Review Article

Role of Klotho in Chronic Calcineurin Inhibitor Nephropathy

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Calcineurin inhibitors (CNIs) are the most popular immunosuppressants in organ transplantation, but nephrotoxicity is a major concern. The common mechanism underlying chronic CNI nephropathy is oxidative stress, and the process of chronic CNI nephropathy is similar to that of aging. Current studies provide evidence that antiaging Klotho protein plays an important role in protecting against oxidative stress, and its signaling is a target for preventing oxidative stress-induced aging process. In this review, we focus on the association between Klotho and oxidative stress and the protective mechanism of action of Klotho against oxidative stress in chronic CNI nephropathy. In addition, we discuss the delivery strategy for Klotho in CNI-induced nephropathy.

1. Overview of Klotho in Human Disease

Klotho is an aging-suppressor gene [1, 2], and it encodes a single-pass transmembrane protein. The extracellular domain of Klotho protein is cleaved on the cell surface by membrane-anchored proteases and is released into the blood [3–5], urine [6–8], and cerebrospinal fluid [4]. Secreted Klotho proteins have diverse functions, including the regulation of multiple ion channels [6, 8–10] and oxidative stress [11–13].

Klotho is involved in various pathologies, such as atherosclerosis, heart failure, hypertension, acute kidney injury, chronic kidney disease, diabetes mellitus, and even cancer [14–17]. Interestingly, Klotho is highly expressed in the kidney [1], and its expression is suppressed under sustained stress conditions in several animal models [18–22] of kidney injury and in patients with chronic renal failure [23]. Thus, the role of Klotho in kidney injury has attracted increasing attention from researchers.

2. Overview of Chronic CNI Nephropathy

Calcineurin inhibitors (CNIs) are the most popular immunosuppressive drugs used for solid organ transplantation, and two CNIs [cyclosporine (CsA) and tacrolimus (TAC)] are available in clinical practice [24]. CNI exerts its immunosuppressive action by inhibiting calcineurin in T-cells. This inhibition then impairs translocation of the nuclear factor of activated T-cells [25–27], which regulates IL-2 transcription and thus T-cell activation [28–30]. Despite the specific inhibition of T-cell activation, long-term treatment with CNIs causes serious adverse effects, and nephrotoxicity is a major issue in solid organ transplantation.

Utilizing a well-established animal model, we and others have demonstrated that CNI causes low-grade ischemic injury by reducing renal blood flow and activating a complex network of proinflammatory and profibrotic mediators (for example, osteopontin [31, 32] and transforming growth factor β 1 [33, 34]), along with the renin-angiotensin system

FIGURE 1: The concept of chronic CNI nephropathy as aging process. Low-grade ischemic injury by long-term CNI treatment decreases antiaging Klotho protein. Thus, renal tubular cells lost its ability to resistance to oxidative stress and subsequent cell death occurs.

[35, 36], apoptosis [37, 38], and endothelial dysfunction [39]. The oxidative stress caused by reactive oxygen species (ROS) is regarded as a common pathway of CNI-induced nephrotoxicity. Antioxidative agents such as statin, angiotensin II blockade, or N-acetylcysteine are known to improve CNI-induced renal injury [40–43].

Chronic CNI nephropathy causes progressive renal failure [44] which is similar to alterations that occur with aging. Indeed, telomere shortening and upregulation of senescence-associated cell cycle inhibitors were reported in CNI-treated renal tubular cells [45] and in renal transplants with graft dysfunction [46]. Thus, we proposed that oxidative stress due to low-grade ischemia accelerates the aging process in chronic CNI nephropathy and the antiaging protein Klotho may be involved in this process (Figure 1).

3. Klotho Expression and Oxidative Stress in Chronic CNI Nephropathy

Using animal model of chronic CNI nephropathy, we firstly reported that CNI treatment decreased Klotho mRNA and protein in the mouse kidney in a dose- and time-dependent manner [43, 47] and Klotho expression was correlated with activity of renin-angiotensin system, tubulointerstitial fibrosis, and marker of oxidative stress (urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) excretion) [48]. This finding suggests that long-term treatment of CNI decreases Klotho expression in the kidney and Klotho is a useful marker to represent chronic CNI nephropathy.

A Klotho-deficient mouse aging model is useful to define the causal relationship between oxidative stress and Klotho.

Kuro-o et al. reported that Klotho deficiency is closely related to cardiovascular diseases [1] and Klotho is an important humoral factor involved in oxidative stress regulation, endothelial dysfunction, cell proliferation, and apoptosis [49–51]. Using Klotho +/- mice, we found that Klotho deficiency renders the kidney more susceptible to TAC-induced injury, which was closely associated with aggravated TAC-induced oxidative stress [47]. These findings suggest strong associations between Klotho and CNI-induced oxidative stress and provide evidence that Klotho plays an important role in protecting against CNI-induced oxidative stress.

4. Protective Mechanism of Action of Klotho against CNI-Induced Oxidative Stress

Klotho is involved in several intracellular signaling pathways (PKC, FGF23, cAMP, TGF- β , p53/p21, Wnt signaling, and PDLIM2/NF- κ B p65 pathway) [52, 53], and many studies have reported the interactions among these pathways [54, 55]. In this review, we focus on the antioxidative function of Klotho via the intracellular phosphatidylinositol 3-kinase (PI3K)-Akt serine-threonine kinase (AKT) signaling pathway.

The PI3K-AKT signaling pathway regulates forkhead box protein O (FoxO) through phosphorylation. The AKT-mediated phosphorylation of FoxO inhibits FoxO activity by promoting its interaction with 14-3-3 proteins and nuclear exportation and also by inducing its proteasomal degradation [56]. FoxO3a can upregulate manganese superoxide dismutase (MnSOD) expression [2, 57, 58]. Thus, FoxO3a functions as a negative regulator of mitochondrial ROS production [59] and thereby closely associates with resistance to oxidative stress. In an experimental model of TAC-induced nephropathy, we found that concomitant Klotho treatment inhibits the PI3K/AKT-mediated phosphorylation of FoxO3a and enhances FoxO3a binding to the MnSOD promoter. Thus, Klotho increases MnSOD mRNA and protein expression in mitochondria and reduces TAC-induced mitochondrial dysfunction and ROS production [60]. Taken together, Klotho protects TAC-induced oxidative stress by negatively regulating the PI3K/AKT pathway and subsequently enhances FoxO3a-mediated MnSOD expression.

5. Role of Klotho in CNI-Induced Cell Death

Endoplasmic reticulum (ER) stress, a common cellular stress, is a potent trigger for autophagy, which is an important protective mechanism against various cellular stresses, including nutrient deprivation, hypoxia, and growth factor deprivation [61, 62]. Thus, the balance between ER stresses and autophagy is important to maintain cell viability, and excessive ER stress or impaired autophagy may cause apoptotic cell death. Recent reports showed that Klotho plays an important role in modulating ER signaling crosstalk between autophagy and apoptosis [49–51] and Klotho treatment alleviates ER stress in unilateral ureteral obstruction or attenuates oxidant-induced alveolar epithelial cell apoptosis [63]. In addition, the association between Klotho and autophagy has been reported in various diseases, such as Alzheimer's disease,

FIGURE 2: The protective mechanism of Klotho in CNI-induced autophagic cell death. Klotho induces nuclear translocation of transcription factor EB (TFEB), a master regulator for lysosomal biogenesis, through inhibition of phosphorylation of glycogen synthase kinase 3 β (GSK3 β). Improved lysosomal function by Klotho increases clearance of autophagosome and resulted in decrease of autophagic cell death.

acute kidney injury, chronic obstructive pulmonary disease, and lung cancer [64–67].

CNI-induced renal injury involves induction of the ER stress response and apoptosis [68, 69]. Kidneys treated with CNI for a short time adapt well to such stress by synthesizing molecular chaperones and activating autophagy process. However, prolonged ER stress by CNI exposure may cause apoptosis by depleting molecular chaperones and overloaded autophagosome [70, 71]. We recently reported that chronic CNI nephropathy is a state of excessive accumulation of autophagosome and impaired autophagy clearance [72] and Klotho treatment reduces the burden of autophagy vacuoles by improving autophagy clearance via activation of lysosomal function in CNI-induced nephrotoxicity [73]. We summarized the mechanism of protective effect of Klotho on CNI-induced autophagy cell death in Figure 2.

6. Delivering Strategy for Klotho

We and other researchers studied how to preserve Klotho against oxidative stress in kidney, and we reported that angiotensin II blockade, statin, and N-acetylcysteine are

effective in preserving Klotho in experimental model of chronic CNI nephropathy [40, 42, 43]. However, it is not certain whether preservation of Klotho by these drugs is casually related to the antioxidant effect.

Accumulating evidence indicates that administration of exogenous Klotho is a rational strategy for the treatment of acute/chronic kidney diseases [74]. However, the half-life of recombinant Klotho is so short (7.2 h) that frequent injection (every day or every alternative day) is needed to achieve therapeutic efficacy [60, 75–77]. To overcome this limitation, we developed minicircle (MC) vector encoding Klotho protein. Using MC delivery, we can detect MC-Klotho until 30 days and MC-mediated Klotho protein until 10 days after single injection via the tail vein and at significantly higher levels than that of conventional vectors [78] (Figure 3). Thus, the MC-mediated vector encoding Klotho provides more long-term and stable Klotho expression than recombinant Klotho protein. We observed the effect of MC in an animal model of ischemia-reperfusion injury and obstructive nephropathy [78]. We expect that MC-mediated Klotho protein production may offer a new approach to Klotho delivery in clinical practice.

FIGURE 3: Strategy of Klotho delivery using minicircle vector system. (a) Production of in vivo Klotho using minicircle vector system. (b) Representative pictures of mice with Klotho protein derived from minicircles in vivo, each day after injection of saline or MC-Klotho using in vivo imaging system. Note that red fluorescence protein signal can be observed at day 30. (c) The plasma level of Klotho by ELISA. Saline-treated group was used as a negative control. pp: parental plasmid DNA; MC: minicircle plasmid DNA. $^{\#}P < 0.05$ vs. the other group. $*P < 0.05$ vs. corresponding pp-Klotho. Scale bar = 100 μm .

7. Conclusions

Klotho plays an important role in protecting against CNI-induced oxidative stress. Klotho and its signaling is an important target of preventing oxidative stress-induced organ injury.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Kang Luo and Sun Woo Lim equally contributed to this paper.

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