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REVIEW

Epigenetic modifications of Klotho expression in kidney diseases

Jinkun Xia[1] · Wangsen Cao[1]

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Abstract

Developments of many renal diseases are substantially influenced by epigenetic modifications of numerous genes, mainly mediated by DNA methylations, histone modifications, and microRNA interference; however, not all gene modifications causally affect the disease onset or progression. Klotho is a critical gene whose repressions in various pathological conditions reportedly involve epigenetic regulatory mechanisms. Klotho is almost unexceptionally repressed early after acute or chronic renal injuries and its levels inversely correlated with the disease progression and severity. Moreover, the strategies of Klotho derepression via epigenetic modulations beneficially change the pathological courses both in vitro and in vivo. Hence, Klotho is not only considered a biomarker of the renal disease but also a potential or even an ideal target of therapeutic epigenetic intervention. Here, we summarize and discuss studies that investigate the Klotho repression and intervention in renal diseases from an epigenetic point of view. These information might shed new sights into the effective therapeutic strategies to prevent and treat various renal disorders.

Keywords Klotho · Epigenetics · DNA methylation · Histone acetylation · miRNA interference · Kidney diseases

Introduction

Klotho (α-Klotho) is a kidney-enriched membrane protein accidently discovered from mice of gene-targeting in 1997 [1]. Mice with Klotho gene knockout display systemic human age-sensitive traits, whereas overexpression or exogenous supplementation of Klotho extends mouse lifespan [1, 2], suggesting that Klotho protein functions primarily as an aging suppressor in mammals. Later, two other paralogs β-Klotho and γ-Klotho were identified based on their homologies to α-Klotho, but less studied [3, 4]. This review will discuss the evidence of epigenetic modifications of α-Klotho, briefly Klotho, in kidney diseases.

Klotho is predominantly expressed in kidney distal tubule, parathyroid gland, and brain choroid plexus [1, 5]. Klotho exists in two general forms, a type 1 transmembrane protein and a secreted form derived from the same gene by alternative mRNA splicing [6]. The membrane-bound Klotho is known to serve as an obligatory co-receptor for fibroblast growth factor 23 (FGF23), through which it plays a critical role in the maintenance of mineral ion and vitamin D homeostasis [7, 8]. The extracellular domain of membrane Klotho consists of two repeated Klotho domains Kl1 and Kl2 that can be cleaved by metalloproteinases and released into blood, urine, and cerebrospinal fluid [9–11], whereby regulating the functions of renal and extrarenal organs. The cleaved KL fragments together with secreted Klotho are jointly called soluble Klotho, which are structurally homologous to family 1 glycosidase and possess purported intrinsic glycosidase activities [12]. Klotho beneficially regulates various cellular processes including aging, renal ion transport, oxidative stress, fibrosis, inflammation, autophagy, and apoptosis by targeting various signaling molecules, cell membrane receptors and ion channels via physical interactions or its enzymatic activities [13, 14]. Hence, Klotho is considered a key gene controlling aging and renal homeostasis and an ideal target of intervention for a number of kidney diseases and the extrarenal complications.

One of the critical features of Klotho expression is that its level drastically declined early in response to almost all renal damaging stimuli under various acute or chronic pathological conditions [15, 16]. Accumulating evidence indicates that acute Klotho repressions can be regulated by epigenetic or non-epigenetic mechanisms [17], but its sustained suppressions in chronic renal disorders mainly involve epigenetic modifications [18]. Epigenetic modifications generally refer

✉ Wangsen Cao
wangsencao@nju.edu.cn

[1] Center for Organ Fibrosis and Remodeling Research, Jiangsu Key Lab of Molecular Medicine, Nanjing University School of Medicine, Nanjing, China

to DNA methylation, protein/histone acetylation, and miRNA interference that influence many physiological and pathological processes such as embryo development, aging, carcinogenesis, and various chronic or degenerative disorders [19–21]. Epigenetic involvements in renal diseases have been evidenced by genome-wide association studies (GWASs), gene-linkage studies, and epigenome analysis [22–24]. Because epigenetic modifications occur in a tissue, cell, or gene-specific manner and epigenetic drugs can reversibly regulate the modifications [25], it is appealing that identification of the key genes of modifications and understanding the underlying regulatory mechanisms might lead to clinical benefits.

Klotho expression regulations by epigenetic or non-epigenetic mechanisms have attracted tremendous research attentions, and its epigenetic regulations also involve other pathological conditions, such as aging, carcinogenesis, and chronic disorders of other organs. This review will only focus on its epigenetic regulations in kidney diseases.

DNA methylation modification of Klotho in kidney diseases

DNA methylation is a process that adds methyl (–CH3) groups onto the C5 position of cytosine to form 5-methylcytosine on cytosine-phosphate-guanine (CpG) dinucleotide [26]. The reaction is catalyzed by maintenance DNA methyltransferase DNMT1 that copies DNA methylation pattern from a parental DNA strand onto newly synthesized daughter strand during DNA replication, and by de novo DNA methyltransferase DNMT3a and DNMT3b that are responsible for the establishment of a new methylation pattern [27]. Conversely, DNA demethylation is processed by Ten-Eleven translocation family protein TET1, TET2, and TET3 in sequential 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC) steps and eventually finished via a base excision repair procedure [28–30]. The methylated cysteine located in CpG island of gene promoters or enhancers can recruit methyl-CpG binding domain (MBD) proteins, histone deacetylases (HDACs), and/or polycomb group (PcG) proteins that form a transcriptional repressor complex and actively silence the downstream gene transcription [31].

Klotho promoter lacks ordinary TATA box but contains evolutionally conserved CpG islands, providing a structural basis for DNA methylation modification [32–35]. Klotho promoter hypermethylation and the associated Klotho repression have been found in several renal diseases of animal models [36–38] and in renal patients [39, 40].

Acute kidney injury

AKI (acute kidney injury) may reflect a transient deficiency of renal Klotho, whose reduction might be an important pathological feature of AKI development and progression to CKD [17, 41, 42]. Intriguingly, the Klotho levels are not alike in all forms of AKI. Urinary Klotho levels were significantly lower in patients with prerenal AKI than that of intrinsic AKI [43], suggesting that the level of Klotho varies under different acute pathological conditions.

It is likely that Klotho suppression is regulated in part by DNA methylation modification during the progression of AKI to CKD. Some demethylating agents such as hydrogen-rich saline is protective against AKI via correcting aberrant DNA methylation of Klotho gene, thus retaining Klotho expression [44]. We found that LPS-induced AKI was effectively alleviated by rhein, a small compound isolated from medicinal plants, via Klotho restoration and subsequent inhibition of TLR4 and the downstream NF-κB signaling [45], which was likely due to Klotho promoter demethylation since rhein possesses DNA demethylating capacity in kidney [38, 46]. It was reported that in LPS triggered AKI, NF-κB interacted with PRMT6 (protein arginine methyltransferase 6) that suppressed Klotho expression, which could be restored by AMI-1, an inhibitor of protein arginine *N*-methyltransferase and demethylating agent 5-Aza-2′-deoxycytidine (decitabine) [47], suggesting that both protein and DNA methylation aberrations are involved in the process. Notably, use of DNA demethylating agents such as decitabine to treat AKI has produced inconsistent results. Decitabine prevented cisplatin-induce nephrotoxicity in rats [48], but another study reported that decitabine enhanced cisplatin-induced apoptosis of proximal tubular cells and mice with renal proximal tubule-specific DNMT1 knockout had more serious AKI after cisplatin treatment [49]. The reason for the discrepancy is currently unclear.

CKD and the extrarenal complications

Chronic kidney disease (CKD) is a systemic disorder of chronic and progressive renal damage in which Klotho loss is considered a biomarker of its progression and severity [50]. Epigenetic Klotho repression due to aberrant DNA methylation is likely a causal event that promotes and expedites the disease course [51]. Renal tissues of CKD animals and blood cells from CKD patients showed marked increases of Klotho promoter methylation that correlated with the Klotho loss. In an animal model of uremic toxin-induced CKD, accumulation of uremic toxins can induce abnormal DNMT expression and Klotho promoter hypermethylation, leading to subsequent Klotho suppression [52]. In particular, indoxyl sulfate (IS) and *p*-cresyl sulfate (PCS), two protein-bound uremic toxins, caused CKD by increasing the expression of DNMT 1,

DNMT3a, and DNMT3b, Klotho promoter hypermethylation, and Klotho repression in the mouse kidney [53]. IS also increased Klotho promoter methylation in vascular smooth muscle cells, exacerbating vascular calcification in the CKD mice [54]. Similarly, urinary cadmium exposure enhanced Klotho promoter methylation to adversely influence liver and kidney functions [40, 55]. These results indicate that DNMT aberration-mediated promoter hypermethylation limits Klotho expression, thus exacerbating CKD pathologies. In addition, persistent inflammation and homocysteinemia in CKD is associated with DNA hypermethylation [56–58], but whether these processes involve Klotho promoter hypermethylation needs further investigation.

Emerging evidence indicates that endogenous Klotho restorations via demethylating agents are beneficial for CKD animals. Downregulation of Klotho in uremic toxin-induced CKD could be reverted by demethylating agent decitabine [52–54]. Rosiglitazone, an antagonist of peroxisome proliferator-activated receptor γ (PPARγ), increased Klotho expression and protected against high phosphate-induced vascular calcification in CKD mice, which might involve reversal of MeCP2 (methyl-CpG binding protein 2) abnormal expression [59]. Several extrarenal CKD complications such as soft-tissue and cardiovascular calcifications, hypertension, cardiomyopathy, disturbed mineral metabolism, and bone injuries could be partially ameliorated via Klotho recovery by demethylating drugs [38, 54, 60, 61]. In studies of CKD mice with mineral and bone disorder (CKD-MBD) incurred by adenine feeding, we observed that aberrant DNMT1/DNMT3a elevations, Klotho promoter hypermethylation, and Klotho suppression were reversed by rhein, a chemical compound isolated from medicinal herb, resulting in improved renal and bone pathologies [38] (Fig. 1), supporting that some components from medicinal plants or other natural sources possess strong epigenetic modulating capacities.

Renal fibrosis

Renal fibrosis is a common pathohistological feature of CKD independent of the underlying etiologies. Its development is characterized by activation of myofibroblast transdifferentiation (MTD) and excessive extracellular matrix (ECM) deposition [62], the processes that are profoundly affected by aberrant DNA methylation modifications [63].

As demonstrated by several studies, Klotho deficiency potentiates renal fibrosis in animal models [64]. Consistently, Klotho reportedly suppressed basic fibroblast growth factor-2 signaling and inhibited TGFβ (transforming growth factor-beta)/Smad and Wnt/β-catenin signaling to relieve EMT (epithelial–mesenchymal transition) and myofibroblast activation, and subsequently improved renal fibrotic lesions [65–68]. Klotho inhibitions of oxidative stress and excessive inflammation also contribute to the anti-fibrotic activities [69, 70]. Administrations of decitabine or medicinal plant–derived products or "epigenetic diets" including rhein, genistein, or curcumin attenuated renal fibrosis by demethylating Klotho promoter. In particular, rhein and genistein were capable of inhibiting aberrant DNMT1/3a elevations, resulting in Klotho restoration and reduced renal fibrosis in UUO (unilateral ureteral obstruction) mice [36, 37, 46, 71]. In mice with renal fibrosis after IRI, hydrogen-rich saline retained Klotho expression and reduced renal fibrotic damage likely via demethylating Klotho promoter [44]. These studies suggest that TGFβ-induced DNMT1/3a aberrations are responsible for the Klotho promoter hypermethylation and Klotho suppression in renal fibrotic mice [37].

TET-mediated hydroxymethylation also plays an important role to induce DNA demethylation on Klotho promoter. Accordingly, High-fidelity CRISPR/Cas9-based gene-specific hydroxymethylation by TET3 catalytic domain was shown to rescue Klotho repression and attenuate renal fibrosis

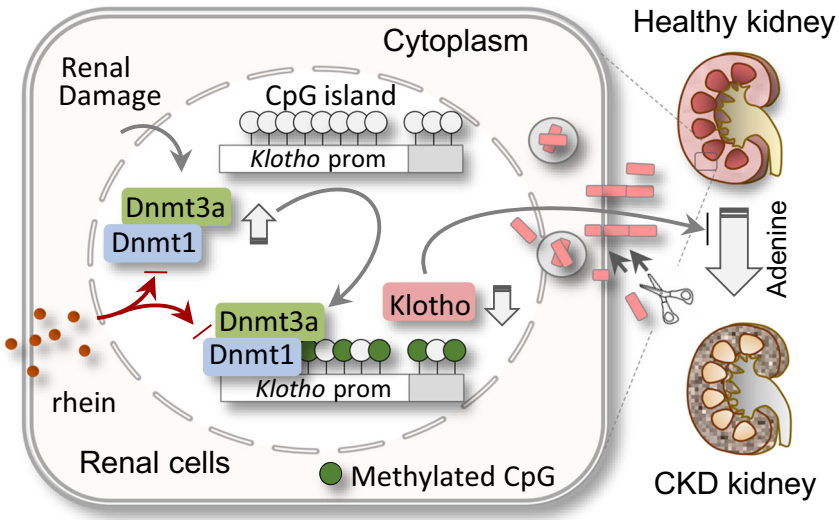

Fig. 1 A diagram illustrating DNMT1/3a aberrations, Klotho promoter hypermethylation, and subsequent Klotho repression as well as rhein intervention in adenine-incurred CKD/MBD mice

[72]. Hydrogen sulfide also attenuates renal fibrosis induced by hypoxia microenvironment by inducing TET-dependent DNA demethylation on Klotho promoter [73]. Those results suggest that TET family proteins can be potential therapeutic targets for reversing Klotho fibrotic suppression.

Diabetic nephropathy

Diabetic nephropathy (DN) is a main cause of CKD [74] and Klotho repressions are observed in DN animals [75]. On the other hand, exogenous Klotho supplementations protected against DN by attenuating glomerular inflammation, fibrosis, oxidative stress, albuminuric activity, and abnormal lipid metabolisms [76–79]. Moreover, epigallocatechin-3-gallate, a green tea extract, reduced DNMT3a binding to Klotho promoter and hypomethylated the promoter, leading to improved DN pathologies [80]. Similarly, administration of baicalin, a flavone glycoside, alleviated the renal injury of DN partially through modulating Klotho promoter methylation [81].

Kidney transplantation

Kidney transplantation is an optimal choice for treatment of patients with the end-stage renal disease (ESRD). Post-transplant ischemic AKI secondary to ischemia reperfusion injury (IRI) is a major problem that affects graft and patient survivals [82]. As seen in AKI, Klotho is also suppressed in patients with kidney transplantation [83]. Patients who experienced delayed graft function were found to have significant lower serum Klotho levels compared with the control group. IRI induces excessive inflammatory responses and oxidative stress [84] that might contribute to the Klotho suppression through aberrant DNA methylation [41, 82]. Clinical studies demonstrated that kidney transplant recipients showed Klotho promoter hypomethylation and Klotho recovery after paricalcitol treatment, which beneficially affected the clinical course [82, 85], suggesting that kidney transplant recipients might benefit from endogenous Klotho restoration via DNA methylation intervention.

It seems that aberrant DNMT elevations are the major causes of most renal diseases reported since DNMT inhibitions by various strategies of DNA demethylation effectively reduced the renal pathologies. It should be also emphasized that DNMTs are not the only factors dictating the methylation status of Klotho promoter. The abnormal expressions of other DNA methylation regulatory proteins such as DNA insulator protein, methylated DNA binding protein, and transcriptional repressors likely play significant roles in the epigenetic Klotho repression, which are important but less explored areas of research.

Histone acetylation modulation of Klotho expression in kidney diseases

Post-translational protein acetylation is another epigenetic regulatory mode that modulates chromatin structure and transcriptional status to affect gene expression. Typically, histone acetylation is regulated by two groups of enzymes of opposite functions, namely histone acetyl-transferases (HAT) and histone deacetylases (HDAC), that consist of at least 4 major classes of 18 members, namely class I (HDAC1, 2, 3, 8), class II (IIa 4, 5, 7, 9, and IIb 6,10), class III (SIRT1–7), and class IV (HDAC11) [86]. Histone acetylation reduces chromatin condensation, allowing access of transcription factors and facilitating gene transcription. On the contrary, HDACs remove the acetyl groups from lysine on the core histone tails and restore the positive charge of lysine, resulting in chromatin compaction and gene transcription inhibition [87]. Comparing to the extensive investigations of histone/protein acetylations and the therapeutic application of HDAC inhibitors in cancer research [88], the protein acetylation modifications in renal diseases are less explored; however, some interesting data are emerging.

Acute kidney injury

In the setting of AKI induced by folic acid, HDAC1 and HDAC2 were aberrantly elevated, which physically interacted with NF-κB, leading to transcriptional downregulation of Klotho [17], whereas HDAC inhibitor trichostatin A and valproate inhibited the downregulation and improved AKI [17, 56]. A number of studies have proposed that persistent inflammation is increasingly recognized as an important determinant factor for AKI progression to CKD [89]. Thus, restoration of Klotho through HDAC inhibitors is considered as potential therapeutic intervention to mitigate the progression of AKI-to-CKD transition [90].

CKD and the extrarenal complications

The specific roles of HDACs in CKD progression are only incompletely understood. It has been reported that Klotho preservation via HDAC inhibition could attenuate CKD and the associated bone injury [91, 92]. In CKD/MBD model of adenine-fed mice, a non-selective HDAC inhibitor trichostatin A (TSA) increased acetylation of PPARγ and PPARγ-dependently alleviated Klotho loss and kidney injury [92]. Moreover, renal HDAC3 was preferentially upregulated in the mouse kidney and a HDAC3-selective inhibitor RGFP966 extent-similarly alleviated Klotho loss and renal injuries as TSA, suggesting that increased HDAC3 is a major driving force for the pathological effects [92].

CKD is often associated with cardiovascular complications [93]. Activation of SIRT1 by SRT1720, a specific SIRT1 activator, could attenuate Klotho deficiency-induced arterial

stiffness and hypertension [94]. Similarly, resveratrol, a known SIRT1 activator, can upregulate Klotho or even reduce vascular calcification in ESRD animals likely through correcting aberrant SIRT1 activities [95, 96].

Renal fibrosis

Aberrant HDAC activities are observed in almost all animal models of renal fibrosis [97, 98]. Various pan- and class-selective inhibitors of histone deacetylase (HDAC) exhibit impressive anti-renal fibrosis properties [99–107]. In the fibrotic kidneys of various animal models, Klotho is markedly depressed due to, at least in a significant part, the aberrant HDAC activities. HDAC inhibitions by known HDAC inhibitor TSA and genistein from soy products with epigenetic modulating capacity displayed anti-renal fibrosis activities by recovering histone acetylation-associated Klotho loss [36, 92]. MC1568, a selective class IIa HDAC inhibitor, increased renal expression of Klotho in UUO mice [103]. Similarly, selective inhibitions of HDAC8 by either a HDAC8-selective inhibitor PCI34051or siRNA interference were reportedly effective in inhibiting multiple profibrotic signaling with concomitant Klotho recovery and renal fibrosis mitigation [108]. Conversely, aldosterone-induced renal fibrosis was accompanied by HDAC1-incurred H3K9 deacetylation and Klotho transcriptional repression [109].

These studies demonstrate that fibrotic Klotho repressions are associated with aberrant expressions or activities of multiple HDAC isoforms; however, the key HDAC isoforms that are causally involved in Klotho repression during renal fibrogenesis might need confirmation by gene-specific deletion investigations. We have recently showed that genomic HDAC3 gene knockout exacerbated renal fibrosis in UUO mice. HDAC3 was aberrantly and preferentially elevated in UUO kidney, likely induced by TGFβ. The upregulated HDAC3 formed a transcriptional repressor complex with NcoR and NF-κB that inhibited Klotho transcription. Conversely, HDAC3-selective inhibitor RGFP966 derepressed Klotho and Klotho dependently mitigated the renal fibrotic damage, suggesting that HDAC3 aberration and the associated Klotho suppuration play essential roles in renal fibrotic pathologies [110] (Fig. 2).

Past research studies have accumulated ample evidence indicating that aberrant protein acetylation alterations due to increased HADC expression or activity contribute significantly to the development of kidney diseases. It is likely that different HDAC isoforms and mediators function at different disease stages of different pathological processes. Identifications of the causal HDAC isoforms and the key mediators causally involved in the processes are the key to fully understand the precise underlying mechanisms.

miRNA modifications of Klotho expression in kidney diseases

Micro-RNA (miRNA) interference represents an additional layer of epigenetic gene expression regulation. MiRNAs are endogenous short non-coding RNAs of 22–25 base pairs that regulate gene expression through post-transcriptional repression of target mRNAs [111, 112]. Generally, miRNA binds to the 3′-untranslational region (UTR) of a target mRNA through base-pairing mechanism to suppress target gene expression by either inhibiting protein translation or mRNA degradation

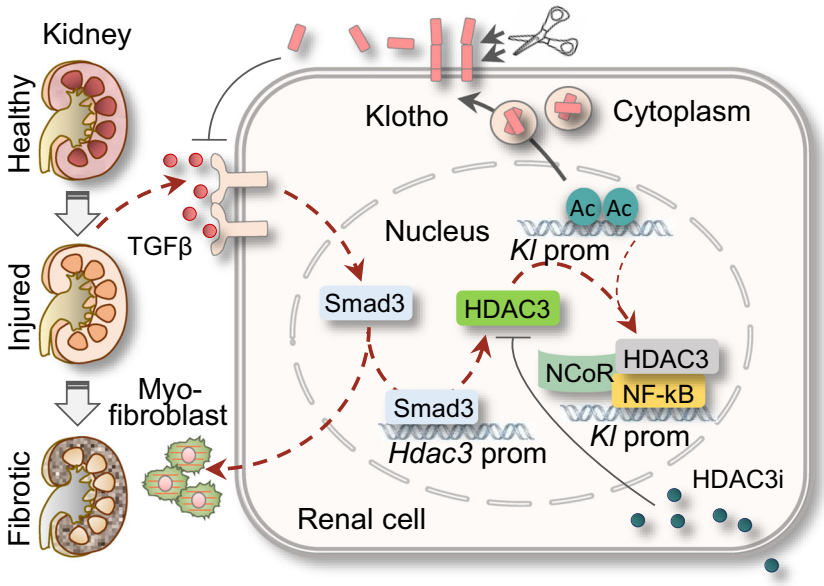

Fig. 2 A schematic of sequential regulations of TGFβ-incurred HDAC3 aberration, Klotho transcription inhibition, and renal fibrogenesis, as well as the epigenetic intervention by HDAC3-selective inhibitor

[112]. Of note, some novel class of RNA regulators for gene expression including long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) have been recently discovered through functional genomics studies [113, 114], adding the complexity of this epigenetic regulatory scheme.

The interplay of miRNAs and Klotho has been extensively investigated in cancer studies [115]. It turned out that Klotho is a target of a diversity of miRNAs as demonstrated by renal cell [116–118] and animal studies [37, 119–127] (see Table 1), suggesting that miRNAs might play essential roles in the expression of Klotho in renal diseases.

AKI

The information regarding miRNA modulation of Klotho in AKI is increasing. Upregulation of miR-29a and miR-34a in mesenchymal stromal cells might act synergistically with Klotho to prevent IRI-induced AKI.[119]. miR-130a promoted PI3K/AKT pathway but inhibited Wnt and NF-κB pathways through upregulation of Klotho to protect against LPS-induced glomerular cell injury [120]. EVs isolated from normal urine transferred their microRNA cargo containing miR-30s/miR-151 to kidney, reduced the endogenous Klotho loss, and exhibited renal protection in a murine model of acute injury generated by glycerol injection [121]. These studies demonstrate that miRNA inhibition of Klotho expression plays critical roles in AKI development and could be targeted for AKI intervention.

CKD and its extrarenal complications

The information regarding altered miRNA regulation of CKD is limited. The evidence for the important role of miRNAs in the physiological regulation of parathyroid function and its dysregulation in the secondary hyperparathyroidism in CKD are recently reported [122, 128]. The studies showed that many miRNAs were aberrantly expressed in experimental uremic hyperparathyroidism, and inhibition of let-7 family increased parathyroid hormone (PTH) secretion in normal and uremic rats. Conversely, inhibition of the upregulated miRNA-148 family blocked the increase of serum PTH in uremic rats, suggesting that miRNA dysregulation represents a crucial step in the pathogenesis of secondary hyperparathyroidism in CKD.

Renal fibrosis

MiRNA aberrations potentially affect renal fibrosis [129]. MiRNA-34a was shown to promote renal fibrosis by directly downregulating Klotho in tubular epithelial cells [123]. On the other hand, miR-152 and miR-30a inhibited by TGFβ can indirectly regulate Klotho expression via targeting DNMT1 and DNMT3a, respectively, leading to Klotho promoter hypomethylation and Klotho recovery in UUO mice, which might serve as a regulatory loop mediating TGFβ's profibrotic activities [37, 130].

Diabetic nephropathy

Diabetic nephropathy (DN) is a main cause of CKD [74] and Klotho repressions are observed in diabetic nephropathy [75], in which altered miRNA expression might be a causal factor. In particular, miRNA-199a-5p functioned as a key mediator of Klotho expression in DN. In renal cell assays, high glucose reportedly increased miRNA-199a-5p expression

Table 1 MiRNA regulation of Klotho expression in various renal cells and kidney diseases

MiRNA	Up/down	Animal model/cell type	Reference
miR-200c	↑	HK-2	[116]
miR-339/miR-556	↑	HEK293T	[117]
miR-335-5p	↑	HUVE	[118]
miR-29a/miR-34a	↑	Rat IRI-AKI/MSC	[119]
miR-130a	↑	Mouse LPS-AKI/HK-2	[120]
miR-30s/miR-151	↑	Mouse glycerol-AKI/MC	[121]
miR-148	↑	Rat uremic-CKD	[122]
miR-34a	↑	Mouse UUO/HK-2	[123]
miR-152/miR-30a	↓	Mouse UUO/HK-2	[37]
miR-199a-5p	↑	Mouse HFD/STZ-DN/HK-2/RMC	[124, 125]
miR-199b-5p	↑	Mouse STZ-DN/HK-2	[126]
miR-199a	↑	Mouse pristine-LN/HEK293T	[127]

MSC mesenchymal stromal cell, *HK-2* human renal tubular epithelial cell, *HUVE* human umbilical vein epithelium, *HFD* high-fat diet, *STZ* streptocozin, *DN* diabetic nephropathy, *LN* lupus nephritis, *RMC* rat mesangial cell, *HEK293T* human embryonic kidney 293T cell

accompanied by significant decrease of Klotho at both mRNA and protein levels. High glucose also activated NF-κB signaling and promoted fibrotic and inflammatory reactions, which could be restrained by inhibition of miR-199a-5p or exogenous addition of Klotho [124]. Likewise, miRNA-199a-5p from HK-2 cell-derived extracellular vesicles (EVs) induced macrophage M1 polarization by targeting the Klotho/TLR4 signaling pathway and further accelerated DN development [125]. In agreement with these, a selective endothelin-A receptor antagonist atrasentan increased Klotho expression by lowering miRNA-199b-5p and prevented renal tubular injury of DN, and overexpression of miR-199b-5p disrupted the influences of atrasentan on Klotho expression and apoptosis of renal tubular cells both in vivo and in vitro [126], suggesting that miRNA-199a-5p might represent a potential target for DN therapeutic intervention.

Nephritis

Lupus nephritis (LN) is considered a life-threatening complication of systemic lupus erythematosus [131] and characterized by exaggerated inflammation and fibrosis progression [132]. As a crucial pathway involved, NF-κB activation is closely related to the initiation and progression of LN through the transcriptional regulation of pro-inflammatory cytokines [133, 134]. Several studies suggested that miRNAs adversely regulated Klotho expression, NF-κB signaling activation, and inflammatory cytokine expression during LN progression [120, 127]. MiR-199a reportedly activated NF-κB signaling and promoted TNF-α and IL-1β expressions by targeting Klotho directly, explaining in part the lupus nephritis pathogenesis [127]. Therefore, Klotho restoration through miRNA intervention is considered a promising treatment option for treatment of nephritis.

Although studies on miRNAs and kidney diseases are continuously expanding, it is noteworthy that analysis of human and mouse Klotho coding regions by miDBD online software (http://mirdb.org) predicts 130 and 72 putative miRNA binding sites, respectively. MiRNA lacks specificity. A single miRNA can regulate many target genes and a single mRNA can be targeted by multiple miRNAs [135]. Therefore, when translating the results from cell and animal assays to a particular clinical setting, the miRNA specificity and the off-target effects on other target gene expressions have to be carefully evaluated.

Other epigenetic modifications of Klotho expression in kidney diseases

Other less-studied epigenetic modifications also affect Klotho expressions in kidney diseases, although the evidence is sparse. A recent study reported that Klotho mRNA was hypermethylated in IS-induced calcified arteries of CKD mice that was mediated by Mettl14 (methyltransferase-like 14) overexpression [136]. Blocking the histone3/lysine 79 methyltransferase DOT1L (disruptor of telomeric silencing-1 like) alleviated renal fibrosis through recovering Klotho loss [137]. H3K27me3 level was increased in kidneys of aged WT and Klotho mutant mice likely due to downregulation of the H3K27 (histone H3 Lys 27)-specific demethylase JMJD3 (the Jumonji domain containing-3). Inhibition of PRC2 (polycomb repressive complex C2; histone trimethyltransferase) decreased the H3K27me3 levels, leading to increased expression of Klotho in cultured primary renal tubule cells [138]. Notably, lncRNA MALAT1 could mediate high glucose-induced glomerular endothelial injury by epigenetically inhibiting Klotho via methyltransferase G9a [139]. It is expected that future investigations of new epigenetic Klotho regulations will bring more details of the molecular mechanisms of Klotho expressions that potentially lead clinical benefits.

Summary and perspectives

Current evidence supports that restoration of endogenous Klotho by epigenetic intervention represents a new direction of therapeutic approaches for kidney disease treatment. Up to now, at least four pan- or class HDAC inhibitors, namely vorinostat, romidepsin, belinostat, and panobinostat, and one demethylating agent decitabine are approved by the US Food and Drug Administration for treating cutaneous and peripheral T-cell lymphomas [140], and myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), respectively, making the applications easier in treatment of kidney diseases. However, long-term uses of synthetic epigenetic drugs inhibiting all HDAC and DNMT activities are potentially cytotoxic and might cause intolerable side effects in certain patients [141]. Moreover, epigenetic modifications are often mechanistically connected. For example, DNMT and HDAC expressions are regulated by miRNAs, which in turn are subjected to DNA methylation and protein acetylation regulations. Methylated CpGs on gene promoter are recognized and bound by methyl-binding proteins complexed with transcription co-repressors and HDACs to facilitate the gene transcriptional silencing [142]. Therefore, a combination of epigenetic drugs targeting multiple epigenetic alterations might lower the drug dosage and incur fewer side effects. In addition, recent studies have demonstrated that many components from natural food products or medicinal plants display previously unrecognized epigenetic modulating capacities with tolerable side effects. It is anticipated that future research identifying the key causal factors and dissecting the contribution of each epigenetic modification to Klotho expression will provide more effective prophylactic and therapeutic options for the managements of various kidney diseases.

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Authors' contributions J.X. searched the literatures drafted the manuscript. W.C. reviewed, edited, and wrote the manuscript.

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Declarations

Competing interests The authors declare that they have no conflict of interest.

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