Ferroptosis Biology

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ABSTRACT

Ferroptosis is a regulated cell death modality triggered by iron-dependent lipid peroxidation. Ferroptosis plays a causal role in the pathophysiology of various diseases, making it a promising therapeutic target. Unlike all other cell death modalities dependent on distinct signaling cues, ferroptosis occurs when cellular antioxidative defense mechanisms fail to suppress the oxidative destruction of cellular membranes, eventually leading to cell membrane rupture. Physiologically, only two such surveillance systems are known to efficiently prevent the lipid peroxidation chain reaction by reducing (phospho)lipid hydroperoxides to their corresponding alcohols or by reducing radicals in phospholipid bilayers, thus maintaining the integrity of lipid membranes. Mechanistically, these two systems are linked to the reducing capacity of glutathione peroxidase 4 (GPX4) by consuming glutathione (GSH) on the one and ferroptosis suppressor protein 1 (FSP1, formerly AIFM2) on the other hand. Notably, the importance of ferroptosis suppression in physiological contexts has been linked to a particular vulnerability of renal tissue. In fact, early work has shown that mice genetically lacking *Gpx4* rapidly succumb to acute renal failure with pathohistological features of acute tubular necrosis. Promising research attempting to implicate ferroptosis in various renal disease entities, particularly those with proximal tubular involvement, has generated a wealth of knowledge with widespread potential for clinical translation. Here, we provide a brief overview of the involvement of ferroptosis in nephrology. Our goal is to introduce this expanding field for clinically versed nephrologists in the hope of spurring future efforts to prevent ferroptosis in the pathophysiological processes of the kidney.

Keywords: acute kidney injury, ferroptosis, GPX4, iron metabolism, lipid peroxidation

Introduction

During the last two decades, multiple cell death modalities have extended the dichotomy of programmed apoptosis and unregulated necrosis, including necroptosis, pyroptosis, parthanatos, and ferroptosis (1). The term ferroptosis (ferrum from the Latin word for iron) was first coined in 2012 and characterized as an iron-dependent form of non-apoptotic cell death in human fibrosarcoma cells expressing oncogenic RAS accompanied by overwhelming lipid peroxidation of polyunsaturated fatty acids (PUFAs) contained in phospholipids (2,3). In 2014, ferroptosis was highlighted as a druggable cell death modality in kidney proximal tubule cells (PTCs) *ex vivo* (4). In the same year, genetic studies performed in mice provided conclusive evidence that the conditional knockout of the glutathione peroxidase 4 (GPX4), the guardian of ferroptosis, causes lethal acute kidney injury (AKI) with histopathological signs of extensive acute proximal tubule necrosis (ATN) (5). Remarkably, the first *in vivo* active ferroptosis inhibitor, generally known as liproxstatin-1 (Lip-1), not only delayed lethal AKI in GPX4 knockout mice but also mitigated hepatic damage inflicted by transient ischemia-reperfusion injury (IRI). Meanwhile, the significance of ferroptosis has been implicated in different AKI models, including cisplatin- and oxalate crystal-induced nephrotoxicity and AKI related to IRI.

Microvascular rarefaction, brush border alterations, tubular necrosis, the activation of myofibroblasts, and the recruitment of immune cells are common histological findings in AKI. Apart from its economic burden, the severity of AKI correlates with a higher overall in-hospital mortality (6) and a higher incidence of developing chronic kidney disease (CKD), independent of the underlying cause (7). In mice, prolonged ischemia-related AKI seems to drive an oxidative stress-mediated AKI-CKD transition (8). Single-cell transcriptome dynamics revealed that PTCs are primarily affected during IRI-mediated AKI and that upregulation of ferroptosis-related genes navigates PTCs towards maladaptive profibrotic tissue repair and CKD (9). By 2040, CKD is expected to be among the top five leading causes of death worldwide (10).

Kidney Disease: Improving Global Outcomes (KDIGO) defines CKD as a functional or structural decline in glomerular filtration rate (GFR) to less than 60 ml/min/1.73m^2 for at least three months and further risk-stratified according to the amount of albuminuria. Each healthy kidney contains approximately one million nephrons as the functional unit, producing up to 180 liters of primary urine and an average output of 1.5 liters daily. Genetically inherited disorders, premature birth, ethnicity, metabolic or cardiovascular diseases, environmental toxins, adverse drug events, bleeding or traumatic injuries, infections, autoimmunity, and malignancies all contribute to an inhomogeneous plethora of risk factors that directly or indirectly affect the individual functional nephron mass and consequently the development of CKD, CKD progression or even end-stage renal disease (ESRD).
Hence, maintaining proper nephron function by inhibiting the cellular demise of endothelial and epithelial cells as critical components is of paramount importance on the one hand. On the other hand, keeping renal homeostasis by selectively inducing cell death to prevent excessive myofibroblast proliferation and tissue inflammation due to maladaptive immune cells is equally essential to ameliorate interstitial fibrosis and tubular atrophy that may result in CKD. In this review, we summarize the most critical aspects of ferroptosis within the complex interplay of redox biology and aim to highlight potential therapeutic targets in the future of nephrology. Immune cell activation following regulated necrotic cell death in renal

disease is beyond the scope of this review, and we refer to Maremonti *et al.* (11) and Mulay *et al.* (12).

General principles of ferroptosis

Superoxide anion ($O_2^{\cdot-}$) is the predominant byproduct of oxidative phosphorylation by incomplete reduction of molecular oxygen. Along with its downstream products, such as hydrogen peroxide (H_2O_2) and hydroxyl radical (OH-), it is summarized under the generic umbrella term “reactive oxygen species” (ROS), which, however, includes many more radical and non-radical oxygen-derived species. While physiological concentrations of ROS are essential for cellular homeostasis (13), excessive ROS production and/or insufficient antioxidant defense mechanisms result in cellular redox imbalance, culminating in oxidative stress (14). Chronic degenerative diseases are often associated with an oxidative cellular environment, which can cause secondary damage to nucleotides, proteins, and lipids. Ferroptosis is a recently described form of regulated cell death executed when cells fail to protect against excessive phospholipid damage. The hallmark of ferroptosis is the uncontrolled occurrence of phospholipid hydroperoxide (PLOOH) in lipid bilayers, which can be facilitated by redox-active iron and can be induced by the disruption of the cellular antioxidant defense system (15). Thus, cells have established surveillance mechanisms to counteract detrimental lipid oxidation and ferroptosis (16) (**Figure 1**).

The cyst(e)ine/glutathione (GSH)/GPX4 axis is the prime defense against ferroptosis. GPX4, meanwhile established as the guardian of ferroptosis, reduces potentially harmful peroxidized phospholipids (i.e., PLOOH) to their corresponding alcohols at the expense of GSH, thereby halting lipid peroxidation and ferroptosis (5,17). Cysteine is the rate-limiting amino acid for the *de novo* synthesis of GSH that acts as an essential cofactor for many GSH-dependent enzymes, including GPX4. At least in cell and tissue culture, the bulk cellular cysteine is imported via the cystine/glutamate antiporter (system X_C^-) in the form of cystine (the oxidized dimeric form of cysteine), which exchanges extracellular cystine for intracellular glutamate (18). Notably, in the kidney, there is an alternate cystine transporter, known as b(0,+)-type amino acid transporter 1 (also b(0,+)AT1), which is encoded by the SLC7A9 gene, and that acts in the reabsorption of cystine in the kidney tubule. The imported cystine is then converted to its reduced form, cysteine, used for GSH biosynthesis. Consequently, cyst(e)ine, GSH, and GPX4 constitute the essential pathway for ferroptosis prevention, particularly in PTCs. In addition to the cysteine/GSH/GPX4 pathway, other GSH-independent surveillance pathways function as backup systems. Ferroptosis suppressor protein 1 (FSP1), encoded by the *AIFM2* (*apoptosis-inducing factor mitochondria associated 2*) gene, has been shown to fully compensate for the loss of GPX4 and protect against lipid peroxidation by neutralizing phospholipid peroxyl radicals in conjunction with extra-mitochondrial ubiquinone (CoQ_{10}) or vitamin K (19–22). Mechanistically, the reduced hydroquinone forms of CoQ_{10} and vitamin K, mediated by FSP1, act as naturally occurring radical-trapping antioxidants, effectively preventing ferroptosis. Furthermore, other radical-trapping antioxidants, such as vitamin E, tetrahydrobiopterin (BH4), squalene, and 7-dehydrocholesterol, have also been shown to efficiently suppress excessive lipid peroxidation and associated ferroptosis (23–26).

As the name implies, iron plays an essential role in ferroptosis. Intracellular redox-active iron promotes ferroptosis by catalyzing the formation of hydroxyl and possibly lipid radicals, thereby initiating the lipid peroxidation chain reaction (27). Consequently, various cellular processes tightly regulate the labile iron pool to lower the cell's susceptibility to lipid peroxidation and ferroptosis. Since PUFAs in cellular membranes are highly susceptible to oxidation and, therefore, the targets of lipid peroxidation, the PUFA content in phospholipid lipid bilayers is critical in regulating ferroptosis (28). Enzymes involved in PUFA and phospholipid metabolism and enzymes responsible for plasma membrane repair can modulate the vulnerability to ferroptosis by controlling the amount of (oxidized) PUFA (29,30). Therefore, the cellular susceptibility to ferroptosis can be influenced by three main factors: i) the efficiency of the lipid peroxidation surveillance system, ii) the extent of PUFA content in the cellular membrane, and iii) the amount of the labile iron pool. Modulating these factors can potentially increase or decrease the cellular vulnerability to ferroptosis, making them promising targets for therapeutic interventions in conditions associated with ferroptosis (31,32).

Ferroptosis in renal disease

The relevance of ferroptosis in renal disease has to be deliberated according to the complex execution of various cell death modalities and their temporal interdependencies, which orchestrate AKI episodes or CKD progression. Therapeutically, dissecting the contribution of ferroptosis to renal disease will also be essential (summarized in **Table 1**).

Cystinuria is an inherited autosomal recessive disorder that results in substantially increased urinary cystine excretion, leading to obstructive kidney and bladder stones. Following glomerular filtration, homodimeric cystine is taken up by PTCs across the brush border via the heterodimer amino acid transporter heavy chain SLC3A1 (encoded by *SLC3A1*) and b(0,+)-type amino acid transporter 1 (encoded by *SLC7A9*) in exchange for intracellular neutral amino acids. Cystinuria can be further subdivided into Type A (*SLC3A1*) and Type B (*SLC7A9)* with variable phenotypes depending on the mutations of the affected genes (33). Since *SLC3A1*-knockout mice exhibit significantly reduced intracellular GSH levels (34) and breast cancer cells overexpressing SLC3A1 accumulate GSH (35), cystinuria could intuitively be a paradigm model for studying ferroptosis in the kidney but has not been linked yet.

Conditional *Gpx4*-knockout mice develop lethal ATN approximately two weeks after induction (5), underpinning the exceptional role of ferroptosis in PTCs and AKI. PTCs are particularly vulnerable to ferroptotic cell death because of the high amount of free and phospholipid-esterified PUFAs (36), high metabolic activity in mitochondria, and oxygen consumption (37).
The implication of GPX4 and ferroptosis in vascular endothelial cells has been nicely demonstrated in endothelium-specific *Gpx4*-knockout mice under reduced vitamin E conditions (38). Multiple lethal microinfarctions and micro bleedings occurred in the kidney, liver, spleen, or spinal cord after conditional deletion of *Gpx4* in nearly 80% of the experimental mice. In contrast, the control group under a regular vitamin E-enriched

breeding diet was unaffected upon tamoxifen induction. On the other hand, the clinical relevance of ferroptosis in glomerules or distal tubule cells still remains ill-defined. Under physiological conditions, circulating ferric (Fe^{3+}) iron is primarily bound to transferrin (Tf), which can be glomerularly filtered despite a low glomerular sieving coefficient and is eventually reabsorbed by the proximal and distal tubule system (39). Non-transferrin-bound iron (NTBI) is defined as circulating iron that is not bound to transferrin, ferritin, heme, or hemoglobin. It encompasses the redox active labile iron pool (LIP) unspecifically bound to small molecular weight plasma proteins (40). Alternatively, inside cells, the LIP is still poorly defined and likely coordinated by GSH and/or cysteine. The daily urinary output of iron is estimated to range between 1-3 µg, rises to 20 µg in patients with Fanconi syndrome, and reaches 500 µg in patients with nephrotic syndrome (37). Independent of the underlying cause, proteinuria is associated with increased iron deposition in PTCs and DTCs, resulting in higher oxidative stress as indicated by elevated heme oxygenase-1 levels (41). A small fraction of redox-active iron may trigger the Fenton reaction, an iron-mediated (typically ferrous iron, Fe^{2+}) production of highly reactive hydroxyl radicals inciting lipid peroxidation and the generation of lipid radicals leading to ferroptosis (42). Systemic iron overload due to ß-thalassemia major also increases Tf-excretion and correlates with urinary levels of kidney injury molecule 1 (KIM-1) (43). Tf has been reported as a regulator of ferroptosis in vitro (41) and the transferrin receptor (TfR), mediating the internalization of Tf via endocytosis, as a specific marker for ferroptosis (44). Increased TfR expression alongside reduced GPX4 levels was observed in passive Heymann`s nephritis rats, a commonly used animal model for studying immune-complex mediated membranous nephropathy (45). AKI is a common feature of extensive intravascular hemolysis and was first reported in 1925 (46). Apart from obstructive hemoglobin cast nephropathy due to extensive intravascular hemolysis, hemoglobinuria resulted in AKI in guinea pigs and beagle dogs with increased hemoglobin autoxidation and lipid peroxidation as shown by increased 4-hydroxynonenal (4-HNE) formation, a surrogate marker of ferroptosis (47). Myoglobin is another iron-binding protein, and increased serum levels emerging from rhabdomyolysis due to exertional heatstroke correlate with the incidence of AKI in humans. Treating human kidney proximal tubular cells with myoglobin was associated with ferroptotic cell death in a heat stress model *in vitro* (48). Deferoxamine (Desferal©) and deferiprone (Ferriprox©) are both FDA-approved iron chelators for treating systemic iron overload that exhibit anti-ferroptotic activity *in vivo* (49,50).

Excessive lipid peroxidation is one of the deteriorating events among the complex pathophysiological processes mediating IRI and is highly relevant for solid organ transplantation (51). Besides acute allograft rejection, IRI is one of the most critical risk factors for developing delayed graft function after transplantation (52). Renal ischemia substantially increases cortical concentrations of esterified cholesterol and triacylglycerides (TGs), fueling ferroptosis, especially de-esterifying TGs into PUFAs (53). A decreased GSH/GSSG (GSSG, the dimeric oxidized form of GSH) ratio and increased lipid peroxidation, indicated by elevated thiobarbituric acid reactive substances (TBARS) levels, has been demonstrated in a murine model of IRI (54). In transgenic mouse models, impaired GPX4 activity and genetic deletion of FSP1 sensitize PTCs to IRI-related ferroptotic cell death (55). IRI further boosts tissue inflammation, amplifying the host's innate immune activation and promoting acute allograft rejection (56). Ferroptosis could, therefore, be inhibited by the selective anti-ferroptotic compounds ferrostatin-1 (Fer-1) and liproxstatin-1 (Lip-1), as well as the iron chelator deferoxamine to mitigate renal and hepatic IRI based on

less lipid peroxidation, minor tissue injury and improved serum parameters (urea/creatinine and ALT/AST, respectively) (57).

Obstructive crystal-induced AKI mainly results from tumor lysis-induced severe hyperuricemia, oxalate nephropathy, or insoluble drug-related intratubular crystal precipitation (58,59). Ferroptosis inhibition significantly improved the histological signs of ATN accompanied by decreased tubular damage markers in a murine model of oxalate-induced AKI (60). Methotrexate (MTX) is prone to tubular precipitation under physiological urine pH and is a standard treatment for autoimmune diseases or cancer therapy in a dose-dependent manner. The molecular mechanism of MTX involves the inhibition of dihydrofolate reductase (DHFR), leading to intracellular depletion of reduced tetrahydrofolate (FH4) and BH4 (61). The deficiency of BH4 may contribute to the renal toxicity of MTX since BH4 is a radical-trapping antioxidant capable of halting the process of lipid peroxidation and ferroptosis (62). Furthermore, MTX treatment sensitizes human cancer cell lines to ferroptosis induced by GPX4 inhibition (23) and is associated with oxidative stress and GSH depletion in the human hepatic cell line HepaRG (63). Folic acid (FA) is the standard supplement in the first-line treatment of rheumatoid arthritis to reduce MTX-related toxicity, but also a frequently used animal model for studying AKI and CKD (64). Supraphysiological concentrations of FA are associated with renal lipid peroxidation, lower GSH levels, and impaired enzymatic antioxidant activity (65), presumably due to increased consumption of the essential redox cofactors NAD(P)H during the reduction process of FA to FH4 (64). Selective inhibition of ferroptosis by Fer-1 improved lipid peroxidation and ATN in a murine model of FA-induced AKI (FA-AKI) (66). But the translational aspect of FA-AKI in humans remains elusive since high dose FA (40 mg/d) was shown to have no impact on overall mortality, major adverse cardiovascular events, or CKD-progression in a large double-blind randomized controlled trial over a median follow-up period of 3.2 years (67). Another notable nephrotoxin is cisplatin, with typical signs of cellular oxidative stress, lipid peroxidation, and proximal tubular damage resulting in AKI and, eventually, CKD (68). Promethazine and rifampicin, as clinically used authorized drugs with anti-ferroptotic potential, improved cisplatin-induced AKI in mice (69).

Diabetes mellitus (DM) is a global burden with an estimated worldwide prevalence of 529 million people, representing 6.1% of the world`s population in 2021 (54). Approximately ⅓ of DM patients develop diabetic nephropathy (DN) with detectable microalbuminuria. DN has turned from an originally noninflammatory glomerulopathy into a multifaceted disease entity originating from metabolic dysregulation, microinflammatory environment, mesangial cell proliferation, and extracellular matrix expansion, leading to interstitial fibrosis and tubular atrophy. Enhanced cellular glucose uptake, increased oxidative phosphorylation, and elevated levels of transforming growth factor ß (TGF-ß) are key features of DN affecting proper endothelial and beta cell function. Furthermore, high glucose and TGF-ß1 treatment sensitized mesangial (70) and kidney tubular cells (71) to ferroptosis via an upregulation of pro-ferroptotic and a downregulation of anti-ferroptotic genes. GPX4 primarily relies on GSH as a reductant and lower GSH levels have been reported in diabetic rats (72) and patients suffering from type 2 diabetes (DM2) (73). Brain homogenates of diabetic rats treated with DM2 first-line metformin displayed increased GSH levels compared to untreated controls (74), likely due to improved glycemic control. Metformin has recently been shown to lower intracellular GSH levels and enhance lipid peroxidation, thereby sensitizing MCF-7 breast cancer cells to ferroptosis (75). The metformin-related lipid peroxidation and ferroptosis

induction occur independent of adenosine monophosphate-activated protein kinase (probably) by destabilizing xCT, which is a critical part of the heterodimeric cystine-glutamate antiporter system x_C^-, essential for GSH synthesis (76).

Besides metabolic control, the family of renin-angiotensin-aldosterone system inhibitors (iRAAS) is the basis for treating DN with apparent microalbuminuria. The beneficial effects are not only related to the reduction of systemic blood pressure and glomerular hyperfiltration but also due to minor proinflammatory gene activation and extracellular matrix production (77). Sodium–glucose cotransporter 2 (SGLT2) is a promising future candidate for lowering renal oxidative stress regarding the glucosuric effect and improved glomerular autoregulation (78). The progressive loss of functional nephrons results in advanced CKD, which is a subsequent risk factor for kidney malignancies, particularly clear cell renal cell carcinoma (ccRCC) (79). ccRCC is the most encountered histotype emerging from the segment S1 of the proximal tubule. On the other hand, papillary renal cell carcinoma (pRCC) develops from the metabolic, highly active, and ischemic-sensitive segment S3 triggered by recurrent AKI episodes, each underpinning the importance of AKI and CKD prevention. Metastatic ccRCC and pRCC have both poor prognoses with limited options for systemic treatment approaches. The discovery of ferroptosis has opened the field for researching and developing selective ferroptosis inducers addressing drug-resistant tumor entities. ccRCC is particularly interesting for upcoming precision therapy strategies owing to an increased susceptibility towards ferroptosis induced by GPX4 inhibition (80).

Future challenges and endeavors: what will the next decade bring?

Nephrology has witnessed significant advancements in recent years, particularly in drug development. One promising avenue of investigation in this field is the inhibition of ferroptosis, a pervasive form of cell death associated with oxidative tissue damage. This section explores recent drug discovery developments, focusing on ferroptosis inhibition and its potential implications for nephrology. Addressing the cellular labile iron pool, sustaining the host antioxidant defense system, and preventing lipid peroxidation by blocking enzymatic oxidation or radical chain propagation are reasonable starting points for future therapeutical intervention. The manipulation of the lipid composition of cellular membranes might also be a future therapeutic approach since ferroptosis induction essentially depends on the PUFA content of lipid bilayers. Decreasing the relative amount of PUFAs by supplementing exogenous monounsaturated oleic acid protects cells and lower organisms from iron-mediated ferroptosis (81).

Tackling the cellular labile iron pool by repurposing FDA-approved iron chelators is a relatively low barrier to entering clinical phase studies. The FAIRPARK-II trial was a 36-week, randomized, double-blinded, multicenter phase II trial evaluating the safety and efficacy of iron chelation in the early stages of Parkinson`s Disease (82) since ferroptosis substantially contributes to the loss of dopaminergic neurons and disease progression (83). Unfortunately, deferiprone was associated with significantly aggravated motor and nonmotor symptoms, which led to the premature discontinuation of deferiprone and the onset of dopaminergic substitution for symptom control. The adverse effects of deferiprone were attributed to a reduced enzymatic activity of the iron-dependent tyrosine hydroxylase

catalyzing the conversion of l-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA). Although there is a reasonable basis for therapeutic iron chelation in various human diseases, results from completed clinical trials addressing the prevention of AKI in the context of cardiopulmonary bypass surgery (CPB), myocardial infarction, or critically ill patients remain elusive (84). DEFEAT-AKI is currently the only clinical trial listed that is actively recruiting patients to reevaluate the efficacy of iron chelation in preventing CPB-related AKI (NCT04633889).

One of the breakthrough discoveries that set the stage for groundbreaking research in nephrology was the observation of tissue detriment resulting from conditional deletion of GPX4 in adult mice. This early finding prompted researchers to explore the development of molecules capable of inhibiting ferroptosis *in vitro* and *in vivo*. The potency of vitamin E to detoxify lipid peroxides has already been described more than half a century ago (85). Vitamin E encompasses a group of eight lipophilic compounds (α-,β-,γ-,δ-tocopherol and α-,β-,γ-,δ-tocotrienol), with α-tocopherol (α-TOH) being the most biologically active form and a naturally occurring RTA (42). Although α-TOH exhibits excellent reactivity in organic solution, the hydroxy group limits the transferability to biological membranes where ferroptosis occurs. Extensive research yielded a novel class of synthetic RTAs with aromatic amines instead of methylated phenols, resulting in superior liposome anti-ferroptotic activity. The aromatic amines Lip-1 and Fer-1 have since become cornerstones in studying ferroptosis, demonstrating efficacy in various cellular and murine models. Optimization efforts of ferroptosis-targeting therapies have led to improved generations of liproxstatin, marking a significant milestone in translating ferroptosis research into therapeutic applications. These efforts include enhancing blood-brain barrier permeability, minimizing off-target effects, and refining anti-ferroptotic mechanisms. Carefully planned phase-I trials in dedicated Early Clinical Trial Units (ECTUs) will serve as a crucial step in assessing the safety and efficacy of next-generation liproxstatins and ferrostatins in human subjects, bringing us closer to tangible therapeutic interventions for ferroptosis-related conditions. The vitamin K group consists of the 2 natural forms phylloquinone (VK1) and menaquinone-4 (VK2), as well as the synthetic product menadione (VK3), each exhibiting anti-ferroptotic activity *in vitro* (VK2 > VK1 > VK3) (21). The most active form of VK2 also attenuated renal and hepatic IRI-related tissue damage in mice with an applied dose of 200 mg/kg. If an equal amount of VK2 is provided for treating IRI in humans, an extrapolated dose of 15.000 mg VK2 would be necessary, considering a body weight of 75 kilograms. Currently, over-the-counter drugs frequently contain either 10 mg of VK1 or 200 µg of VK2. Clearly, all these findings put to the fore an intertwined metabolic network determining renal cell death propensity and amenable to therapeutic targeting.

Concluding remarks

This innovative research branch holds great potential for preventing tissue damage and organ failure in nephrology and other medical fields. The development of novel drugs focusing on ferroptosis inhibition holds immense promise for the field of nephrology. The discovery of liproxstatins and their optimization, in combination with the potential application of gene therapy approaches, opens up unprecedented possibilities for therapeutic interventions in kidney diseases and related disorders. With further research and clinical trials on the horizon, the future of nephrology looks increasingly bright.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

None declared.

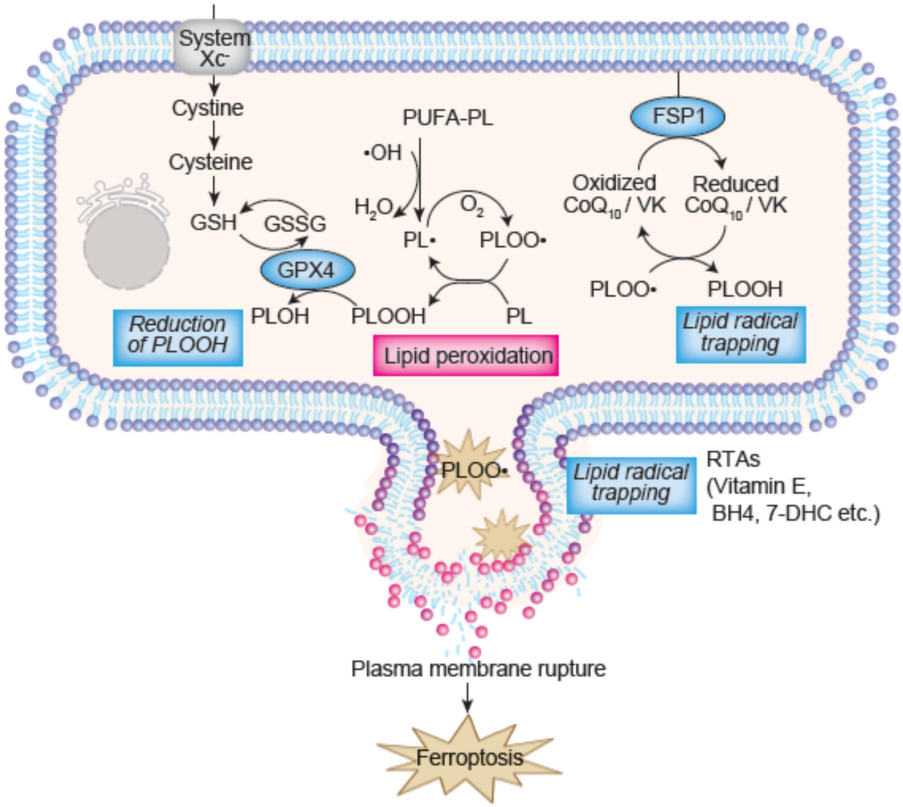

Figure 1. Principles of the most important cellular anti-ferroptotic defense mechanisms. Detrimental lipid peroxidation and ferroptosis execution are mainly controlled by the cyst(e)ine/GSH/GPX4 and CoQ_{10}/FSP1 axis. PLOOH is reduced to PLOH by GPX4, which, in turn, is reduced by GSH. Cysteine is the rate-limiting amino acid for the de novo GSH synthesis and is transported into cells as homodimeric cystine by the amino acid antiporter system Xc^-. The FSP1-mediated reduction of CoQ_{10} and VK generates potent RTAs capable of reducing PLOO• to PLOOH. (Abbreviations: 7-DHC, 7-dehydrocholesterol; BH_4, tetrahydrobiopterin; GPX4, glutathione peroxidase 4; GSH, glutathione; GSSG, glutathione disulfide; PL•, phospholipid radical; PLOH, phospholipid alcohol; PLOO•, phospholipid peroxyl radical; PLOOH, phospholipid hydroperoxide; PUFA, polyunsaturated fatty acid; PUFA-PL, PUFA-containing phospholipid; VK, vitamin K.) The figure was adopted from Mishima et al. 2024.

REFERENCES

1. Vanden Berghe T, Linkermann A, Jouan-Lanhouet S, Walczak H, Vandenabeele P. Regulated necrosis: the expanding network of non-apoptotic cell death pathways. *Nature reviews. Molecular cell biology* 2014; 15: 135–147.

2. Dixon SJ, Lemberg KM, Lamprecht MR et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012; 149: 1060–1072.

3. Kagan VE, Mao G, Qu F et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nature chemical biology* 2017; 13: 81–90.

4. Skouta R, Dixon SJ, Wang J et al. Ferrostatins inhibit oxidative lipid damage and cell death in diverse disease models. *Journal of the American Chemical Society* 2014; 136: 4551–4556.

5. Friedmann Angeli JP, Schneider M, Proneth B et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nature cell biology* 2014; 16: 1180–1191.

6. Abebe A, Kumela K, Belay M, Kebede B, Wobie Y. Mortality and predictors of acute kidney injury in adults: a hospital-based prospective observational study. *Scientific reports* 2021; 11: 15672.

7. Ide S, Kobayashi Y, Ide K et al. Ferroptotic stress promotes the accumulation of pro-inflammatory proximal tubular cells in maladaptive renal repair. *eLife [Internet]* 2021; 10. Available from: http://dx.doi.org/10.7554/eLife.68603

8. Zhao ZB, Marschner JA, Iwakura T et al. Tubular Epithelial Cell HMGB1 Promotes AKI-CKD Transition by Sensitizing Cycling Tubular Cells to Oxidative Stress: A Rationale for Targeting HMGB1 during AKI Recovery. *Journal of the American Society of Nephrology: JASN* 2023; 34: 394–411.

9. Balzer MS, Doke T, Yang Y-W et al. Single-cell analysis highlights differences in druggable pathways underlying adaptive or fibrotic kidney regeneration. *Nature communications* 2022; 13: 4018.

10. Ortiz A, Asociación Información Enfermedades Renales Genéticas (AIRG-E), European Kidney Patients' Federation (EKPF), Federación Nacional de Asociaciones para la Lucha Contra las Enfermedades del Riñón (ALCER), Fundación Renal Íñigo Álvarez de Toledo (FRIAT), Red de Investigación Renal (REDINREN), Resultados en Salud 2040 (RICORS2040), Sociedad Española de Nefrología (SENEFRO) Council, Sociedad Española de Trasplante (SET) Council, Organización Nacional de Trasplantes (ONT). RICORS2040: the need for collaborative research in chronic kidney disease. *Clinical kidney journal*2022; 15: 372–387.

11. Maremonti F, Meyer C, Linkermann A. Mechanisms and Models of Kidney Tubular Necrosis and Nephron Loss. *Journal of the American Society of Nephrology: JASN* 2022; 33: 472–486.

12. Mulay SR, Linkermann A, Anders H-J. Necroinflammation in Kidney Disease. *Journal of the American Society of Nephrology: JASN* 2016; 27: 27–39.

13. Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. *Molecular cell* 2012; 48: 158–167.

14. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. *Current biology: CB* 2014; 24: R453-62.

15. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nature reviews. Molecular cell biology* 2021; 22: 266–282.

16. Mishima E, Conrad M. Nutritional and Metabolic Control of Ferroptosis. *Annual review of nutrition* 2022; 42: 275–309.

17. Yang WS, SriRamaratnam R, Welsch ME et al. Regulation of ferroptotic cancer cell death by GPX4. *Cell* 2014; 156: 317–331.

18. Sato H, Tamba M, Ishii T, Bannai S. Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. *The Journal of biological chemistry* 1999; 274: 11455–11458.

19. Doll S, Freitas FP, Shah R et al. FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* 2019; 575: 693–698.

20. Bersuker K, Hendricks JM, Li Z et al. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* 2019; 575: 688–692.

21. Mishima E, Ito J, Wu Z et al. A non-canonical vitamin K cycle is a potent ferroptosis suppressor. *Nature* 2022; 608: 778–783.

22. Mishima E, Wahida A, Seibt T, Conrad M. Diverse biological functions of vitamin K: from coagulation to ferroptosis. *Nature metabolism* 2023; 5: 924–932.

23. Soula M, Weber RA, Zilka O et al. Metabolic determinants of cancer cell sensitivity to canonical ferroptosis inducers. *Nature chemical biology* 2020; 16: 1351–1360.

24. Garcia-Bermudez J, Baudrier L, Bayraktar EC et al. Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death. *Nature* 2019; 567: 118–122.

25. Sun Q, Liu D, Cui W et al. Cholesterol mediated ferroptosis suppression reveals essential roles of Coenzyme Q and squalene. *Communications biology* 2023; 6: 1108.

26. Carlson BA, Tobe R, Yefremova E et al. Glutathione peroxidase 4 and vitamin E cooperatively prevent hepatocellular degeneration. *Redox biology* 2016; 9: 22–31.

27. Galy B, Conrad M, Muckenthaler M. Mechanisms controlling cellular and systemic iron homeostasis. *Nature reviews. Molecular cell biology [Internet]* 2023;Available from: http://dx.doi.org/10.1038/s41580-023-00648-1

28. Aldrovandi M, Fedorova M, Conrad M. Juggling with lipids, a game of Russian roulette. *Trends in endocrinology and metabolism: TEM* 2021; 32: 463–473.

29. Doll S, Proneth B, Tyurina YY et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nature chemical biology* 2017; 13: 91–98.

30. Sun W-Y, Tyurin VA, Mikulska-Ruminska K et al. Phospholipase iPLA2β averts ferroptosis by eliminating a redox lipid death signal. *Nature chemical biology* 2021; 17: 465–476.

31. Mishima E, Nakamura T, Zheng J et al. DHODH inhibitors sensitize to ferroptosis by FSP1 inhibition. *Nature*2023; 619: E9–E18.

462 32. Nakamura T, Hipp C, Santos Dias Mourão A et al. Phase separation of FSP1 promotes
463 ferroptosis. *Nature* 2023; 619: 371–377.

464 33. Kowalczyk NS, Zisman AL. Cystinuria: Review of a life-long and frustrating disease. *The*
465 *Yale journal of biology and medicine* 2021; 94: 681–686.

466 34. Woodard LE, Welch RC, Veach RA et al. Metabolic consequences of cystinuria. *BMC*
467 *nephrology* 2019; 20: 227.

468 35. Jiang Y, Cao Y, Wang Y et al. Cysteine transporter SLC3A1 promotes breast cancer
469 tumorigenesis. *Theranostics* 2017; 7: 1036–1046.

470 36. Wenzel SE, Tyurina YY, Zhao J et al. PEBP1 Wardens Ferroptosis by Enabling
471 Lipoxygenase Generation of Lipid Death Signals. *Cell* 2017; 171: 628-641.e26.

472 37. van Swelm RPL, Wetzels JFM, Swinkels DW. The multifaceted role of iron in renal
473 health and disease. *Nature reviews. Nephrology* 2020; 16: 77–98.

474 38. Wortmann M, Schneider M, Pircher J et al. Combined deficiency in glutathione
475 peroxidase 4 and vitamin E causes multiorgan thrombus formation and early death in
476 mice. *Circulation research* 2013; 113: 408–417.

477 39. Norden AG, Lapsley M, Lee PJ et al. Glomerular protein sieving and implications for
478 renal failure in Fanconi syndrome. *Kidney international* 2001; 60: 1885–1892.

479 40. Angoro B, Motshakeri M, Hemmaway C, Svirskis D, Sharma M. Non-transferrin bound
480 iron. *Clinica chimica acta; international journal of clinical chemistry* 2022; 531: 157–167.

481 41. van Raaij S, van Swelm R, Bouman K et al. Tubular iron deposition and iron handling
482 proteins in human healthy kidney and chronic kidney disease. *Scientific reports* 2018; 8:
483 9353.

484 42. Conrad M, Pratt DA. The chemical basis of ferroptosis. *Nature chemical biology* 2019;
485 15: 1137–1147.

486 43. van Raaij SEG, Rennings AJ, Biemond BJ et al. Iron handling by the human kidney:
487 glomerular filtration and tubular reabsorption both contribute to urinary iron excretion.
488 *American journal of physiology. Renal physiology* 2019; 316: F606–F614.

489 44. Feng H, Schorpp K, Jin J et al. Transferrin Receptor Is a Specific Ferroptosis Marker.
490 *Cell reports* 2020; 30: 3411-3423.e7.

491 45. Shi X, Zhang Q, Chang M et al. Ferroptosis is involved in passive Heymann nephritis in
492 rats. *Heliyon* 2023; 9: e21050.

493 46. Bancroft FW. ANURIA FOLLOWING TRANSFUSION: EFFECT OF DECAPSULATION
494 OF BOTH KIDNEYS. *Annals of surgery* 1925; 81: 733–738.

495 47. Deuel JW, Schaer CA, Boretti FS et al. Hemoglobinuria-related acute kidney injury is
496 driven by intrarenal oxidative reactions triggering a heme toxicity response. *Cell death &*
497 *disease* 2016; 7: e2064.

498 48. Luan Y, Huang E, Huang J et al. Serum myoglobin modulates kidney injury via inducing
499 ferroptosis after exertional heatstroke. *Journal of translational internal medicine* 2023;
500 11: 178–188.

501 49. Jia H, Liu X, Cao Y et al. Deferoxamine ameliorates neurological dysfunction by

inhibiting ferroptosis and neuroinflammation after traumatic brain injury. *Brain research* 2023; 1812: 148383.

50. Rayatpour A, Foolad F, Heibatollahi M, Khajeh K, Javan M. Ferroptosis inhibition by deferiprone, attenuates myelin damage and promotes neuroprotection in demyelinated optic nerve. *Scientific reports* 2022; 12: 19630.

51. Conrad M, Angeli JPF, Vandenabeele P, Stockwell BR. Regulated necrosis: disease relevance and therapeutic opportunities. *Nature reviews. Drug discovery* 2016; 15: 348–366.

52. Ponticelli C, Reggiani F, Moroni G. Delayed Graft Function in Kidney Transplant: Risk Factors, Consequences and Prevention Strategies. *Journal of personalized medicine [Internet]* 2022; 12. Available from: http://dx.doi.org/10.3390/jpm12101557

53. Erpicum P, Rowart P, Defraigne J-O, Krzesinski J-M, Jouret F. What we need to know about lipid-associated injury in case of renal ischemia-reperfusion. *American journal of physiology. Renal physiology* 2018; 315: F1714–F1719.

54. Stoppe C, Averdunk L, Goetzenich A et al. The protective role of macrophage migration inhibitory factor in acute kidney injury after cardiac surgery. *Science translational medicine [Internet]* 2018; 10. Available from: http://dx.doi.org/10.1126/scitranslmed.aan4886

55. Tonnus W, Meyer C, Steinebach C et al. Dysfunction of the key ferroptosis-surveilling systems hypersensitizes mice to tubular necrosis during acute kidney injury. *Nature communications* 2021; 12: 4402.

56. Uehara M, Solhjou Z, Banouni N et al. Ischemia augments alloimmune injury through IL-6-driven CD4+ alloreactivity. *Scientific reports* 2018; 8: 2461.

57. Ito Y, Lim DK, Nabeshima T, Ho IK. Effects of picrotoxin treatment on GABAA receptor supramolecular complexes in rat brain. *Journal of neurochemistry* 1989; 52: 1064–1070.

58. Perazella MA. Crystal-induced acute renal failure. *The American journal of medicine* 1999; 106: 459–465.

59. Ejaz AA, Johnson RJ, Shimada M et al. The Role of Uric Acid in Acute Kidney Injury. *Nephron* 2019; 142: 275–283.

60. Linkermann A, Skouta R, Himmerkus N et al. Synchronized renal tubular cell death involves ferroptosis. *Proceedings of the National Academy of Sciences of the United States of America* 2014; 111: 16836–16841.

61. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. *Nature reviews. Rheumatology* 2020; 16: 145–154.

62. Nakamura T, Hipp C, Santos Dias Mourão A et al. Phase separation of FSP1 promotes ferroptosis. *Cell research* 2023; 619: 371–377.

63. Schmidt S, Messner CJ, Gaiser C, Hämmerli C, Suter-Dick L. Methotrexate-Induced Liver Injury Is Associated with Oxidative Stress, Impaired Mitochondrial Respiration, and Endoplasmic Reticulum Stress In Vitro. *International journal of molecular sciences [Internet]* 2022; 23. Available from: http://dx.doi.org/10.3390/ijms232315116

64. Yan L-J. Folic acid-induced animal model of kidney disease. *Animal models and experimental medicine* 2021; 4: 329–342.

65. Gupta A, Puri V, Sharma R, Puri S. Folic acid induces acute renal failure (ARF) by enhancing renal prooxidant state. *Experimental and toxicologic pathology: official journal of the Gesellschaft fur Toxikologische Pathologie* 2012; 64: 225–232.

66. Martin-Sanchez D, Ruiz-Andres O, Poveda J et al. Ferroptosis, but Not Necroptosis, Is Important in Nephrotoxic Folic Acid-Induced AKI. *Journal of the American Society of Nephrology: JASN* 2017; 28: 218–229.

67. Jamison RL, Hartigan P, Kaufman JS et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA: the journal of the American Medical Association* 2007; 298: 1163–1170.

68. Tang C, Livingston MJ, Safirstein R, Dong Z. Cisplatin nephrotoxicity: new insights and therapeutic implications. *Nature reviews. Nephrology* 2023; 19: 53–72.

69. Mishima E, Sato E, Ito J et al. Drugs Repurposed as Antiferroptosis Agents Suppress Organ Damage, Including AKI, by Functioning as Lipid Peroxyl Radical Scavengers. *Journal of the American Society of Nephrology: JASN* 2020; 31: 280–296.

70. Wu Y, Zhao Y, Yang H-Z, Wang Y-J, Chen Y. HMGB1 regulates ferroptosis through Nrf2 pathway in mesangial cells in response to high glucose. *Bioscience reports [Internet]* 2021; 41. Available from: http://dx.doi.org/10.1042/BSR20202924

71. Kim S, Kang S-W, Joo J et al. Characterization of ferroptosis in kidney tubular cell death under diabetic conditions. *Cell death & disease* 2021; 12: 160.

72. Ewis SA, Abdel-Rahman MS. Effect of metformin on glutathione and magnesium in normal and streptozotocin-induced diabetic rats. *Journal of applied toxicology: JAT* 1995; 15: 387–390.

73. Lutchmansingh FK, Hsu JW, Bennett FI et al. Glutathione metabolism in type 2 diabetes and its relationship with microvascular complications and glycemia. *PloS one* 2018; 13: e0198626.

74. Correia S, Carvalho C, Santos MS et al. Metformin protects the brain against the oxidative imbalance promoted by type 2 diabetes. *Medicinal chemistry (Shariqah (United Arab Emirates))* 2008; 4: 358–364.

75. Chen J, Qin C, Zhou Y, Chen Y, Mao M, Yang J. Metformin may induce ferroptosis by inhibiting autophagy via lncRNA H19 in breast cancer. *FEBS open bio* 2022; 12: 146–153.

76. Yang J, Zhou Y, Xie S et al. Metformin induces Ferroptosis by inhibiting UFMylation of SLC7A11 in breast cancer. *Journal of experimental & clinical cancer research: CR* 2021; 40: 206.

77. Kishi S, Nagasu H, Kidokoro K, Kashihara N. Oxidative stress and the role of redox signalling in chronic kidney disease. *Nature reviews. Nephrology [Internet]* 2023;Available from: http://dx.doi.org/10.1038/s41581-023-00775-0

78. Yaribeygi H, Butler AE, Atkin SL, Katsiki N, Sahebkar A. Sodium-glucose cotransporter 2 inhibitors and inflammation in chronic kidney disease: Possible molecular pathways. *Journal of cellular physiology* 2018; 234: 223–230.

79. Peired AJ, Lazzeri E, Guzzi F, Anders H-J, Romagnani P. From kidney injury to kidney

cancer. *Kidney international* 2021; 100: 55–66.

80. Zou Y, Palte MJ, Deik AA et al. A GPX4-dependent cancer cell state underlies the clear-cell morphology and confers sensitivity to ferroptosis. *Nature communications* 2019; 10: 1617.

81. Mann J, Reznik E, Santer M et al. Ferroptosis inhibition by oleic acid mitigates iron-overload-induced injury. *Cell chemical biology* 2024; 31: 249-264.e7.

82. Devos D, Labreuche J, Rascol O et al. Trial of Deferiprone in Parkinson's Disease. *The New England journal of medicine* 2022; 387: 2045–2055.

83. Do Van B, Gouel F, Jonneaux A et al. Ferroptosis, a newly characterized form of cell death in Parkinson's disease that is regulated by PKC. *Neurobiology of disease* 2016; 94: 169–178.

84. Sharma S, Leaf DE. Iron Chelation as a Potential Therapeutic Strategy for AKI Prevention. *Journal of the American Society of Nephrology: JASN* 2019; 30: 2060–2071.

85. Schwarz K, Foltz CM. Factor 3 activity of selenium compounds. *The Journal of biological chemistry* 1958; 233: 245–251.

Compounds	Mechanism-of-action	Kidney injury mouse models	References
Liproxstatin-1	RTA	*Gpx4*$^{fl/fl}$; *Rosa26*CreERT2 *Gpx4*$^{fl/fl}$; *Cdh16*CreERT2 Kidney IRI	Refs. (1) Refs. (2) Refs. (3)
UAMC-3203	RTA	*Gpx4*$^{fl/fl}$; *Cdh16*CreERT2	Refs. (2)
Ferrostatin-1	RTA	Folic Acid-AKI *Pkd1*$^{fl/fl}$:*Pkhd1*Cre	Refs. (4) Refs. (5)
SRS16-86 (next generation ferrostatin)	RTA	Kidney IRI	Refs. (6)
Menaquinone-4 (vitamin K2)	RTA	Kidney IRI	Refs. (7)
Vitamin K1	RTA	Kidney IRI	Refs. (8)
Promethazine	RTA	Cisplatin-AKI	Refs. (9)
Curcumin	RTA	Rhabdomyolysis-AKI	Refs. (10)
Quercetin	RTA	Kidney IRI and Folic acid-AKI	Refs. (11)
Nuciferine	RTA	Folic acid-AKI	Refs. (12)
Glycyrol	RTA	Folic acid-AKI	Refs. (13)
AY9944	Inhibition of DHCR7	Kidney IRI	Refs. (14)

Abbreviations: RTA, radical trapping antioxidant; IRI, ischemia-reperfusion injury; AKI, acute kidney injury.

Table 1. Antiferroptotic compounds exhibiting renoprotective activity in animal models.

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References

1. Friedmann Angeli JP, Schneider M, Proneth B et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nature cell biology* 2014; 16: 1180–1191.

2. Van Coillie S, Van San E, Goetschalckx I et al. Targeting ferroptosis protects against experimental (multi)organ dysfunction and death. *Nature communications* 2022; 13: 1046.

3. Ide S, Kobayashi Y, Ide K et al. Ferroptotic stress promotes the accumulation of pro-inflammatory proximal tubular cells in maladaptive renal repair. *eLife [Internet]* 2021; 10. Available from: http://dx.doi.org/10.7554/eLife.68603

4. Martin-Sanchez D, Ruiz-Andres O, Poveda J et al. Ferroptosis, but Not Necroptosis, Is Important in Nephrotoxic Folic Acid-Induced AKI. *Journal of the American Society of Nephrology: JASN* 2017; 28: 218–229.

5. Zhang X, Li LX, Ding H, Torres VE, Yu C, Li X. Ferroptosis promotes cyst growth in autosomal dominant polycystic kidney disease mouse models. *Journal of the American Society of Nephrology: JASN* 2021; 32: 2759–2776.

6. Linkermann A, Skouta R, Himmerkus N et al. Synchronized renal tubular cell death involves ferroptosis. *Proceedings of the National Academy of Sciences of the United States of America* 2014; 111: 16836–16841.

7. Mishima E, Ito J, Wu Z et al. A non-canonical vitamin K cycle is a potent ferroptosis suppressor. *Nature* 2022; 608: 778–783.

8. Kolbrink B, von Samson-Himmelstjerna FA, Messtorff ML et al. Vitamin K1 inhibits ferroptosis and counteracts a detrimental effect of phenprocoumon in experimental acute kidney injury. *Cellular and molecular life sciences: CMLS* 2022; 79: 387.

9. Mishima E, Sato E, Ito J et al. Drugs Repurposed as Antiferroptosis Agents Suppress Organ Damage, Including AKI, by Functioning as Lipid Peroxyl Radical Scavengers. *Journal of the American Society of Nephrology: JASN* 2020; 31: 280–296.

10. Guerrero-Hue M, García-Caballero C, Palomino-Antolín A et al. Curcumin reduces renal damage associated with rhabdomyolysis by decreasing ferroptosis-mediated cell death. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology* 2019; 33: 8961–8975.

11. Wang Y, Quan F, Cao Q et al. Quercetin alleviates acute kidney injury by inhibiting ferroptosis. *Journal of advanced research* 2021; 28: 231–243.

12. Li D, Liu B, Fan Y et al. Nuciferine protects against folic acid-induced acute kidney injury by inhibiting ferroptosis. *British journal of pharmacology* 2021; 178: 1182–1199.

13. Cao L, Han K, Fan L, Zhao C, Yin S, Hu H. Glycyrol alleviates acute kidney injury by inhibiting ferroptsis. *International journal of molecular sciences* 2024; 25: 2458.

14. Li Y, Ran Q, Duan Q et al. 7-Dehydrocholesterol dictates ferroptosis sensitivity. *Nature* 2024; 626: 411–418.