see original article on page 640

KLOTHO methylation is linked to uremic toxins and chronic kidney disease

Guang-Huar Young[1,2] and Vin-Cent Wu[1,3]

Epigenetic regulation plays a major role in uremic toxin-induced chronic kidney disease (CKD) progression. The KLOTHO protein is a key modulator of homeostasis in renal function. Uremic toxin accumulation can induce DNA methyltransferase (DNMT) protein expression, which is involved in the silencing of *KLOTHO* through hypermethylation. Treatment with DNMT inhibitors can induce a hypermethylated status of *KLOTHO* and suppress mRNA and protein expression. Epigenetic targeting of specific genes may become an effective strategy to prevent progression of uremia-related CKD.

Kidney International (2012) **81,** 611–612. doi:10.1038/ki.2011.461

In eukaryotes, DNA methylation is an epigenetic mechanism that facilitates the transfer of methyl (-CH3) groups onto cytosine, affecting the expression of CpG-rich genes. During times of metabolic stress and inflammation, a coordinated transcriptome remodeling process occurs in order to maintain cellular homeostasis. Emerging evidence indicates that several transcriptional regulators, such as hypoxia-inducible factor, nuclear factor-κB, activating protein-1, stimulatory protein-1, p53, Mys, and interleukin-6, are essential mediators of cellular stress and cellular homeostasis.[1]

Although epigenetic mechanisms play important roles in the pathogenesis of various human diseases, their role in chronic kidney disease (CKD) is unclear; however, the plethora of metabolic alterations and coexisting inflammation that occur in CKD could potentially involve and induce diverse epigenetic changes. During progression of CKD, kidneys lose their ability to effectively remove toxic compounds from the body. The accumulation of these uremic toxins is associated with the progression of glomerular and interstitial fibrosis at the transcriptional level. The increased levels of uremic toxins can stimulate the production of reactive oxygen species, deteriorate renal function, and promote progression of CKD. Most studies have focused on how these uremic toxins have affected the phenotype, but few have focused on the underlying molecular mechanism.

Several factors, such as inflammation, oxidative stress, and uremic toxins, are characteristic of CKD[2] and progressive kidney function deterioration, all of which could contribute to epigenetic alternation. Stenvinkel *et al.*[3] demonstrated the correlation between DNA methylation and inflammation among CKD patients. DNA hypermethylation with lower ratios of HpaII to MspI is associated with cardiovascular disease. The same phenomenon has also been observed in end-stage renal disease patients. The connection between inflammatory factors and hypermethylation could be due to interleukin-6-mediated upregulation of DNA methyltransferase (DNMT) proteins and therefore has an impact on the cellular epigenome in a kidney undergoing an inflammatory response.[4,5]

Recently, the importance of the phosphaturic hormone fibroblast growth factor-23 and its necessary cofactor KLOTHO in the process of CKD was reported.[6] KLOTHO is a transmembrane protein, but because of alternative splicing, two non-membrane-bound circulating protein subtypes also exist. The predominant distribution of KLOTHO is in the kidneys and in the parathyroid. Although the biological functions of KLOTHO are not entirely understood, KLOTHO has been identified as having important roles in anti-aging, mineral metabolism, and vitamin D metabolism. Several *in vivo* experiments using animal models that correspond with human kidney diseases have indicated that KLOTHO mRNA and protein levels are reduced during acute or chronic kidney diseases in response to reactive oxygen species.[7] Conversely, the overexpression of *Klotho* in a transgenic mouse model, compared with the wild-type mice, exhibited better renal function, less calcification, and increased suppression of the insulin-like growth factor-1 (IGF-1) signaling pathway. The IGF-1 signaling pathway is one of the most important pathways for compensatory renal hypertrophy after uninephrectomy.[8] Taken together, these published data indicate that KLOTHO is a potential key factor in response to renal dysfunction.

Sun *et al.*[9] (this issue) used *in vitro* cell models and *in vivo* animal models of uremia-related CKD under oxidative stress conditions in an attempt to examine DNMT protein expression patterns and their role in DNA hypermethylation of important CpG islands. This information is important because of its novelty in the CKD field as well as illustrating important physiological functions of KLOTHO in cancer.

Indoxyl sulfate and *p*-cresyl sulfate are two protein-bound molecules that are carried into the renal tubular lumen by multiple organic anion transporters.[10] In an experimental CKD mouse model, indoxyl sulfate and *p*-cresyl sulfate were used as a source of uremic toxins. Despite the continuous infusion of these uremic toxins, renal function indicators including blood, urine, nitrogen, and creatinine were not significantly different. The remnant

[1]*National Taiwan University Study Group on Acute Renal Failure, National Taiwan University Hospital, Taipei, Taiwan;* [2]*Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan and* [3]*Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan*
Correspondence: *Vin-Cent Wu, Department of Internal Medicine, National Taiwan University Hospital, Room 735, Clinical Research Building, 7 Chung-Shan South Road, Taipei 100, Taiwan. E-mail: q91421028@ntu.edu.tw*

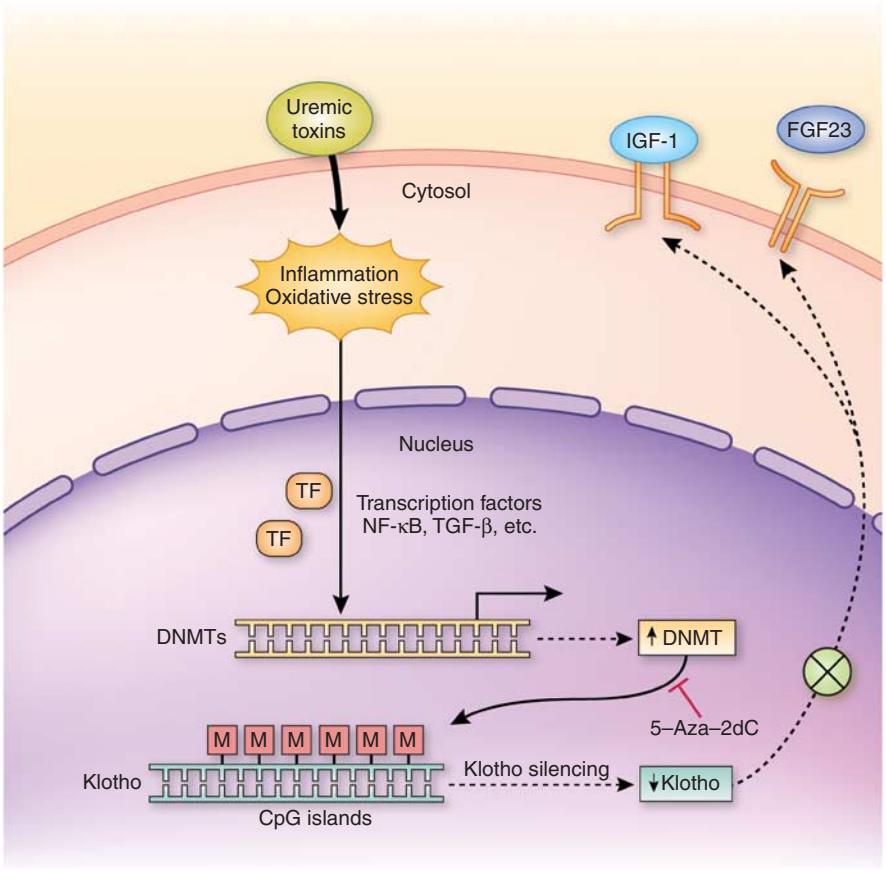

Figure 1 | Potential mechanisms of uremic toxin-induced silencing of the *Klotho* gene in chronic kidney disease involve DNA methyltransferase-mediated regulation. 5-Aza-2dC, 5-aza-2′-deoxycytidine; DNMT, DNA methyltransferase; FGF23, fibroblast growth factor-23; IGF-1, insulin-like growth factor-1; TF, transcription factor; TGF-β, transforming growth factor-β.

kidney exhibited a severe degree of fibrosis and high serum levels of uremic toxins. Taken together, these results parallel the progression symptoms of CKD. The modified model also mimicked the progressive nephron loss and maintained the high level of uremic toxins caused by a perpetual inflammatory state, which often occurs in human CKD.[9] On the basis of the analysis of DNMT expression and the methylated status of the *Klotho* gene by methylation-specific PCR, the authors hypothesized that the uremic toxin-induced oxidative stress could potentially stimulate DNMT upregulation and eventually result in the alternative splicing of the *Klotho* gene. It will be interesting to use the *in vivo* experimental model to investigate uremic toxin-induced injury in the remnant kidney during CKD and to determine whether *Klotho* is an effective biomarker for CKD.

In support of this hypothesis, the combination of uremia-related cell and animal model manipulations with decitabine (5-aza-2′-deoxycytidine), an inhibitor of DNMT activity, which is also used clinically in patients with myelodysplastic syndrome,[11] resulted in a reduction of the methylated status of the *Klotho* gene and improved the expression of the KLOTHO protein significantly (Figure 1). Mechanism-based studies suggest that decitabine is a cytidine analog that forms irreversible covalent bonds with DNMT at cytosine sites targeted for methylation, and could modulate the uremic toxin-induced DNMTs.

In conclusion, the work by Sun *et al.*[9] provides a new window in research on uremia-related CKD and the epigenetic effect of uremic toxins such as indoxyl sulfate and *p*-cresyl sulfate on the *Klotho* gene. Uremic toxin-induced DNMT expression was involved in the silencing of the *Klotho* gene through hypermethylation. Treatment with DNMT inhibitors may alter the hypermethylated status of the *Klotho* gene and thus reverse the expression level. Although recent studies have indicated that the KLOTHO protein acts as a key modulator in the homeostasis of renal function, including mineral and vitamin D metabolism, this study provides us with new insight into the regulation of KLOTHO in the progressive decline of CKD via epigenetic modification. With increasing advances in our understanding of epigenetic mechanisms involved in CKD, more opportunities for preventive and therapeutic intervention will arise. Epigenetic targeting of specific genes may become an important therapeutic strategy to prevent the progression of uremia-related CKD.

DISCLOSURE

The authors declared no competing interests.

ACKNOWLEDGMENTS

This study was supported by the Ta-Tung Kidney Foundation and the Taiwan National Science Council (grants NSC 96-2314-B-002-164, NSC 96-2314-B-002-033-MY3, NSC 97-2314-B-002-155-MY2, NSC 98-2314-B-002-155-MY4, NTUH.098-001177, and NTUH 100-001667.

REFERENCES

1. Safronova O, Morita I. Transcriptome remodeling in hypoxic inflammation. *J Dent Res* 2010; **89**: 430–444.
2. Niwa T. Uremic toxicity of indoxyl sulfate. *Nagoya J Med Sci* 2010; **72**: 1–11.
3. Stenvinkel P, Karimi M, Johansson S *et al.* Impact of inflammation on epigenetic DNA methylation: a novel risk factor for cardiovascular disease? *J Intern Med* 2007; **261**: 488–499.
4. Dwivedi RS, Herman JG, McCaffrey TA *et al.* Beyond genetics: epigenetic code in chronic kidney disease. *Kidney Int* 2011; **79**: 23–32.
5. Hodge DR, Xiao W, Clausen PA *et al.* Interleukin-6 regulation of the human DNA methyltransferase (HDNMT) gene in human erythroleukemia cells. *J Biol Chem* 2001; **276**: 39508–39511.
6. Hofman-Bang J, Martuseviciene G, Santini MA *et al.* Increased parathyroid expression of klotho in uremic rats. *Kidney Int* 2010; **78**: 1119–1127.
7. Torres PU, Prie D, Molina-Bletry V *et al.* Klotho: an antiaging protein involved in mineral and vitamin D metabolism. *Kidney Int* 2007; **71**: 730–737.
8. Nagasu H, Satoh M, Kuwabara A *et al.* Overexpression of klotho protein modulates uninephrectomy-induced compensatory renal hypertrophy by suppressing IGF-I signals. *Biochem Biophys Res Commun* 2011; **407**: 39–43.
9. Sun C-Y, Chang S-C, Wu M-S. Suppression of Klotho expression by protein-bound uremic toxins is associated with increased DNA methyltransferase expression and DNA hypermethylation. *Kidney Int* 2012; **81**: 640–650.
10. Miyamoto Y, Watanabe H, Noguchi T *et al.* Organic anion transporters play an important role in the uptake of p-cresyl sulfate, a uremic toxin, in the kidney. *Nephrol Dial Transplant* 2011; **26**: 2498–2502.
11. Issa JP. Optimizing therapy with methylation inhibitors in myelodysplastic syndromes: dose, duration, and patient selection. *Nat Clin Pract Oncol* 2005; **2**(Suppl 1): S24–S29.