Epigenetics of kidney diseases.

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Abstract
Epigenetics has been recently recognized as an essential mechanism for pathogenesis in many diseases, including kidney diseases. Epigenetic modifications are inherited nuclear characteristics, or molecular changes that can affect gene expression without altering DNA sequences, including DNA methylation, histone modification, and non-coding RNAs. The kidney has an intricate structure and its origin is very complex despite sharing the same DNA. Indeed, epigenetic modulation of the kidney is diverse; furthermore, the extracellular environment after birth may affect various aspects of renal epigenetic modification. Epigenetic modifications in patients with acute kidney injury (AKI) or chronic kidney disease (CKD) are actively undergoing investigation. However, there have been few reports relating epigenetic changes to mineral homeostasis and CKD–mineral and bone disorder (CKD-MBD), particularly in the text of parathyroid diseases.
In this review, we describe epigenetic modification and subsequently discuss the roles and mechanisms of epigenetic modification in the pathogeneses of AKI, AKI-to-CKD transition, CKD, and CKD–MBD.

Key Words
Acute kidney injury, Chronic kidney disease, Chronic kidney disease–mineral and bone disorder, Epigenetics, Calcium-sensing receptor, Vitamin D receptor
1. Epigenetic modification

Epigenetics is a discipline that was advocated by Conrad Hal Waddington\(^1\)-\(^3\), which is now attracting great attention. Epigenetic modifications are inherited nuclear characteristics, or molecular changes that can affect gene expression without altering DNA sequences. Waddington emphasized that just one cell, a fertilized egg, differentiated into cell lineages via unidirectional differentiations\(^4\). The epigenome differs among cell types and integrates various information regarding the functional identity of each cell type during development or disease\(^5\). Thus, epigenetic modifications contribute to embryogenesis\(^6\), and cell differentiation; moreover, they affect the incidence of diseases, such as imprinting disorders\(^7\)-\(^10\), cancer, neurological disease\(^11\)-\(^14\), autoimmune disorders\(^15\),\(^16\), and various lifestyle-related diseases\(^17\),\(^18\).

The most common epigenetic modifications are DNA methylation, histone modification, and non-coding RNA-associated gene silencing (Figure 1). These modifications influence gene expression; thus, epigenetics promotes biological adaptation by permitting functional variability in response to environmental changes\(^19\)-\(^21\), such as diet, inflammation, metabolic changes and toxins\(^21\)-\(^24\). Notably, epigenetic changes may be reversible\(^25\),\(^26\). Accordingly, investigation of the epigenome contributes to the elucidation of mechanisms of numerous diseases as well as the development of innovative therapeutic targets, including kidney diseases.

![Figure 1](image_url)

**Figure 1:** Model for the regulation of epigenetic modifications. Schematic representation of euchromatin and heterochromatin as accessible or condensed nucleosome fibers that are acetylated (Ac), phosphorylated (ph), or methylated (me). Typical transcriptional control by histone modification is complex; for example, active histone markers include the methylation of H3K4, H3K36, H3K79, and H3R3; acetylation of H2AK15, H2BK12, H2BK15, H4K5, H4K8, H4K12, H4K16, H3K9, H3K14, and H3K18; ubiquitination of H2BK123; and phosphorylation of H3S10. Inactive histone markers include the methylation of H3K9, H3K27, and H3R8; ubiquitination of H2AK119; and phosphorylation of H2AS1. In addition, there are complicated histone modifications, such as dimethylated lysine and trimethylated lysine.
2. Epigenetics of AKI

AKI has received increasing attention due to the high incidence of adverse events, such as mortality rates and the risk of chronic kidney disease (CKD)\textsuperscript{27,28}. The involvement of epigenetics in AKI has recently attracted great interest\textsuperscript{29,30}, such that the number of reports regarding epigenetics of AKI has increased.

Regarding the role of DNA methylation in AKI, using a mouse ischemia-reperfusion renal injury (IRI) model, Huang reported that the global level of 5-hydroxymethylcytosine was reduced in the injured kidney; however, the 5-methylcytosine level did not significantly change. Furthermore, Huang showed decreased expression of \textit{Tet1} and \textit{Tet2} genes but not \textit{Tet3}\textsuperscript{31}. Guo showed that the DNA methylation inhibitor 5-aza-2'-deoxycytidine increased the number of apoptotic cells in a rat kidney proximal tubular cell line upon exposure to cisplatin\textsuperscript{32}. These reports indicated that DNA methylation plays an essential role in AKI.

Regarding the role of histone modification in AKI, chromatin immunoprecipitation with sequencing (ChIP-seq) enable the determination of the gene expression profile in target tissues. Nangaku reported that gene expression is intricately regulated by DNA methylation; histone modification; chromosome conformation changes; and ncRNA in various types of diseases, including kidney disease\textsuperscript{33}. Marumo demonstrated that reduction of histone deacetylases 5 (HDAC5) contributed to histone re-acetylation; furthermore, BMP7, a renoprotective factor, induced the onset of the recovery phase in the mouse IRI model\textsuperscript{34}. Ruiz-Andres demonstrated that histone lysine crotonylation increased during AKI; this increased crotonylation may positively impact the onset of AKI owing to the altered gene expression of peroxisome-proliferator-activated receptor gamma coactivator-1\textalpha (PGC-1\textalpha) and sirtuin-3\textsuperscript{35}. Naturally, the investigation of the modulators of histone modification as a therapeutic target has made progress. Obstruction (blocking) class I HDAC activity and inhibition of HDAC6, a class IIb HDAC, are protective against AKI\textsuperscript{36-38}. Therefore, histone modification appears to have an important role in AKI.

In regards to the role of ncRNA in AKI, many reports have shown relationships between AKI and each of the following: miRNAs, lncRNAs, and circular RNAs (cirRNAs)\textsuperscript{39}. Using various mouse models (IRI, cisplatin-induced AKI, contrast-induced AKI, and miRNA knockout) and in human studies using blood samples and urine samples of AKI patients with AKI, many miRNAs such as miR-10a, miR-21, miR-24, and miR-217, have been identified related to AKI\textsuperscript{40-45}. Few studies have addressed the roles of lncRNAs in AKI\textsuperscript{46-48}. Based on these results, ncRNAs have the
potential to serve as diagnostic and therapeutic tools.

3. Epigenetics of AKI-to-CKD transition
Previously AKI was considered a transient event for patients who recovered from the acute phase of AKI and regained complete kidney function on a long-term basis. However, recent epidemiological studies and meta-analyses have shown that apparent recovery from AKI can lead to CKD, including end-stage kidney disease. If aberrant repair mechanisms and/or injury-induced stimuli persist, AKI can progress to CKD, characterized by fibrosis and inadequate organ remodeling. Histological features include glomerulosclerosis, vascular sclerosis and tubulointerstitial fibrosis regardless of etiology; this fibrosis exacerbates the aberrant renal function through capillary rarefaction and tissue hypoxia. Nangaku demonstrated the importance of hypoxia associated with the mechanism of AKI-to-CKD transition. Hypoxia-inducible factor (HIF), a master regulator of adaptive responses against hypoxia, is important in the transcriptional regulation of target genes (e.g., erythropoietin, vascular endothelial growth factor, glucose transporters, and CCAAT/enhancer-binding protein δ). Furthermore, Nangaku showed epigenetic changes in AKI-to-CKD transition, namely hypoxic memory; these include decreased dimethylation of lysine 9 (H3K9me2), caused by increased KDM3A; increased H3K27me3, caused by decreased KDM6B; and increased IncRNA DARS-AS1. Zager has also showed that posts ischemic inflammation, ongoing lipid accumulation (lipotoxicity), and increasing histone acetylation at proinflammatory/profibrotic genes may sustain the injured state; based on those results, he concluded that AKI can trigger CKD. Notably, there have been several reports regarding the epigenetic regulation of fibrosis. In the kidney fibrosis model of unilateral ureteral obstruction (UUO), Hewitson showed that global kidney H3K9me3 and H3K9Ac were increased. In a similar mouse model, Zhou demonstrated that the expression of enhancer of zeste homolog 2 (EZH2)—a methyltransferase that induces H3K27me3—was increased and suggested that the inhibition of EZH2 could be a therapeutic target for the treatment of CKD. There have been a few reports of epigenetic silencing via miRs in kidney fibrosis. Some miRs have specific roles in fibrosis; miR-21 and -130 have profibrotic activity, whereas miR-29 has anti-fibrotic activity; notably, miR-192 has both profibrotic and anti-fibrotic activities. There is some evidence that DNA methyltransferase inhibitors may be beneficial in the treatment of renal fibrosis. TGF-β1 caused hypermethylation of RASAL1 through increased
Dnmt1 expression in fibrotic kidney, leading to Ras hyperactivity and proliferation. Hydralazine, a demethylating agent, led to RASAL1 promoter demethylation and renal fibrosis attenuation. These findings were confirmed in COL4A3-deficient Aloport mice as well as murine models of diabetic nephropathy, UUO, subtotal nephrectomy, and folic acid nephropathy. However, in the IRI model mouse, the hypermethylation of RASAL1 could not be confirmed. Accordingly, hypermethylation of RASAL1 in the kidney may be important in the process of AKI-to-CKD transition to irreversible fibrosis. Importantly, TGF-β1 has been shown to promote the differentiation of erythropoietin-secreting pericytes into myofibroblasts as well as to support erythropoietin hypermethylation in experimental kidney fibrosis; this change was prevented by low-dose 5-aza. Therefore, specific demethylation therapy may prevent AKI-to-CKD transition.

4. Epigenetics of CKD
Several groups have identified associations between CKD and epigenetic modulation. Ingrosso demonstrated global hypomethylation in peripheral mononuclear cells in a group of male hemodialysis patients with hyperhomocysteinemia compared with mononuclear cells from control subjects. However, with respect to different stages of CKD (stage 2–4), there were no significant correlations between global DNA methylation levels and homocysteinosis or arteriosclerosis. Epigenetic regulation occurs in stage-specific and tissue-specific manners; thus, these aspects should be considered when investigating epigenetic modifications. Smyth showed that 23 genes have differential methylation patterns in CKD patients compared to their patterns in controls. These genes included several strong biologic candidates for CKD, such as CUX1, ELMO1, FKBP5, INHBA-AS1, PTPRN2, and PRKAG2. These genes have been implicated in pathways involving kidney development, extracellular matrix accumulation, cell growth, cell differentiation, apoptosis, hypertension, and type 1 diabetes mellitus. These results suggest that the CKD environment causes the particular DNA methylation changes in specific genes related to its pathophysiology. In addition, diabetes mellitus and obesity, both strongly associated with CKD and risk factors for the onset of CKD, involve differential methylation patterns, therefore, further studies regarding causes of CKD are necessary to clearly understand DNA methylation in CKD.

Regarding the role of histone modification in CKD, Van Beneden showed that valproic acid, a class I HDAC inhibitor, abrogated the reduction in glomerular acetylation and halted glomerulosclerosis through podocyte detachment, epithelial to mesenchymal...
transition, apoptosis, and proliferation in mice with adriamycin-induced nephropathy. Furthermore, H3K4me in podocytes was found to have an important role in CKD; H3S10ph in proximal tubular epithelial cells prevented regeneration and proliferation in CKD; and histone H3.3 and histone cell cycle regulation defective homolog A (HIRA) were implicated in TGF-β1-induced renal fibrosis. These findings suggest that changes in histone modification may be a common mechanism in the process of CKD; thus, they may provide therapeutic targets for treatment of CKD.

5. Epigenetics of CKD–MBD

Epigenetics of CKD-MBD is more complex than AKI, AKI-to-CKD transition, and CKD because there are four intricate main axes that involve complex interactions, namely kidney, intestine, bone, and parathyroid gland. Furthermore, epigenetic modifications of these organs may differ depending on the origins of CKD. Epigenetic modifications in patients with AKI and CKD are actively undergoing investigation; however, few reports are available concerning epigenetic changes to mineral homeostasis and CKD–MBD.

The parathyroid gland plays a crucial role in mineral homeostasis, particularly when there is the loss of kidney function. However, it is difficult to elucidate the maintenance mechanisms and pathogenesis of parathyroid diseases because the parathyroid glands in animal models are too small and stable cell lines of parathyroid glands are not provided due to difficulties in operatively extracting the normal human parathyroid glands as control samples. Klotho, which is mainly expressed in parathyroid glands, kidney, and pituitary glands and considerably important for mineral homeostasis, functions as an essential subunit of the high-affinity receptor for fibroblast growth hormone 23 (FGF23). There have been a few reports regarding epigenetic changes of Klotho in CKD; however, Klotho is not a mineral regulator, but functions as a multifunctional protein through inhibition of multiple signaling pathways (TGF-β1, Wnt, and insulin/insulin-like growth factor-1). Klotho deficiency is associated with the progression of renal fibrosis as well as secondary hyperparathyroidism (SHPT), vascular calcification, and cardiac hypertrophy. Various reports have shown that CKD causes the epigenetic changes in Klotho expression, thereby inducing renal fibrosis. Sun reported that hypermethylation of Klotho in renal tubules induced uremic toxin associated with renal fibrosis; Irifuku then demonstrated that TGF-β1 induced G9a, an H3K9 methyltransferase with an important role in the progression of renal fibrosis, which reduced klotho expression through H3K9me. There is no
report of epigenetic modification in Klotho gene as a mineral regulator. However, several studies have reported the epigenetic changes in parathyroid diseases. Parathyroid cancers are rare malignancies and have shown a prevalence of 0.005% in all cancers. Approximately 90% of parathyroid cancers are hormonally hypersecreting parathyroid hormone (PTH). Somatic inactivating mutations of the HRPT2/CDC73 and MEN1 genes are identified in parathyroid cancers; moreover, recent reports showed the epigenetic change of parathyroid cancers. Parathyroid cancers showed hypermethylation of the promoter region of genes such as CDKN2B/p15, CDKN2A/p16, SFRPs, RASSF1, HIC1 and APC. The H3K27 methyltransferase EZH2 is overexpressed in parathyroid cancers; thus, global H3K27me3 status may be obtained. Furthermore, miRNA expressions, such as miR-517c and -372-3p, have been identified in parathyroid cancers.

Pseudohypoparathyroidism type 1B (PHP1B) is an imprinting disorder characterized by renal resistance to PTH, namely decreased or absent urinary phosphate excretion and cAMP responses to PTH injection. The pathogenesis of PHP1B is complex. One of the causes is DNA methylation changes involves the region of the GNAS gene, including the loss of methylation at the A/B, GANS-AS1, and XLαs differentially methylation regions (DMRs) and gain of methylation at the NESP55 DMR. Elli showed that epimutation in PHP1B patients occurred in early post-zygotic phases and that the partial GNAS imprinting defects were mosaics. Thus, as mentioned above, there are some epigenetic disorders in parathyroid glands; therefore, epigenetic modifications occur in secondary hyperparathyroidism (SHPT) caused by CKD. Calcium-sensing receptor (CaSR) and vitamin D receptor (VDR) expression levels in PTGs are characteristically decreased in the progression of SHPT. Although many regulatory factors are involved in mineral homeostasis, CaSR and VDR are regarded as major determinants of parathyroid function in CKD. However, the underlying mechanism remains largely unknown. Lewin previously reported no change in CaSR and VDR methylation in SHPT rats. However, Lewin used melting curve analysis and the severity of SHPT in those CKD rats was moderate; we investigated the methylation status of CaSR and VDR genes in the parathyroid glands using more severe SHPT rat model and quantitative analysis of DNA methylation using real-time PCR (qAMP). CKD rats fed a normal phosphate diet or high phosphate diet and in sham-operated rats fed a normal phosphate diet or high phosphate diet; the CKD model comprised two-step 5/6 nephrectomy rats. The results showed that although CaSR and VDR hypermethylation occurred in the
PTGs of CKD rats fed a high phosphate diet, the extent of hypermethylation was insufficient to support clear relationship between hypermethylation and down-regulation of gene expression because of the low percentage of methylation. Consequently, we concluded that mechanisms other than DNA hypermethylation were responsible for the reduction in mRNA and protein levels of CaSR and VDR in the PTGs of CKD rats fed a high phosphate diet\textsuperscript{86}. Further studies are needed to clarify the precise mechanism concerning CKD-MBD in PTGs as well as kidney, bone, and intestine.

**Conflict of interest** All the authors have declared no competing interest.
References


35. Ruiz-Andres O, Ortiz A, Sanz AB,


50. Nangaku M, Hirakawa Y, Tanaka T, Epigenetic changes in the acute kidney


63. Tikoo K, Ali Y, Gupta C, 5-azacitidine prevents cisplatin induced nephrotoxicity and potentiates anticancer activity of cisplatin by involving inhibition of metallothionein,


77. Irifuku T, Doi S, Masaki T, Inhibition of


