been identified as a major driver of ferroptosis (Yang and Stockwell, 2016). This process can be induced by a variety of oxidants (superoxides, H2O2, and highly reactive hydroxyl radicals) or by exposure to xenobiotics and environmental pollutants. As the main component of cellular membranes, lipids have an indispensable role in maintaining cell structural integrity. Polysaturated fatty acids (PUFAs), especially arachidonic acid (AA) or arachidonic acid (AA), are most susceptible to peroxidation, which is quite important for the maintenance of cellular membrane fluidity (Figure 1; de Carvalho and Caramujo, 2018).

In the non-enzymatic pathway, lipid peroxidation can be mediated by carbon- and oxygen-centered radicals initiated by Fenton reaction (see below) or it can also be carried out in an orderly manner by enzyme pathway. Acyl-CoA Synthetase Long Chain Family Member 4 (ACSL4) catalyzes the addition of CoA to the long-chain polysaturated bonds of AA or AD to produce AA or AD acyl-CoA derivatives, thereby promoting the esterification of PUFA into phospholipids (Kagan et al., 2017). It incorporates cellular membranes with PUFAs and drives ferroptosis through the accumulation of oxidized cellular membrane phospholipids (Doll et al., 2017). Then these derivatives are esterified into phosphatidylethanolamines (PEs) containing AA or DA (AA-PE or DA-PE) by Lysophosphatidylethanolamine Phospholipase A2 (LPAT) and lysophosphatidylcholine Acyltransferase 3 (LPCAT3). Lipoxygenases (LOXs) form a family of iron-containing lipid peroxide enzymes with highly conserved Fe-coordination sphere positioned in a helical core and shows the substrate specificity (Newcomer and Brash, 2015). According to the location specificity of arachidonic acid oxidation, lipoxygenase was named and classified as ALOXE3 (arachidonate lipoxygenase 3), ALOX5 (arachidonate 5-lipoxygenase), ALOX12 (arachidonate 12-lipoxygenase, 12S type), ALOX12B (arachidonate 12-lipoxygenase, 12R type), ALOX15 (arachidonate 15-lipoxygenase), and ALOX15B (arachidonate 15-lipoxygenase type B) in humans. Finally, AA-PE and DA-PE, were oxidized by lipoxygenases (LOXs) to generate lipid hydroperoxides, thus proximately execute ferroptosis. The antioxidant α-tocopherol and α-tocotrienol could regulate ferroptosis by suppressing the LOX, then generating doubly and triply-oxygenated (15-hydroperoxy)-diacylated phosphatidylinositolamine (PE) species (Kagan et al., 2017). Furthermore, cytochrome P450 oxidoreductase (POR) were recently reported to generate lipid hydroperoxides by an ALOX-independent manner (Figure 1; Zou et al., 2020).

The GPX4, an antioxidant enzyme and a central blocker of ferroptosis, is the only glutathione peroxidase that uses GSH as a co-substrate to reduce phospholipid hydroperoxides to hydroxyl phospholipid in the membranes (Yang et al., 2014). Decreased detoxification of GPX4, depletion of GSH and increased LOXs activity are the main driving factors of ferroptosis (Seiler et al., 2008; Yang and Stockwell, 2016). Selenium is a component of selenocysteine at the catalytic site of GPX4. Similar to ferrostatin-1, selenium has a strong inhibitory effect on cerebral hemorrhage and can reduce the concentration of ferroptosis, which is a
specific ferroptosis inhibitor (Ingold et al., 2018). In addition, lipophilic antioxidant ubiquinone (CoQ10) exerts a fundamental role in the mitochondrial electron transport chain and can hamper ferroptosis in a GSH-independent manner (Doll et al., 2019). Glutamate is converted from glutamine by glutaminase and is required for the induction of ferroptosis during serum-induced injury after amino acid starvation (Gao et al., 2015; Altman et al., 2016). Then glutamate can also be exchanged for cystine via xCT. Ferroptosis suppressor protein 1 (FSP1), also named as apoptosis-inducing factor mitochondria associated 2 (AIFM2), acts as an NADH-dependent oxidoreductase to catalyze oxidase ubiquinone into reduced ubiquinol and prevents lipid peroxides and incorporation into membranes and lipoproteins, in parallel to the canonical glutathione-based GPX4 pathway (Bersuker et al., 2019; Doll et al., 2019). The newly found Dihydroorotate dehydrogenase (DHODH), inhibits ferroptosis in the mitochondrial intima by reducing the ubiquitin ketone to ubiquitin alcohol, operating in parallel to mitochondrial GPX4, but not to cytosolic GPX4 or FSP1 (Mao et al., 2021; Figure 1).

2.2 Iron Homeostasis and Ferroptosis

Iron is an important trace element that plays an important role in many key biological processes. Iron is a key cofactor in electron transfer processes and redox reactions because of its ability to switch between specific oxidative forms; however, iron’s redox potential might lead to cytotoxicity when iron is overloaded (Gozzelino and Arosio, 2016).

2.2.1 Iron Overload Induces Ferroptosis

The excessive intracellular labile iron can directly participate in the redox cycle, providing electrons to expedite ROS production and lipid peroxidation via the Fenton reaction, a series of reactions in which this labile iron reacts with endogenous hydrogen peroxide or superoxide to form oxygen centered...
radicals (\(\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot \text{OH}\)) (Koppenol, 1993; Neyens and Baeyens, 2003). GSH is necessary for the assembly of Fe-S clusters and can act as a coordinating ligand for \([2\text{Fe}-2\text{S}]\) clusters when bound to a glutaredoxin scaffold (Hider and Kong, 2013). Interestingly, studies in yeast support the prime actor of GSH in maintaining iron balance during iron-sulfur cluster assembly, challenging the traditional view of its primary role in redox balance (Kumar et al., 2011).

Compelling evidence indicated that ferroptosis can be motivated by elevated iron levels and inhibited by iron chelation (Dixon et al., 2012). Several proteins impacting iron homeostasis or transportation can influence the sensitivity of ferroptosis (Figure 2; Dixon et al., 2012; Sun et al., 2015; Yuan et al., 2016; Kim et al., 2018). Ferritin plays a major role in protecting against ferroptosis through storing intracellular iron (Fang et al., 2020). It further emphasizes the essentiality of iron in ferroptosis, which requires transferrin and TFR1 as specific markers of ferroptosis (Gao et al., 2015; Feng et al., 2020). DMT1 inhibition could block the translocation of lysosomal iron, leading to the accumulation of lysosomal iron and cell death with ferroptotic features (Song et al., 2021). NCOA4-mediated ferritinophagy promotes ferroptosis by degradation of ferritin (Hou et al., 2016). In kidney, the liver-derived hormone hepcidin serves as a promising therapeutic target in conditions such as post-cardiopulmonary bypass AKI and delayed graft function after renal transplantation (Scindia et al., 2019). Reportedly, it could promote erastin-induced ferroptosis by degrading ferroportin, the sole iron export protein (Geng et al., 2018). Recently, prominin 2 has been found to facilitate the formation of ferritin-containing multivesicular bodies (MVB) and exosomes in mammalian epithelial, thus driving ferroptosis resistance (Belavgeni et al., 2019; Brown et al., 2019). Heat shock protein beta-1 (HSPB1) can inhibit TR1 recycling (Chen et al., 2006), its up-regulation can confine erastin-induced iron uptake through suppressing the ferroptosis (Sun et al., 2015). In addition to participating in Fenton reaction, iron is also transported to several iron-containing enzymes involved in lipid peroxidation, thus in turn actuating ferroptosis. For instance, phosphorylase kinase G2 (PHKG2) plays a significant role in promoting iron accumulation and regulating iron availability to LOXs (Yang et al., 2016). CDGSH iron-sulfur domain (CISD) has also been shown to bind a redox-active \([2\text{Fe}-2\text{S}]\) cluster and participates in regulating iron storage and redox reactions (Fidai et al., 2016). Down-regulating CISD1 level can promote iron-mediated intramitochondrial lipid peroxidation contributing to erastin-induced ferroptosis (Yuan et al., 2016). Silencing CISD2 gene can raise lipid ROS production and mitochondrial iron content, making drug-resistant head and neck cancer cells sensitive to sulfasalazine-induced ferroptosis (Kim et al., 2018). Accumulation of iron responsive element binding protein 2 (IREB2) can induce iron aggregation, thus contributing to the ferroptosis (Li Y. et al., 2020), while the silencing of IREB2 gene leads to mutual changes in the expression of known iron absorption, metabolism and storage genes TFR1, iron-sulfur cluster assembly enzyme (ISCU), FTH1, FTL and in erasin sensitivity (Salahudeen et al., 2009; Vashisht et al., 2009).

### 3 Ferroptosis in Chronic Renal Injury and Fibrosis

#### 3.1 Lipid Peroxidation in Chronic Kidney Disease

The final common pathological denominator in the case of chronic kidney injury is renal fibrosis, and the extent of tubulointerstitial fibrosis is the best predictor of renal survival for CKD patients (Zeisberg and Neilson, 2010). Oxidative stress is present from the early stages of CKD and continues to increase as kidney function deteriorate due to antioxidant consumption and increased ROS production (Sureshbabu et al., 2015; Daenen et al., 2019; Duni et al., 2019; Gyuraszova et al., 2019).

Diverse renal abnormalities can trigger oxidative stress and accelerate the kidney disorders. Fibrotic and damaged tubular and glomerular cells in CKD may enhance ROS production, which is a major agent of oxidative stress (Daenen et al., 2019). Compared with healthy control group, the levels of GPX and GSH in renal cortex of CKD model rats and CKD patients were significantly decreased, while the levels of ROS and MDA were significantly increased (Zachara et al., 2004; Tbahriti et al., 2013). Lipid aldehydes, the end product of many oxidation reactions, including 4-hydroxy-2-hexenal (4-HHE) and 4-hydroxy-2-nonenal (4-HNE) (Himmelbär, 2005), and their plasma concentrations are significantly higher in CKD patients (Soulage et al., 2020). Malondialdehyde (MDA), is the final product of peroxidation of polyunsaturated fatty acids and an indicator of ferroptosis (Kim et al., 2018), which is commonly raised in the serum of CKD patients (Taccone-Gallucci et al., 1989; Pei et al., 2016). These markers, including 4-HHE, 4-HNE, and MDA have all been widely used in the detection of ferroptosis recently. In HEK-293T cells, GPX4 was shown to block TNFα-mediated activation of NF-κB (Li et al., 2018). Moreover, recent researches have reflected that ferroptosis could facilitate renal damage in diabetic nephropathy (DN), and lipid peroxidation is increased in DN mice, and the expressions of ACSL4, SLC7A11, and SLC3A2 are up-regulated, while GPX4 is down-regulated (Wang Y. et al., 2020). ACSL4 inhibitor rosiglitazone alleviated renal impairment in DN by suppressing ferroptosis (Wang Y. et al., 2020). It is established that pathological changes of DN include renal hypertrophy, enlargement of glomerular capillaries, mesangial expansion, and glomerular basement membrane thickening caused by ECM deposition, which ultimately lead to inflammation, endothelial dysfunction, and renal fibrosis (Jha et al., 2016).

#### 3.2 Iron Metabolism in Kidney

##### 3.2.1 Iron Homeostasis in Kidney

Normally, nutritional iron, which consists of both ferric iron (\(\text{Fe}^{3+}\)) and ferrous iron (\(\text{Fe}^{2+}\)), is absorbed by duodenal enterocytes. \(\text{Fe}^{3+}\) could bind to transferrin (TF) on the cell membrane to form transferrin-bound iron (TFI), which is captured by transferrin receptor 1 (TFR1) into the endosomes. Intracellular \(\text{Fe}^{2+}\) is reduced to \(\text{Fe}^{3+}\) by iron reductase (represented by Steap3). \(\text{Fe}^{2+}\) would then be released into the labile iron pool (LIP) in the cytoplasm by the divalent metal transporter 1 (DMT1) (Dhungana et al., 2004; Knutson, 2007).
Endosomal Fe$^{3+}$ is quickly reduced to Fe$^{2+}$ by an oxidoreductase activity. Excess iron ions are either released through the basal membrane protein ferroportin 1 (FPN1) to the circulation or stored in ferritin, the major iron storage protein (Arosio et al., 2009). Ferritin is a spherical heteropolymer capable of storing 4,500 iron atoms, consisting of a ferritin heavy chain (FTH1) and a ferritin light chain (FTL), both of which can sequestrate and store iron in a non-toxic and bioavailable form. Iron is incorporated into ferritin via ferritin iron pores as Fe$^{2+}$, which could be formed by intestinal absorption or erythrocyte degradation. FTH1 catalyzes the oxidation of Fe$^{2+}$ to Fe$^{3+}$, while FTL promotes the nucleation of ferrihydrite, enabling inert deposits of Fe$^{3+}$, which can hardly be used in cells or generate ROS (Philpott, 2018). Poly(rC) binding proteins (PCBP) (especially PCBP1 and PCBP2), are important iron chaperones to regulate the metalation of iron-containing proteins and the storage and export of Fe$^{2+}$ (Shi et al., 2008). They directly bind ferritin and deliver labile irons to ferritin for storage (Philpott et al., 2017). For intracellular utilization, iron needs to be released from ferritin and reduced to Fe$^{2+}$ again. Ferritin degradation mediated by nuclear receptor coactivator 4 (NCOA4) is required for the maintenance of mitochondrial respiratory activity, respiratory chain complex assembly, mitochondrial iron storage, and membrane potential (Figure 3; Fujimaki et al., 2019). However, it has been reported that iron excess in red blood cells in turn triggers the ubiquitin-mediated NCOA4 degradation by the E3-9 ligase HERC2 (Ryu et al., 2018).

In the kidney, circulating iron can be filtered through the glomeruli into the tubules under physiological conditions (Zhang et al., 2007; Smith and Thevenod, 2009). The filtered iron is almost completely reabsorbed in the renal tubules, where it would be then stored in ferritin, utilized in the mitochondria or exported from the cell. TBI, transferrin-bound iron; NTBI, non-transferrin-bound iron; NGAL, neutrophil gelatinase-associated lipocalin; HO-1, heme oxygenase-1; PCBP1, Poly(rC) binding protein 1; NCOA4, nuclear receptor coactivator 4; FPN1, protein ferroportin 1; HCP1, heme carrier protein 1; DMT1, divalent metal transporter 1; Zip8, zinc transporter Zip8; Zip14, zinc transporter Zip14; PRNP, prion protein; NGALR, neutrophil gelatinase-associated lipocalin receptor; TFR1, transferrin receptor 1.

![FIGURE 3](image)
parenterally induced iron loading (Weiss et al., 2018), but increased under low iron conditions (Smith et al., 2019). NGAL is a promising biomarker for acute or chronic kidney disease, where iron binds to NGAL and is engulfed by neutrophil gelatinase-associated lipid calcin receptor (NGALR) in distal tubules (Langelueddeke et al., 2012). Heme is reabsorbed through the heme importer, namely, heme carrier protein 1 (HCP1), in proximal and distal tubules (Le Blanc et al., 2012). Transporters in different nephron segments that promote the uptake of other non-transferrin proteins binding iron on the plasma membrane has not been fully elucidated (Thévenod and Wolff, 2016). Reportedly, zinc transporter ZIP8 (Zip8), zinc transporter ZIP14 (Zip14) and DMT1 were present in the apical membrane of human proximal and distal tubules (Canonne-Hergaux and Gros, 2002; Veuthey et al., 2014; van Raaij et al., 2018b). The endocytic iron is released from the transport protein, and is then reduced and exported to the cytosol mainly through DMT1, where Fe3+ is excluded under low iron conditions (Smith et al., 2019). In addition, iron transport and release from the proximal tubules (Canonne-Hergaux and Gros, 2002; Veuthey et al., 2014; van Raaij et al., 2018b). The endocytic iron is released from the transport protein, and is then reduced and exported to the cytosol mainly through DMT1, where Fe3+ is excluded under low iron conditions (Smith et al., 2019). In addition, iron transport and release from the proximal tubules (Canonne-Hergaux and Gros, 2002; Veuthey et al., 2014; van Raaij et al., 2018b). The endocytic iron is released from the transport protein, and is then reduced and exported to the cytosol mainly through DMT1, where Fe3+ is excluded under low iron conditions (Smith et al., 2019).

There is no evidence for basolateral uptake of TBI, heme, hemoglobin, or non-transferrin-bound iron (NTBI) in the basolateral renal tubules so far, whereas the iron exporter-ferroportin exists in the basolateral membrane of proximal tubules (van Raaij et al., 2018a). As a master regulator of iron homeostasis, hepcidin regulates this ferroportin-mediated iron export and intracellular H-ferritin levels (Vyoral and Jiri, 2017). In podocytes, hemoglobin can be taken up by megalin-cubilin endocytosis catabolized by heme oxygenase-1 (HO-1), thus resulting in increased oxidative stress (Rubio-Navarro et al., 2018; Duann et al., 2019). Transporters in different nephron segments that promote the uptake of other non-transferrin proteins binding iron on the plasma membrane has not been fully elucidated (Thévenod and Wolff, 2016). Reportedly, zinc transporter ZIP8 (Zip8), zinc transporter ZIP14 (Zip14) and DMT1 were present in the apical membrane of human proximal and distal tubules (Canonne-Hergaux and Gros, 2002; Veuthey et al., 2014; van Raaij et al., 2018b). The endocytic iron is released from the transport protein, and is then reduced and exported to the cytosol mainly through DMT1, where Fe3+ is excluded under low iron conditions (Smith et al., 2019).

There is no evidence for basolateral uptake of TBI, heme, hemoglobin, or non-transferrin-bound iron (NTBI) in the basolateral renal tubules so far, whereas the iron exporter-ferroportin exists in the basolateral membrane of proximal tubules (van Raaij et al., 2018a). As a master regulator of iron homeostasis, hepcidin regulates this ferroportin-mediated iron export and intracellular H-ferritin levels (Vyoral and Jiri, 2017). In podocytes, hemoglobin can be taken up by megalin-cubilin endocytosis catabolized by heme oxygenase-1 (HO-1), thus resulting in increased oxidative stress (Rubio-Navarro et al., 2018; Duann et al., 2019). In addition, iron transport and regulation also exist in mesangial cells, since ferritin, TFR1, HO-1, and IRP1 are all expressed in these cells (Cheng et al., 2015; Liu and Templeton, 2015).

### 3.2.2 Iron Overload and Toxicity in Kidney

Iron plays a key role in mitochondria, promoting oxidative stress and ROS production, leading to cell apoptosis, mitochondrial damage and vascular calcification (Nakanishi et al., 2020). In 1988, the deleterious role of iron in renal disease was first described as being involved in free radical mediated renal damage after ischemia and lipid peroxidation, while iron chelators can reduce these injury (Paller and Hedlund, 1988), suggesting the existence of ferroptosis. Mitochondria are extremely dependent on iron metabolism and are the target of iron transport in these cells. The tubular epithelium possesses abundance of mitochondria to support active transport, but quite susceptible to hypoxia, toxic compounds, proteinuria, metabolic disorders. Kidneys can be exposed to toxic levels of bound iron (e.g., transferrin), or free iron, due to redundant filtration of iron into renal tubules, alterations in cellular iron localization or compartmentalization in the kidney (Martines et al., 2013; van Raaij et al., 2018b; Figure 4).

It was found that kidney and urinary iron content are elevated in patients with CKD (Sears et al., 1966; Wang et al., 2001; Shah et al., 2007; van Raaij et al., 2018b) and animal models of CKD (Nankivell et al., 1994; Ueda et al., 1996; Harris and Tay, 1997; Naito et al., 2015). Altered molecular iron handling may lead to iron deposition in kidney (van Raaij et al., 2018b). When these iron or iron-containing molecules exceed the storage capacity of ferritin or the cell's ability to export iron, they damage renal tubular cells (Sponsel et al., 1996; Zager and Burkhart, 1997; Martines et al., 2013; Naito et al., 2015). Exposure to either the Fe2+ or Fe3+ (100 μM, 30–80 h) may impair healing of denuded areas made within confluent monolayers of renal tubular epithelial cell lines (Sponsel et al., 1996). Fe2+ (FeSO4 100 μM) can stimulate H2O2 production in renal tubular cells in vitro (Zager and Burkhart, 1997). Fe2+ can also cause cytotoxic changes including numerous iron-particles-containing lysosomes and distortional mitochondria (Sponsel et al., 1996; Figure 4). Apart from the tubules, cultured human glomerular endothelial cells (HGECs) have been shown to express TFR1, FPN1 and DMT1. The endothelial injury factor-Ang II could induce iron incorporation followed by an increase in labile iron in HGECs (Tajima et al., 2010). Iron chelators have been confirmed to improve renal function in patients with glomerular disease, and minimize the reactivity of iron and remove iron from the kidneys or relieving iron-related oxidative stress (Rajapurkar et al., 2013). Iron restriction can also delay the progress of DN in diabetic rats (Matsumoto et al., 2013). In addition, administration of iron...
chelation can alleviate renal interstitial fibrosis and suppress the increments of collagen type III and TGF-β (Naito et al., 2015).

Iron-mediated cell injury or ferroptosis is considered as a core of the pathogenesis of acute kidney injury (Scindia et al., 2019). Iron homeostasis is becoming the focus of new therapeutic interventions for kidney diseases. Although iron overload or toxicity are not equivalent to ferroptosis, there is reason to believe that these “iron induced oxidative stress or lipid peroxidation” are at least related to ferroptotic events.

### 3.3 Ferroptosis in Renal Fibrosis

ROS play a wide role in the oxidation of lipids, proteins, and DNA, resulting in damage to the tubules and vascular endothelium, thus secreting profibrotic factors (Pisani et al., 2013; Daenen et al., 2019). Inhibition of oxidative stress has always been an anti-fibrotic target (Wang et al., 2019). Oxidative stress can cause fibroblast/pericyte activation (Bondi et al., 2010), tubular epithelial-mesenchymal transition (EMT) (He et al., 2015; Song et al., 2018), fibrocyte/macrophage recruitment (Wagner et al., 2012; Lee et al., 2016), and endothelial-mesenchymal transition (EndMT) (Echeverria et al., 2013; Montorfano et al., 2014). Reportedly, HK2 (human kidney tubular cell) has been reported to secrete profibrotic factors and facilitate fibroblast proliferation and activation during ferroptosis (Zhang et al., 2021). Oxidative stress induced glomerular endothelial cell dysfunction (Sol et al., 2020), mesangial sclerosis (Lu et al., 2018), podocyte abnormally (Okamura and Pennathur, 2015; Zschiedrich et al., 2017), and parietal epithelial cell injury (Odaci et al., 2015; Su et al., 2015) can lead to glomerulosclerosis (Tang et al., 2011; Su et al., 2019). Increased production of ROS (including OH−, H2O2, lipid peroxides, and superoxide anion free radicals) in podocytes can directly damage podocytes themselves, which consequently mediate glomerular injury (Gwinner et al., 1997; Marshall et al., 2006). Numerous pathologic processes mediated by oxidative stress associated factors can stimulate glomerular endothelial cells to undergo EndMT, which is consistent with increased mesenchymal marker expression including α-smooth muscle actin (αSMA) and fibroblast specific protein 1, and the production of ECM proteins (Liang et al., 2016; Dejana et al., 2017; Figure 4).

Although oxidative stress is known as an inducer for ferroptosis, we have to notice it is the “lipid ROS” and subsequently membrane rupture that represent the occurrence of ferroptosis, instead of the “ROS” (Dixon et al., 2012). Ferroptosis triggers inflammatory and immune responses through the release and activation of damage-related molecular patterns (DAMPs) signals, which makes it to be an attractive therapeutic target for renal fibrosis especially renal graft fibrosis (Linkermann et al., 2013; Martin-Sanchez et al., 2017). Synchronous tubule necrosis can be induced in renal ischemia/reperfusion (I/R) injury model, oxalate-induced AKI model and diabetic nephropathy (DN) tubule cell death model (Linkermann et al., 2014; Wang Y. et al., 2020; Li S. et al., 2021). Erastin or TGF-β can induce ferroptosis, promote fibroblast-to-myofibroblast differentiation resulting in fibrosis (Gong et al., 2019). Recent studies have shown that ureteral obstruction can induce ferroptosis in renal tubular epithelial cells in vivo, and HK-2 cells (human proximal tubular cell line) to secrete various profibrotic factors which further promoted the proliferation and activation of fibroblast during ferroptosis (Zhang et al., 2021).

#### 3.3.1 HO-1

HO-1, an inducible enzyme, catalyzes the heme group into carbon monoxide (CO) and biliverdin, which can further convert to bilirubin, and ferrous iron. HO-1 induction could protect the kidney from oxidative stress showing anti-inflammatory and anti-apoptotic activity in DN (Abo El Gheit and Emam, 2016), which was also proved to reduce glomerular sclerosis and intimal hyperplasia, being a therapeutic target to improve the long-term outcomes after kidney transplantation (Bedard et al., 2005; Corona et al., 2020). Additionally, compared with wild-type mice, HO-1−/− mice had higher levels of renal fibrosis (Kovtunovych et al., 2010), and more susceptible to myocardial I/R (Abo El Gheit and Emam, 2016). Absence of HO-1 was associated with immune response deficiency, such as Th1-weighted shift in cytokine responses (Kapturczak et al., 2004), and defects in erythropagocytosing macrophages (Kovtunovych et al., 2010), thus supporting it a protective role in kidney diseases. However, activation of HO-1 triggers ferroptosis through iron overloading and excessive lipid peroxides, thus promoting renal fibrosis area and collagen I in DN mice (Chang et al., 2018; Feng et al., 2021). Increased HIF-1α/HO-1 level, iron content, ferritin level and lipid peroxides were detected in this DN kidneys, indicating that ferroptosis might enhance DN and damage renal tubules through HIF-1α/HO-1 pathway (Feng et al., 2021).

Researches on neurodegenerative diseases and neoplastic disorders also demonstrated HO-1 has pro-ferroptotic effects on cells through iron accumulation and pro-oxidant effects (Chang et al., 2018; Li R. et al., 2020; Fernández-Mendivil et al., 2021).

Resultingly, though induction of HO-1 has been a potential therapeutic target in kidney diseases, attention must be paid to the safe range of HO-1 induction, owing to the pro-ferroptotic role of HO-1 in raising intracellular ferrous load and redox homeostasis (Chang et al., 2018; Li R. et al., 2020; Fernández-Mendivil et al., 2021).

#### 3.3.2 NOX4

NADPH oxidase (NOXs) plays the most important role in many enzyme systems involved in ROS production in the kidney (Jha et al., 2016). NOX4 is a major source of ROS in the renal cells, linking with myofibroblast differentiation and tubulointerstitial fibrosis through various pathways. Although NOx4 is expressed in a variety of kidney cells, including endothelial, epithelial, podocytes, mesangial cells, and fibroblasts, its role in fibrosis is controversial (Yang et al., 2018). Inhibition of NOX4 could protect from ferroptosis in various cells via suppressing H2O2 and lipid peroxides production (Dixon et al., 2012; Wang et al., 2018; Chen et al., 2019). Manipulating NOx4 expression in mice proximal tubules cells could upregulate renal fibronectin and TGF-β level (Sedek et al., 2010). It is confirmed that rapamycin can decrease MDA and NOX4 levels, inhibit the NADPH oxidase activity, and elevate the GSH level and SOD activity, thus...
inhibiting the ROS production of mesangial cells in DN (Lu et al., 2018). Nrf2 is known to regulate the antioxidant response and the activity of several ferroptosis-related proteins, involving iron homeostasis, intermediate metabolism, and GSH metabolism; Nrf2-mediated up-regulation of HO-1, as described above, could facilitate heme degradation, free iron release, and lipid peroxidation, thus inducing ferroptosis (Dodson et al., 2019; Fang et al., 2019). The up-regulation of Nrf2 expression by Fenofibrate can inhibit iron droop, thus alleviating renal pathological changes in DN mice (Li S. et al., 2021). HIF-1α could negatively regulate ferroptosis induction in human cancer cells (Yang M. et al., 2019), which is also mechanically antithetical to oxidative stress mentioned above (Nlandu Khodo et al., 2012). This is contrary to the traditional view that NOXs promotes ferroptosis by oxidative stress-induced lipid peroxidation (Xie et al., 2016a; Wang J. et al., 2020; Park et al., 2021). Different subcellular localization, cell types, molecular concentrations, disease types or/and stages may be responsible for this functional variability, and more research is warranted on cell-specific and inducible overexpression or knockout systems in vivo.

3.3.3 TP53
TP53 (p53) is considered as a tumor suppressor due to its ability to promote cell death or permanently inhibit cell proliferation. P53 can negative regulate SLC7A11, leading to reduced glutathione production and increased ROS (Jiang et al., 2015), target SAT1 which increase the level of ALOX15 (Ou et al., 2016), suggesting that p53 represents a novel inducer of ferroptosis. However, p53 can also regulate GSL2 (glutaminase 2) to influence glutamine metabolism and block dipeptidyl-peptidase-4 (DPP4) activity, which finally defense lipid peroxidation and ferroptosis (Hu et al., 2010; Xie et al., 2017). Reportedly, CRISPR/Cas9 mediated p53 deletion cells were sensitive to ferroptosis, implying that p53 hold a pro-survival function in ferroptosis, which requires p53 targeting CDKN1A (P21) expression (Tarangelo et al., 2018). These context-dependent roles of p53 in ferroptosis may result from fine-tuning its phosphorylation, ubiquitination, methylation, acetylation, and other modifications.

TP53 also plays a critical role in non-neoplastic diseases including renal fibrosis and CKD, which acts as a critical co-factor for several key fibrotic and cell cycle effectors including TGF-β1, CTGF, PAI-1, P21 (Kortlever et al., 2006; Yang et al., 2010; Samarakoon et al., 2012). P53 inhibitor piñiflirin-α was proved to alleviate renal experimental fibrogenesis consistent with decreased CTGF and TGF-β1 levels (Yang et al., 2010). Interestingly, the p53-dependent P21, shows both effect in ferroptosis and renal fibrosis (Tarangelo et al., 2018; Das et al., 2020), but whether a direct link exists between ferroptosis, renal fibrosis, and P21 has not been reported.

4 FERROPTOSIS REGULATORS AND RENAL FIBROSIS

4.1 Ferroptosis Inhibitors
Many drugs or compounds have been reported to inhibit renal fibrosis in vitro (Table 1) or in vivo (Table 2) and have also been proved to inhibit ferroptosis (Table 3).

4.1.1 Antioxidants
Several antioxidants can suppress ferroptosis through limiting lipid peroxidation and have been verified to alleviate renal fibrosis from different causes in vivo and/or in vitro through different targets and mechanisms, including Vitamin E, CoQ10, N-acetylcysteine (NAC), Trolox, Diphenylene iodonium, and Zileuton (Jenkins et al., 2001; Ho et al., 2007; Piao et al., 2013; Shen et al., 2016; Atmaca et al., 2017; Giam et al., 2017; Gonzalez et al., 2017; Helmy et al., 2018; Zhao et al., 2018; Li C. et al., 2019; Montford et al., 2019; Farias et al., 2020).

4.1.2 Iron Chelators
Reportedly, deferoxamine prevents renal interstitial fibrosis in the UUO mice (Ikeda et al., 2014). Long-term oral administration of deferiprone have been shown to have renal protective effects in both diabetic and non-diabetic glomerular disease (Rajapurkar et al., 2013). These two iron chelating agents have anti-iron toxicity at 100 μM (Do Van et al., 2016).

4.1.3 CYP450 Substrate
Certain cytochrome P450 substrate drugs have the anti-ferroptotic effect by scavenging activity against lipid peroxyl radicals in various cell lines including NRK-49F (rat kidney fibroblast), NRK52E (rat kidney tubular cell), and HK2 (Mishima et al., 2020). These drugs inhibit the ferroptotic pathological changes of different renal cell types and ameliorates renal damage, indicating the therapeutic promise of this repurposed drugs (e.g., Triiodothyronine, and Carvedilol) (Wong et al., 2001; Jovanovic et al., 2005; Lu et al., 2013; Adedoyin et al., 2018; El Agaty, 2018).

4.1.4 Thiazolidinediones
The PPAR-γ agonists thiazolidinediones (TZDs), including rosiglitazone, troglitazone, and pioglitazone, could prevent ferroptosis probably by inhibiting ACSL4, even in PPARγ KO cells (Doll et al., 2017; Kagan et al., 2017; Wang Y. et al., 2020). However, non-thiazolidinediones PPARγ agonists failed to rescue cells from RSL3-induced ferroptosis, suggesting that thiazolidinediones might exert anti-ferroptosis effect regardless of PPARγ (Doll et al., 2017). Reportedly, these drugs can alleviate the glomerulosclerosis and tubulointerstitial fibrosis of various renal disease models (McCarthy et al., 2000; Chung et al., 2005; Panchapakesan et al., 2005; Omasu et al., 2007; Kawai et al., 2009; Han et al., 2010; Bilan et al., 2011; Koch et al., 2012; Shui et al., 2017; Sun et al., 2017; Németh et al., 2019; Sun et al., 2019; Wei et al., 2020), and ameliorate chronic renal allograft dysfunction (Kiss et al., 2010; Deng et al., 2019).

4.1.5 DPP4 Inhibitors
In the absence of TP53, ferroptosis can be inhibited by downregulation of DPP4 or administration of DPP4 inhibitors (e.g., alogliptin, linagliptin, and vildagliptin) (Xie et al., 2017). Additionally, DPP4 represents a precise druggable target in CKD. These DPP4 inhibitors were proved to exhibit renoprotective effects and ameliorate the glomerulosclerosis and tubulointerstitial fibrosis in different diseases (Kanasaki et al., 2014; Vavrinec et al., 2014; Tanaka et al., 2016; Tsuprykov et al.,...
TABLE 1 | Evidence of ferroptosis inhibitors alleviates renal fibrosis in vitro.

| Agents | Concentration | Cell line model | Effect | References |
|--------|---------------|-----------------|--------|------------|
| Rosiglitazone | 1 μM | NRK-52E (Rat renal tubule epithelial cells)/HK2 Cells (Human Kidney 2) | Blocks ferroptosis by inhibiting ACSL4-mediated lipid peroxidation | Wang Y et al. (2020) |
| | 1-50 μM | Rat mesangial cells | Causes an anti-fibrotic effect in renal mesangial cells by increasing sphingosine kinase 1 levels | Koch et al. (2012) |
| Troglitazone | 1-20 μM | Rat mesangial cells | Causes an anti-fibrotic effect in renal mesangial cells by increasing sphingosine kinase 1 levels | Koch et al. (2012) |
| Pioglitazone | 10 μM | High glucose-induced profibrotic HK-2 Cell model | Reverses the high glucose-induced profibrotic by intervention of AP-1, TGF-β1, and the extracellular matrix protein fibronectin | Panchapakesan et al. (2005) |
| | 5 μM | TGF-β1-induced HK-2 Cell model | Improves mitochondrial functions and protects against renal fibrosis | Sun et al. (2017) |
| Vitamin E | 100 μM | TGF-β1-induced HK-2 Cell model | Suppresses the TGF-β1 signaling and inducing mitochondrion-associated myofibroblast apoptosis | Wang et al. (2010); Wang et al. (2016); Hu et al. (2017) |
| | 20-80 μM | TGF-β1-induced NRK-49F cells | Inhibits TGF-β1-induced expression of α-SMA, col1α1 and fibronectin | Li et al. (2022) |
| Ferrostatin-1 | 10 μM | TGF-β1-induced HK-2 Cell model | Inhibits TGF-β1-induced expression of fibronectin | Li et al. (2022) |
| Diphenylene iodonium | 20 μM | RMC (Rat kidney mesangial cells) | Suppresses methylglyoxal-induced renal fibrosis by inhibiting superoxide, and expression of TGF-β1 and fibronectin | Ho et al. (2007) |
| N-acetylcysteine | 10 mM | Cisplatin-induced NRK-52E | Decreases cisplatin-induced renal interstitial fibrosis via sirtuin1 activation and p33 deacetylation | Li C et al. (2019) |
| Puerarin | 50 μM or 100 μM | HK2 | Ameliorates renal fibrosis by reducing oxidative stress induced-epithelial cell apoptosis via MAPK signal pathways | Zhou et al. (2017) |
| Trolox | 1 μM | Mouse renal CD cell line (M-1) | Blunts prorenin-dependent ROS formation and augmentation of profibrotic factors | Gonzalez et al. (2017) |
| Triiodothyronine | 1 nM | HK2 | Inhibits TGFβ1 induced renal tubular epithelial to mesenchymal transition by increasing miR34a expression | Lu et al. (2013) |
| Linagliptin | 100-251 μM | Mouse proximal tubular cell | Attenuates FFA-bound albumin-induced tubular inflammation, fibrosis and apoptosis | Tanaka et al. (2016) |
| | 10 μM | HRPTECs (human renal proximal tubular epithelial cells) | Reverses interleukin-17A induces renal fibrosis | Weng et al. (2020) |
| | 20 mM | Primary PTECs from mice kidneys and pig kidney proximal tubule (LLC-PK1) cell line | Inhibits TGF-β1-induced renal fibrosis | Chen et al. (2014) |
| Chloroquine | 50 μM | HKC-II cells (human tubular epithelial cell line) | Reduces renal fibrosis via increasing extracellular uronase and collagen degradation | Fox et al. (2016) |
| Liproxstatin-1 | 0.5 μM | HK2 and fibroblasts | Blocks the proliferation and differentiation of fibroblasts by inhibiting the release of profibrotic factors released by ferroptotic tubular epithelial cells | Zhang et al. (2021) |

2016; Uchida et al., 2017; Oraby et al., 2019; Pengrattanachot et al., 2020; Tang et al., 2021).

4.1.6 Others

Recent studies have demonstrated that lipoxstatin-1 attenuates renal fibrosis induced by UUO through inhibiting ferroptosis in renal tubular epithelial cells (Zhang et al., 2021). Ferrostatin-1 is a widely used ferroptosis blocker, which exhibits renoprotective effects in obesity-induced renal fibrosis, inflammatory cell infiltration by inhibiting ferroptosis in high-fat diet mice (Li et al., 2022). It might reverse pathological parameters and improve renal function of folic acid-induced AKI mice, which is a model for studying nephroptotic tubule damage and gradual progression of renal fibrosis (Li X. et al., 2020). Puerarin has been demonstrated to protect against heart failure via mitigating ferroptosis (Liu et al., 2018), which also ameliorates renal fibrosis by reducing oxidative stress induced-epithelial cell apoptosis via MAPK signal pathways (Zhou et al., 2017).

Baecalein restrains ferroptosis by abrogating ALOX-mediated lipid peroxidation (Xie et al., 2016b; Yang et al., 2016), and can inhibit renal fibrosis in a variety of ways (Wang W. et al., 2015; Wang et al., 2016; Hu et al., 2017). U0126, the ERK inhibitor, was proved to protect against cell death induced by erastin in MEFs (Gao et al., 2015). It has also been reported to ameliorate renal fibrosis in different renal proximal tubular epithelial cells (Chen et al., 2014; Weng et al., 2020). FG-4592 pretreatment plays a protective role at the early stage of folic acid-induced renal fibrosis and other kidney injury through alleviating ferroptosis (Li X. et al., 2020) However, in the UUO mice model, even high dose treatment of FG-4592 has little effect on renal fibrosis (Kabei et al., 2020).

4.2 Ferroptosis Inducers

Interventions preventing cell death could initially prevent parenchymal cell loss thus protecting the kidney early in the course of kidney damage, but they may also facilitate fibrosis by
**TABLE 2 | Evidence of ferroptosis inhibitors alleviates renal fibrosis in vivo.**

| Agents | Dosage regimen | Animal or patient model | Effects | References |
|--------|----------------|-------------------------|---------|------------|
| Rosiglitazone | 5 mg/kg/day, for 12 weeks; 3 or 30 mg/kg/day for 8 weeks | Chronic renal allograft dysfunction rats | Inhibits TGF-β signaling and the renal tubular EMT | Kiss et al. (2010); Deng et al. (2019) |
| | 3 mg/kg/day, for 4 weeks | Cyclosporine-induced renal injury rats | Inhibits TGF-β signaling and apoptosis | Chung et al. (2005) |
| | 5 mg/kg/day in drinking water, for 24 weeks | Diabetic nephropathy (DN) rats | Alleviates the glomerulosclerosis and tubulointerstitial fibrosis | Bilan et al. (2011) |
| | 5 mg/kg/day, for 4 weeks | Aristolochic acid nephropathy mice | Reduces renal fibrosis | Shui et al. (2017) |
| Troglitazone | 150 or 300 mg/kg/day, for 14 days | UUO mice | Attenuates renal interstitial fibrosis and inflammation through reduction of TGF-β | Kawai et al. (2009) |
| | 6 mg/g chow for the first 12 weeks and 12 mg/g chow between weeks 13 and 25 | Type 2 diabetes mellitus rats | Ameliorates mesangial expansion thus preventing development of glomerulosclerosis | McCarthy et al. (2000) |
| Pioglitazone | 20 mg/kg/day, for 5 weeks | TGF-β overexpressing transgenic mice | Prevents TGF-β induced renal fibrosis by repressing ESR1 and STAT3 | Németh et al. (2019) |
| | 10 mg/kg/day, for 2 weeks | UUO mice | Reduces renal tubulointerstitial fibrosis via miR-21-5p modulation | Han et al. (2010); Sun et al. (2019) |
| | 10 mg/kg/day, for 8 weeks | 5/6 nephrectomized rats | Reduces renal fibrosis by downregulating TGF-β1, fibronectin and collagen I | Sun et al. (2017) |
| | 5 mg/kg/day, for 24 weeks | Spontaneously hypercholesterolemic rats | Exerts renoprotective effects and inhibits plasminogen activator inhibitor-1 level | Omasu et al. (2007) |
| Vitamin E | 350 mg/kg, for 2 weeks | UUO mice | Inhibits the TGF-β1/Smad2/3 during EMT | Farias et al. (2020) |
| | 100 mg/kg, for 9 weeks | Renal interstitial fibrosis rats | Inhibits renal interstitial fibrosis via alleviation of autophagic stress | Zhao et al. (2018) |
| | 25 mg/kg | Cyclosporine nephrotoxicity rats | Inhibits renal interstitial fibrosis by suppressing renal mRNA expression of COX II, HO I, TGF-β, and osteopontin | Jenkins et al. (2001) |
| Baicalein | 50 or 100 mg/kg/day, for 7 days | UUO mice | Attenuates renal fibrosis by inhibiting inflammation via down-regulating NF-kappaB and MAPK signaling mitochondrial-associated myofibroblast apoptosis | Wang W et al. (2015) |
| CoQ10 (also known as CoQ6) | 20 and 30 mg/kg/day in 2–3 divided doses | Patients with ADCK4-related glomerulopathy | Appeared renoprotective effects with preserved eGFR and reduced proteinuria | Atlamca et al. (2017) |
| | 500 mg/kg/day, for 9–11 weeks | Cisplatin-induced mice | Decrease cisplatin-induced renal interstitial fibrosis via siurin1 activation and p53 deacetylation | Li C et al. (2019) |
| | 40 mg/kg/day, 8 weeks | Dilated Cardiomyopathy mice | Attenuates tubulointerstitial and glomerular fibrosis and renal oxidized glutathione levels | Giam et al. (2017) |
| | 250 mg/kg/day, for 7 days | UUO mice | Alleviate angiotensin II-mediated renal fibrosis | Shen et al. (2016) |
| | 150 mg/kg/day, for 4 weeks | Chronic cyclosporine nephropathy mice | Attenuated tubulointerstitial fibrosis in p-AKT/p-FoxO1 pathway | Piao et al. (2013) |
| Puerarin | 50 mg/kg or 100 mg/kg, for 7 days | UUO mice | Ameliorates renal fibrosis by reducing oxidative stress induced-epithelial cell apoptosis via MAPK signal pathways | Zhou et al. (2017) |
| Zileuton | 30 mg/kg/day, for 7 or 14 days | UUO mice | Reduced renal fibrosis through manipulation of 5-lipoxygenase pathway | Montford et al. (2019) |
| | 10 mg/kg, given twice; 1 h before and 12 h after cisplatin | Cisplatin-induced mice | Alleviates cisplatin nephropotoxicity, including tubular necrosis and interstitial fibrosis via renal oxidative/inflammatory/caspase-3 axis amplification | Helmy et al. (2018) |
| Deferoxamine | 100 mg/kg/day, for 7 days | UUO mice | Prevents renal tubulointerstitial fibrosis by regulating TGF-β1-Smad signaling, oxidative stress, and inflammatory responses | Ikeda et al. (2014) |
| Deferiprone | 60 mg/kg/day; for 6 months or 150 mg/kg/day, for 9 months | Patients with diabetes and non-diabetic glomerular disease | Exhibits renoprotective effect in human | Rajapaksa et al. (2013) |
| Ferrostatin-1 | 5 mg/kg, single use | FA-induced mice | Exhibits renoprotective effects | Li Y et al. (2020) |
| Triiodothyronine | 100 μg/kg/day, for 2 weeks | 5/6 nephrectomized rats | Attenuates renal fibrosis with reduced NF-κB level and increased HO-1 level | El Agaty, (2018) |
| FG-4592 | 10 mg/kg/day, for 2 days | FA-induced mice | Plays a protective role at the early stage of FA-induced renal fibrosis through alleviating ferroptosis via Akt/GSK-3β-mediated Nrf2 activation | Li Y et al. (2020) |
| Alogliptin | 40 mg/kg/day for 10 days | UUO mice | Exhibits renoprotective effects by modulating macrophages M1/2 | Uchida et al. (2017) |
| Linagliptin | 3 mg/kg/day, for 16 weeks | 5/6 nephrectomized rats | (Continued on following page) | Tsypunykov et al. (2016) |
TABLE 2 | (Continued) Evidence of ferroptosis inhibitors alleviates renal fibrosis in vivo.

| Agents          | Dosage regimen          | Animal or patient model | Effects                                      | References                  |
|-----------------|-------------------------|-------------------------|----------------------------------------------|-----------------------------|
| Liproxstatin-1  | 5 mg/kg/day, for 4 weeks| DN mice                 | Decreased interstitial fibrosis with decreased urine albumin-to-creatinine ratio | Kanasaki et al. (2014); Oraby et al. (2019); Tang et al. (2021) |
| Vildagliptin    | 3 mg/kg/day, for 4 weeks| High-fat diet-induced obese rats | Attenuated renal fibrosis by inhibiting EndMT, apoptosis and blocking the DPP4/CD32b/NF-κB signaling circuit | Pengrattanachot et al. (2020) |
| Carvedilol      | 3 mg/kg/day, for 15 weeks| Zucker Diabetic Fatty (ZDF) rat | Attenuate renal lipid accumulation-induced lipotoxicity and reduced the TGF-β level | Vavrinc et al. (2014) |
| Chloroquine     | 20 mg/kg, 3 times a week, for 15 days | Spontaneously hypertensive stroke-prone (SHR-Sp) rats, Hypertensive rats | Shows renoprotective effects by inhibiting TGF-β | Wong et al. (2001) |
| Liproxstatin-1  | 10 mg/kg/d for 14 consecutive days | UUO mice and chronic ischemia reperfusion injury mice | Shows renoprotective effects by inhibiting TGF-β | Jovanovic et al. (2005) |

blocking the downregulation of fibroblast numbers later in the course of the renal disease. EMT, characterized by loss of E-cadherin, can facilitate ferroptosis, and upregulate transcription factors, including SNAI1, ZEB1, and, TWIST1, inducing EMT-mediated tumor metastasis, elevates ferroptosis sensitivity in turn (Wu et al., 2019), while E-cadherin-mediated cell-cell contacts has been demonstrated to protect against ferroptosis (Yang W.-H. et al., 2019; Wenz et al., 2019; Wu et al., 2019). Furthermore, human cancer cell lines and organoids with a highly mesenchymal-like cell state are selectively vulnerable to ferroptosis (Viswanathan et al., 2017). It is speculated that EMT might confer susceptibility to ferroptosis-based therapies in cancers (Chen et al., 2021). Analogizing to cancer cells, it is possible that induction of ferroptosis could also attenuate renal fibrosis by selectively killing mesenchymal-like cells transited from epithelium and endothelium.

Interestingly, some ferroptosis inducers (e.g., Acetaminophen, artemisinin and its derivatives, sorafenib, sulfasalazine, and withaferin A) have also been demonstrated to alleviate renal fibrosis in various models, suggesting a complex relationship between ferroptosis and renal fibrosis (Table 4). Reportedly, sorafenib, an xCT inhibitor, can inhibit the TGF-β/Smad3-induced EMT signaling (Jia et al., 2015) and suppress the CXCR3/CXCL11-mediated macrophage infiltration (Ma et al., 2016), ameliorating renal fibrosis. Besides, an xCT inhibitor-sulfasalazine also play a protective role against renal fibrosis in the UUO rats, with decreased level of TGF-β1, ROS, and lipid peroxides (Demirbilek et al., 2007). Acetaminophen, a resistance indicator of fibrosis, and a reduction of tubular EMT in glomerular sclerosis may prevent renal oxidative stress in an obese Zucker mouse model (Wang et al., 2013; Lőrincz et al., 2015), and exerts renoprotective effects (Wang C. et al., 2015). In different models, artemisinin and its derivatives have been reported to induce ferroptosis by triggering ROS, promoting ferritin degradation, and regulating xCT/GPX4 axis (Louandre et al., 2013; Louandre et al., 2015; Sun et al., 2016), thus alleviating renal fibrosis (Cao et al., 2016; Zhang et al., 2017; Wen et al., 2019; Zhang et al., 2019; Liu et al., 2020). Withaferin A can down-regulate pro-fibrotic factors, inflammatory signaling molecules, and ER stress-related molecules and improve kidney function by decreasing uric acid (Chen et al., 2020; Zhao et al., 2021), it could also induce ferroptosis via the NRF2 pathway by targeting KEAP1 or inactivating GPX4 (Hassannia et al., 2018).

Besides, erastin and sorafenib could induce ferroptosis in hepatic stellate cells and are potential drugs for the treatment of hepatic fibrosis (Zhang et al., 2018). Neratinib, a demonstrated ferroptosis inducer in breast cancer (Nagpal et al., 2019), has also been shown to inhibit liver fibrosis (Park et al., 2020). Fluvastatin suppressed interstitial renal fibrosis and oxidative stress in UUO mice model (Moriyama et al., 2001) and NRK-49F cells (Ikeuchi et al., 2004). Overall, these highlight the possibility of inducing ferroptosis to achieve remission of renal fibrosis.

5 OTHER PROGRAMMED CELL DEATH AND FERROPTOSIS IN RENAL FIBROSIS

It is considered that ferroptosis interacts extensively with other modes of cell death, and even distinct from other types of programmed cell death (PCD). Multiple types of PCD have been discovered and well-studied, including apoptosis, necroptosis, pyroptosis and ferroptosis, which we would like to briefly overview their relationship with renal fibrosis.

Apoptosis is regarded as a mechanism of self-protection that eliminates dead or damaged cells that form during the physiological process of aging, and is the best-known form of PCD which was first described in 1972 (Argüelles et al., 2019). Inhibition of apoptosis has been shown to reduce kidney fibrosis (Tanaka et al., 2003; Portilla, 2014), and a number of molecules or drugs have been reported to protect the kidneys by inhibiting apoptosis (Tanaka et al., 2005).
TABLE 3 | Mechanisms of ferroptosis inhibitors restraining ferroptosis.

| Agents       | Work concentration | Effects                                                                 | Model                      | References                                                   |
|--------------|--------------------|------------------------------------------------------------------------|----------------------------|--------------------------------------------------------------|
| Rosiglitrone | 1 μM               | Inhibits ACSL4-mediated lipid peroxidation                             | NRK-52E/HK2 Cells          | Wang Y et al. (2020); Doll et al. (2017); Kagan et al. (2017) |
|              | 10–30 μM           | Inhibits ACSL4-mediated lipid peroxidation                             | Pa1 cells (Breast cancer cell line) |                                                          |
|              | 0.4–0.5 mg/kg/day  | Inhibits ACSL4-mediated lipid peroxidation                             | Ischemia/reperfusion (I/R) injury mice | Li Y et al. (2019); Xu et al. (2020) |
| Troglitzone  | 10–30 μM           | Inhibits ACSL4-mediated lipid peroxidation                             | Breast cancer cell lines   | Doll et al. (2017)                                          |
| Pioglitzone  | 10–30 μM           | Inhibits ACSL4-mediated lipid peroxidation                             | Breast cancer cell lines   | Doll et al. (2017)                                          |
|              | 10 μM              | Suppresses sulfasalazine-induced ferroptosis                          | HNC/SNU cell lines         | Kim et al. (2018)                                           |
| Vitamin E    | 100 μM             | Inhibits lipid peroxidation by scavenging ROS                          | HT-1080/SJ cell lines      | Yang et al. (2014); Shimada et al. (2016)                   |
| Bacacain     | 10 μM              | Inhibits ALOX-mediated lipid peroxidation                             | G-401 cell line (a human renal carcinoma cell line) | Yang et al. (2016)                                          |
| CoQ10        | 10 μM              | May involve reprogramming of lipid metabolism in a way that is not conducive to the execution of ferroptosis | HT-1080 cells              | Shimada et al. (2016)                                       |
| Diphenylene  | 1 μM               | Inhibits NOX-mediated lipid peroxidation                               | SHSY5Y cells               | Hou et al. (2019)                                           |
| iodonium     | 1 mM               | Inhibits toxic arachidonic acid products of nuclear ALOX5              | Primary cortical neurons    | Karappagounder et al. (2018)                               |
| N-acetylcysteine | 10 mg/kg/day, for 7 days | Inhibits toxic arachidonic acid products of nuclear ALOX5              | Collagenase-induced intracerebral hemorrhage mice | Karappagounder et al. (2018) |
|             | 1 mM               | Inhibits erastin-induced ferroptosis through supplementing GSH         | HT-1080 cells              | Yang et al. (2014)                                          |
| Puerarin     | 20 μM              | Suppresses erastin- or isoprenaline-induced ferroptosis               | H9c2 cells                 | Liu et al. (2018)                                           |
|             | 100 mg/kg/day or 200 mg/kg/day, for 12 weeks | Suppresses erastin- or isoprenaline-induced ferroptosis               | Heart failure rats         | Liu et al. (2018)                                           |
| Trolox       | 100 μM             | Inhibits lipid peroxidation                                            | HT-1080 cells              | Dixon et al. (2012)                                         |
| Zileuton     | 10–100 μM          | Inhibits lipid peroxidation by suppressing ALOX5                      | HT-22 neuronal cells        | Liu et al. (2015)                                           |
| Diferoxamine | 100 μM             | Inhibits iron-mediated lipid peroxidation                             | HT-1080 cells              | Dixon et al. (2012)                                         |
| Diferoxamine | 100 mg/kg/day, for 7 days | Inhibits lipid peroxidation via ferroptotic-specific mitochondria genes | Spinal cord injury rats    | Yao et al. (2019)                                           |
| Diferiprone  | 100 μM             | Inhibits iron-mediated lipid peroxidation                             | LUHMES cells (Lund human mesencephalic cells) | Do Van et al. (2016)                                        |
| Ferrostatin-1| 0.1–10 μM          | Scavenge initiating alkoxyl radicals produced by ferrous iron from lipid hydroperoxides | HT-1080/HEK293T/LUHMES cells | Dixon et al. (2012); Do Van et al. (2016); Miotto et al. (2020) |
| Triiodothyronine | 10 μM            | Inhibits lipid peroxidation                                            | H9c2 cells                 | Mishima et al. (2020)                                       |
| FG-4592      | 10 mg/kg           | Alleviates ferroptosis via Akt/GSK-3β-mediated Nrf2 activation        | FA-induced mice            | Li Y et al. (2020)                                          |
| Alogliptin   | 10 μM              | inhibits NOX1-mediated lipid peroxidation                             | TP53−/− or TP53-deficient CRC cells (HCT116 and SW48) | Xie et al. (2017)                                           |
| Linagliptin  | 10 μM              | inhibits NOX1-mediated lipid peroxidation                             | TP53−/− or TP53-deficient CRC cells (HCT116 and SW48) | Xie et al. (2017)                                           |
| Vildagliptin | 10 μM              | inhibits NOX1-mediated lipid peroxidation                             | TP53−/− or TP53-deficient CRC cells (HCT116 and SW48) | Xie et al. (2017)                                           |
| U0126        | 10 μM              | Inhibits lipid peroxidation independent from MEK activity              | MEFs (mouse embryonic fibroblasts) | Gao et al. (2015)                                           |
| Carvedilol   | 0.1. 1, and 1 μM   | Inhibits lipid peroxidation                                            | H9c2 cells                 | Mishima et al. (2020); Gao et al. (2016)                    |
| Chloroquine  | 50 μM              | Blocks ferroptosis at earlier time points, but the inhibitory effect will be gradually lost at later time points | MEFs and HT1080 cells   | Mishima et al. (2020); Gao et al. (2016)                    |

Necroptosis, a mode of cell death dependent on the activity of RIPK1 and RIPK3 kinases, which is characterized by permeable and rupture of cell membranes can also trigger necroinflammation and release of DAMPs (Belavgeni et al., 2020). Inhibiting necroptosis was demonstrated to attenuate different type of renal fibrosis (Zhu et al., 2015; Zhu et al., 2016; Xiao et al., 2017; Dai et al., 2020; Huang et al., 2020).

Pyroptosis is caused by the cleavage of Gasdermins by various caspases, and is subsequently executed by the insertion of the N-terminal fragment of cleaved Gasdermins into the plasma membrane, facilitate plasma membrane rupture and the release of pro-inflammatory products, which has been considered as a key fibrotic mechanism in the kidney pathology development (Kesavardhana et al., 2020; Cuevas and Pelegrin, 2021). It is a form of lytic programmed cell death...
TABLE 4 | Evidence of ferroptosis inducers alleviates renal fibrosis in vitro and in vivo.

| Agents         | Dosage Model                                      | Effects                                                                                     | References |
|----------------|---------------------------------------------------|-----------------------------------------------------------------------------------------------|------------|
| Acetaminophen  | 30 mg/kg/day via drinking water for 26 weeks Obese Zucker rat model | Prevents tubulointerstitial fibrosis and glomerulosclerosis by alleviating tubular EMT and inhibiting renal oxidative stress | Wang et al. (2013) |
|                | 3 µM or 10 µM Human renal mesangial cells (HRMCs) | Exerts renoprotective effects by diminishing renal oxidative stress and p38/MAPK hyper-phosphorylation | Wang et al. (2013) |
| Artemisinin    | 100 mg/kg/day for 16 weeks Rats with 5/6 nephrectomy | Attenuates tubulointerstitial inflammation and fibrosis via the NF-kappaB/NLRP3 pathway       | Wen et al. (2019) |
|                | 25, 50, 100 µM Ang II-induced HK2                 | Attenuates tubulointerstitial inflammation and fibrosis via the NF-kappaB/NLRP3 pathway       | Wen et al. (2019) |
| Artesunate     | 30 or 60 mg/kg/day for 2 weeks UUO rats           | Attenuates renal fibrosis with the up-regulation of BMP-7 and down-regulation of USAG-1      | Cao et al. (2016) |
|                | 0.01, 0.1, and 1 µg/ml TGF-β1-induced NRK-52E cells | Ameliorates TGF-β1-induced renal interstitial fibrosis by inhibition of EMT.                   | Zhang et al. (2017) |
| Dihydroartemisinin | 40 mg/kg/day for 2 weeks UUO mice              | Attenuates renal fibrosis through regulation of fibroblast proliferation and differentiation via PI3K/AKT pathway and MALAT1/mir-145/FAK axis | Zhang et al. (2019); Liu et al. (2020) |
|                | 10 µM Primary human kidney fibroblasts-HK2 cells | Attenuates renal fibrosis through regulation of fibroblast proliferation and differentiation via PI3K/AKT pathway and MALAT1/mir-145/FAK axis | Zhang et al. (2019); Liu et al. (2020) |
| Sorafenib      | 20, 40, 80 mg/kg/day, for 14 or 21 days UUO rats | Ameliorates renal fibrosis by inhibiting TGF-β-Smad3-induced EMT signaling                    | Jia et al. (2015) |
|                | 5 mg/kg/day, for 10 days UUO mice               | Ameliorates renal fibrosis by inhibition of macrophage infiltration via the CXCR3/CXCL11 pathway | Ma et al. (2016) |
|                | 1, 5, and 10 µM NRK-52E cells                    | Ameliorates renal fibrosis by inhibiting TGF-β-Smad3-induced EMT signaling                   | Jia et al. (2015) |
| Sulfasalazine  | 100 mg/kg/day, for 7 days UUO rats               | Protects against the renal interstitial inflammation and tissue damage                        | Demirbilek et al. (2007) |
| Wilfoferin A   | 3 mg/kg/day, for 14 days UUO mice                | Reverses the increases in the protein levels of pro-fibrotic factors, inflammatory signaling molecules, and ER stress-related molecules | Chen et al. (2020) |
|                | 0.1, 0.5, and 1 µM UA-induced NRK-52E cells      | Improves kidney function by decreasing uric acid via regulation of xanthine oxidase and transporter genes in renal tubular cells | Zhao et al. (2021) |
|                | 3, 5, 10 mg/kg/day, for 7 days Hypouricemic mouse model | Improves kidney function by decreasing uric acid via regulation of xanthine oxidase and transporter genes in renal tubular cells | Zhao et al. (2021) |

initiated by inflammasomes, and HMGB1 released from pyroptotic renal tubular cells amplified inflammatory responses, which recruits immune cells to promote tissue healing and contributes to renal fibrogenesis (Raupach et al., 2006; Li Y. et al., 2021). GSDME-mediated pyroptosis is responsible for renal tubule injury and subsequently fibrosis induced by ureteral obstruction, 5/6 nephrectomy, and DN (Li W. et al., 2021; Li Y. et al., 2021; Wu et al., 2021). For more details, interested readers may refer to these excellent recently published reviews.

In general, apoptosis is a non-lytic form of cell death and is described as an active programmed cell division process to avoid triggering inflammation to remain immunologically silent, which may confine the contribution of subsequently renal fibrosis (Fink and Cookson, 2005). However, ferroptosis, pyroptosis, and necroptosis are all considered as lytic cell death, which can contribute to the release of several immune-stimulatory DAMPs, that trigger immune responses (Tang et al., 2020). Different lytic cell deaths serve distinct purposes, determine different profiles of the inflammatory infiltrate and thus determine the association between cell death and long-term renal fibrosis (Belavengi et al., 2020).

The critical role of necroptotic pathway lies in the host response to pathogen infection, which is triggered when apoptosis is hindered (Upton et al., 2010; Brault and Oberst, 2017). Pyroptosis is a primary cellular response following the sensing of pathogens as well as danger signals, which mainly includes DAMPs, pathogen-associated molecular patterns (PAMPs). The most significant initiator of pyroptosis is NLRP3 inflammasome which can be activated by various stimuli (Jo et al., 2016). However, as mentioned above, a key feature of ferroptosis is intracellular overload iron and catalysis of lipid peroxidation, which are both common and worsen in CKD with disease progression (Naito et al., 2015). In addition, proactive high-dose iron supplementation due to renal anemia also led to renal iron toxicity (Tanaka and Tanaka, 2015; van Raaij et al., 2018b). Thus, diverse chronic kidney disease creates an appropriate condition for the occurrence of ferroptosis. Although these different types of PCD have multiple functions in various physiological states and in a host’s response to external damage, there is clear evidence of crosstalk or interactions (Wang and Kanneganti, 2021).

6 CONCLUSION AND PERSPECTIVE

We have witnessed an outbreak of ferroptosis research in biomedicine over the past decades. Generally, it is a form of iron-dependent, lipid peroxidation-mediated regulated necrosis, which is strictly under controlled. This type of cell death may play a fairly important role...
in a wider range of physiological and pathophysiological processes (Qiu et al., 2020). On the other hand, a variety of studies on ferroptosis have detected only a few non-specific indicators, such as glutathione (GSH) levels and GPX4 levels, to demonstrate the presence of ferroptosis, which may possibly exaggerate the role of ferroptosis to some extent due to the variety of oxidative stress.

Despite a large number of recent studies have implicated that ferroptosis is related to renal fibrosis, its complex and finely concrete mechanism is still far from being resolved. More research is warrant to elucidate whether and how pharmaceuticals or chemicals mentioned in this article affect renal fibrosis by regulating ferroptosis.

REFERENCES

Abo El Geith, R., and Emam, M. (2016). Targeting Heme Oxygenase-1 in Early Diabetic Nephropathy in Streptozotocin-Induced Diabetic Rats. Acta Physiol. Hung 103 (4), 413–427. doi:10.1556/2060.103.2016.4.001
Azeddin, O., Bouda, R., Traylor, A., Lever, J. M., Bolisetty, S., George, J. F., et al. (2018). Heme Oxygenase-1 Mitigates Ferroptosis in Renal Proximal Tubule Cells. Am. J. Physiology-Renal Physiol. 314 (5), F702–F714. doi:10.1152/ajprenal.00044.2017
Altman, B. J., Stine, Z. E., and Dang, C. V. (2016). From Krebs to Clinic: Glutamine Ameliorates Renal Fibrosis in Rats. Cell Physiol. Biochem. 427. doi:10.1556/2060.103.2016.4.001
Allman, B. J., Stine, Z. E., and Dang, C. V. (2016). From Krebs to Clinic: Glutamine Metabolism to Cancer Therapy. Nat. Rev. Cancer. 16 (10), 619–634. doi:10.1038/nrc2016.71
Argüelles, S., Guerrero-Castilla, A., Cano, M., Muñoz, M. F., and Ayala, A. (2019). Mechanisms of Ferroptosis. J. Am. Soc. Nephrol. 21 (1), 93–102. doi:10.1681/ASN. 2009020146
Brault, M., and Oberst, A. (2017). Controlled Detonation: Evolution of Necroptosis in Pathogen Defense. Immunol. Cell Biol. 95 (2), 131–136. doi:10.1038/icb. 2016.117
Brittenham, G. M. (2011). Iron-Chelating Therapy for Transfusional Iron Overload. N. Engl. J. Med. 364 (2), 146–156. doi:10.1056/NEJMc1004810
Brown, C. W., Amante, J. J., Chhoy, P., Elaimy, A. L., Liu, H., Zhu, L. J., et al. (2019). Prominin2 Drives Ferroptosis Resistance by Stimulating Iron Export. Developmental Cell 51 (5), 575–586. doi:10.1016/j.devcel.2019.10.007
Canonne-Hergaux, F., and Gros, P. (2002). Expression of the Iron Transporter DMT1 in Kidney from Normal and Anemic Mk Mice. Kidney Int. 62 (1), 147–156. doi:10.1046/1523-1755.2002.0045x
Cao, J., Wang, W., Li, Y., Xia, J., Peng, Y., Zhang, Y., et al. (2016). Artesunate Attenuates Unilateral Ureteral Obstruction-Induced Renal Fibrosis by Regulating the Expressions of Bone Morphogenetic Protein-7 and Uterine Sensitization-Associated Gene-1 in Rats. Int. Urol. Nephrol. 48 (4), 619–629. doi:10.1007/s11255-016-1232-0
Cao, J. Y., and Dixon, S. J. (2016). Mechanisms of Ferroptosis. Cell. Mol. Life Sci. 73 (11-12), 2195–2209. doi:10.1007/s00018-016-2194-1
Chang, L., Chiang, S.-K., Chen, S.-E., Yu, Y.-L., Chou, R.-H., and Chang, W.-C. (2018). Heme Oxygenase-1 Mediates BAY 11-7085 Induced Ferroptosis. Cancer Lett. 416, 124–137. doi:10.1016/j.canlet.2017.12.025
Chen, C.-M., Chung, Y.-P., Liu, C.-H., Huang, K.-T., Guan, S.-H., and Chiang, W.-C. (2020). Ferritin Bound to Heme-Chelating Agent Protects Against Endoplasmic Reticulum Stress-Associated Apoptosis, Inflammation, and Fibrosis in the Kidney of a Mouse Model of Unilateral Ureteral Obstruction. PLoS One. 15(12):e0245332.
Chen, X., Kang, R., Kroemer, G., and Tang, D. (2021). Broadening Horizons: the Role of Ferroptosis in Cancer. Nat. Rev. Clin. Oncol. 18, 280–296. doi:10.1038/s41571-020-0246-0
Cheng, H.-T., Yen, C.-J., Chang, C.-C., Huang, K.-T., Chen, K.-H., Zhang, R.-Y., et al. (2019). Ferritin Heavy Chain Mediates the Protective Effect of Heme Overload. N. Engl. J. Med. 381 (12), 2506–2517. doi:10.1056/NEJMc1902015
Chen, W.-C., Lin, H.-H., and Tang, M.-J. (2014). Regulation of Proximal Tubular Cell Differentiation and Proliferation by Matrix Stiffness and ECM Components. Am. J. Physiology-Renal Physiol. 307 (6), F695–F707. doi:10.1152/ajprenal.00684.2013
Chen, X., Kang, R., Kroemer, G., and Tang, D. (2021). Broadening Horizons: the Role of Ferroptosis in Cancer. Nat. Rev. Clin. Oncol. 18, 280–296. doi:10.1038/ s41571-020-0246-0
Chen, X., Xu, S., Zhang, Y., Liu, B. (2019). Role of TLR4/NADPH Oxidase 4 in Promoting Cell Death Through Autophagy and Ferroptosis During Heart Failure. Biochem. Biophysical Res. Commun. 516 (1), 37–43. doi:10.1016/j. biocel.2019.02.006
Cheng, H.-T., Yen, C.-J., Chang, C.-C., Huang, K.-T., Chen, K.-H., Zhang, R.-Y., et al. (2015). Ferritin Heavy Chain Mediates the Protective Effect of Heme Oxygenase-1 Against Oxidative Stress. Biochim. Biophys. Acta (Bba) - Gen. Subjects. 1850 (12), 2506–2517. doi:10.1016/j.bbagen.2015.09.018
Chung, B. H., Li, C., Sun, B. K., Lim, S. W., Ahn, K. O., Yang, J. H., et al. (2005). Rosiglitazone Protects against Cyclosporine-Induced Pancreatic and Renal Injury in Rats. Am. J. Transpl. 5 (8), 1856–1867. doi:10.1111/j.1600-6143.2005.00979.x

AUTHOR CONTRIBUTIONS

RYT, MG, YHZ and YZ developed the theoretical formalism. ZJW, JJZ, and CJS contributed to the final version of the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (Grant no. 82070769).
Zhang et al. Ferroptosis in Renal Fibrosis

Tang, D.-q., Wei, Y.-q., Yin, X.-x., Lu, Q., Hao, H.-h., Zhai, Y.-p., et al. (2011). Vavrinec, P., Henning, R., Landheer, S., Wang, Y., Deelman, L., Dokkum, R., et al. (2014).

Tang, P. M.-K., Zhang, Y.-Y., Hung, J. S.-C., Chung, J. Y.-F., Huang, X.-R., To, K.-M., Veuthey, T., Hoffmann, D., Vaidya, V. S., and Wessling-Resnick, M. (2014). Therapeutic Potential of Hepcidin

van Swelm, R. P. L., Wetzels, J. F. M., and Swinkels, D. W. (2020). The Multifaceted Role of CRP in the Pathogenesis of Nephropathy.

Vyoral, D., and Jiri Petrak, P. (2017). Biochemical and Biophysical Research Communications. 470 (3), 539–545. doi:10.1016/j.bbrc.2016.01.109

Tang, D.-q., Wei, Y.-q., Yin, X.-x., Lu, Q., Hao, H.-h., Zhai, Y.-p., et al. (2011). In Vitro suppression of Quercetin on Hypertrophy and Extracellular Matrix Accumulation in Rat Glomerular Mesangial Cells Cultured by High Glucose.

Tang, P., Yuan, J.-L., Tao, Y.-Y., Zhang, Y., Liu, P., and Liu, C.-H. (2010). Am. J. Pathol. 176, 1570–1611.

Wagner, B., Tan, C., Barnes, J. L., Ahuja, S., Davis, T. L., Gorin, Y., et al. (2012). Nephrogenic Systemic Fibrosis. Am. J. Pathol. 181 (6), 1941–1952. doi:10.1016/j.ajpath.2012.08.026

Wang, W., Zhou, P.-h., Xu, C.-g., Zhou, X.-j., Hu, W., and Zhang, J. (2015). Baicalin Attenuates Renal Fibrosis by Inhibiting Inflammation via Down-Regulating NF-Kb and MAPK Signaling Pathways. J. Mol. Hist. 46 (3), 283–290. doi:10.1007/s10735-015-9621-8

Wang, C., Blough, E., Arvapalli, R., Dai, X., Triest, W. E., Leidy, J. W., et al. (2015). Metabolic Syndrome-Induced Tubulointerstitial Injury: Role of Oxidative Stress and Preventive Effects of Acetaminophen.

Wang, K., Lin, J., and Zhou, H. (2019). Fenoldopam Inhibits Furosemide-Induced Myocyte Necrosis in Vitro.

Wang, J., Deng, B., Liu, Q., Huang, Y., Chen, W., Li, J., et al. (2020). Pyroptosis and Ferroptosis Inhibited by Mixed Lineage Kinase 3 (MLK3) Signaling in Cardiomyocytes Are Essential for Myocardial Fibrosis in Response to Pressure Overload.

Wang, Y., Bi, R., Quan, F., Cao, Q., Lin, Y., Yue, C., et al. (2020). Ferroptosis Involves in Renal Tubular Cell Death in Diabetic Nephropathy. Eur. J. Pharmacol. 888, 173574. doi:10.1016/j.ejphar.2020.173574

Wang, Q.-L., Yuan, J.-L., Tao, Y.-Y., Zhang, Y., Liu, P., and Liu, C.-H. (2010). Fuzheng Huayu Recipe and Vitamin E Reverse Renal Interstitial Fibrosis through Countering TGF-B1-Induced Epithelial-To-Mesenchymal Transition. J. ethnomedecology apocalypse 127 (3), 631–640. doi:10.1002/1jep.2009.12.011

Wang, W., Zhou, P.-h., Xu, C.-g., Zhou, X.-j., Hu, W., and Zhang, J. (2016). Baicalin Ameliorates Renal Interstitial Fibrosis by Inhibiting Myofibroblast Apoptosis Vivotio. BJU Int. 118 (1), 145–152. doi:10.1111/bju.13219

Wang, Y., and Kanneganti, T. D. (2021). From Pyroptosis, Apoptosis and Ferroptosis to PAnoptosis: A Mechanistic Compendium of Programmed Cell Death Pathways. Comput. Struct. Biotechnol. J. 19, 4641–4657. doi:10.1016/j.csbj.2021.07.038

Wang, Y., Pang, L., Zhang, Y., Lin, J., and Zhou, H. (2019). Fenoldopam Improved Interstitial Fibrosis of Renal Allograft through Inhibited Epithelial-Mesenchymal Transition Induced by Oxidative Stress. Medicale. 2019, 1–12. doi:10.1016/j.sj.2019.08.033

Wang, Z., Ding, Y., Wang, X., Lu, S., Wang, C., He, C., et al. (2018). Pseudolipid Acid B Triggers Ferroptosis in Glioma Cells via Activation of Nox4 and Inhibition of xCT. Cancer Lett. 428, 21–33. doi:10.1016/j.canlet.2018.04.021

Wei, S., Xu, C., Zhang, Y., Shi, Z., Wu, M., and Yang, B. (2020). Ultrasound Assisted a Peroxisome Proliferator-Activator Receptor (PPAR)γ agonist-Loaded Nanoparticle-Microbubble Complex to Attenuate Renal Interstitial Fibrosis. Int. J. nanomedicine. 15, 7315–7327. doi:10.2147/IJN.S262052

Weiss, A., Spektor, L., A. Cohen, L., Magid Gold, L., Zhang, D.-L., Truman-Rosentsvir, M., et al. (2018). Orchestrated Regulation of Iron Trafficking Proteins in the Kidney during Iron Overload Facilitates Systemic Iron Retention. Proc. Acm. 10 (12), e024471. doi:10.1371/journal.pone.024471

Wen, Y., Pan, M. M., Lv, L. L., Tang, T. T., Zhou, L. T., Wang, B., et al. (2019). Artemisinin Attenuates Tubulointerstitial Inflammation and Fibrosis via the NF-kB/NLRP3 Pathway in Rats with 5/6 Subtotal Nephrectomy. J. Cell Biochem. 120 (3), 4291–4300. doi:10.1002/jcb.27714

Weng, C.-H., Li, Y.-J., Wu, H.-H., Liu, S.-H., Hsu, H.-H., Chen, Y.-C., et al. (2020). Interleukin-17A Induces Renal Fibrosis through the ERK and Smad Signaling Pathways. Biomed. Pharmacother. 123, 109741. doi:10.1016/j.biopha.2019.109741

Wenz, C., Faust, D., Linz, B., Turmann, C., Nikolova, T., and Dietrich, C. (2019). Cardiac Cell-Contact Protection against T-BuOOH-Induced Cellular Damage and Ferroptosis In Vitro. Arch. Toxicol. 93 (5), 1265–1279. doi:10.1007/s00204-019-02413-w

Wong, Y. N., Lapin, N. J., Nelson, A. H., Contino, L. C., Olson, B. A., Gygielko, E., et al. (2001). Renoprotective Effects of Carvedilol in Hypertensive-Stroke Prone}

Frontiers in Molecular Biosciences | www.frontiersin.org

May 2022 | Volume 9 | Article 890766

20
