Nephrol Dial Transplant (2018) 33: 1875–1886
doi: 10.1093/ndt/gfy009
Advance Access publication 12 March 2018

ndt
Nephrology Dialysis Transplantation

Targeting epigenetic DNA and histone modifications to treat kidney disease

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ABSTRACT

Epigenetics refers to heritable changes in gene expression patterns not caused by an altered nucleotide sequence, and includes non-coding RNAs and covalent modifications of DNA and histones. This review focuses on functional evidence for the involvement of DNA and histone epigenetic modifications in the pathogenesis of kidney disease and the potential therapeutic implications. There is evidence of activation of epigenetic regulatory mechanisms in acute kidney injury (AKI), chronic kidney disease (CKD) and the AKI-to-CKD transition of diverse aetiologies, including ischaemia-reperfusion injury, nephrotoxicity, ureteral obstruction, diabetes, glomerulonephritis and polycystic kidney disease. A beneficial *in vivo* effect over preclinical kidney injury has been reported for drugs that decrease DNA methylation by either inhibiting DNA methylation (e.g. 5-azacytidine and decitabine) or activating DNA demethylation (e.g. hydralazine), decrease histone methylation by inhibiting histone methyltransferases, increase histone acetylation by inhibiting histone deacetylases (HDACs, e.g. valproic acid, vorinostat, entinostat), increase histone crotonylation (crotonate) or interfere with histone modification readers [e.g. inhibits of bromodomain and extra-terminal proteins (BET)]. Most preclinical studies addressed CKD or the AKI-to-CKD transition. Crotonate administration protected from nephrotoxic AKI, but evidence is conflicting on DNA methylation inhibitors for preclinical AKI. Several drugs targeting epigenetic regulators are in clinical development or use, most of them for malignancy. The BET inhibitor apabetalone is in Phase 3 trials for atherosclerosis, kidney function being a secondary endpoint, but nephrotoxicity was reported for DNA and HDAC inhibitors. While research into epigenetic modulators may provide novel therapies for kidney disease, caution should be exercised based on the clinical nephrotoxicity of some drugs.

Keywords: acetylation, crotonylation, epigenetics, kidney, methylation

INTRODUCTION

Acute kidney injury (AKI) is defined as a rapid loss of renal function, with mortality around 40% and no evidence of treatment that accelerates recovery [1]. The initial insult to injury, which includes cell death and inflammation, is followed by a recovery phase that may recapitulate kidney development and lead to functional and structural recovery or result in transition to chronic kidney disease (CKD) [2]. Epigenetic changes have been suggested to contribute to AKI, CKD and the AKI-to-CKD transition [3–7]. Epigenetics refers to heritable changes in gene expression patterns that are not caused by an alteration of the DNA nucleotide sequence itself [8]. This information is not only heritable and self-perpetuating, but also dynamic and reversible in response to the environment. When transcription factors are available, the epigenome determines the transcriptional outcome, allowing certain genes to be expressed while others are not accessible to transcription factors [6, 9]. Epigenetics encompasses non-coding RNAs and covalent modifications of DNA and histones. We now review the functional evidence for a role of histone and DNA modifications in the pathogenesis of kidney disease. In particular, we will focus on the therapeutic approaches that have been tested *in vivo*. Non-coding RNA, including miRNAs, have been recently reviewed and are beyond the scope of this review [10].

EPIGENETIC MODIFICATIONS: ENZYMES AND MODULATORS

DNA and histone modifications are key regulators of gene expression. Histones (H1 through H4) are small basic

proteins that wrap the DNA to form nucleosomes and guide transcription factor binding (Figure 1). More than 100 different types of modifications have been described, including methylation, acetylation, crotonylation, phosphorylation, sumoylation and ubiquitination [6]. In 2011, 67 previously undescribed histone modifications were identified in a single report, increasing the number of known histone marks by about 70% [11]. At least eight of these modifications are short-chain lysine acylations (propionylation, butyrylation, 2-hydroxyisobutyrylation, succinylation, malonylation, glutarylation, crotonylation and β-hydroxybutyrylation), which together with histone acetylation, are partially regulated by the metabolism and availability of their respective acyl-coenzyme A (CoA) [12]. However, the functional significance of most of these modifications remains unknown. In the context of kidney diseases, the bulk of available information relates to DNA methylation and to histone methylation, acetylation and crotonylation. Protein writers, erasers and readers for these modifications are recognized.

DNA methylation

Methylation of DNA promoter regions is a general silencing mechanism that blocks transcription factor binding by recruiting co-repressors or by packaging chromatin [8]. DNA methyltransferases (DNMT) promote methylation at the 5-cytosine of CpG dinucleotides (i.e. a cytosine C followed by a guanine G in the DNA sequence), which are found most commonly at CpG islands in the first exons or near gene promoters [13]. The most abundant is Dnmt1, which maintains established CpG methylation patterns through mitosis, while Dnmt3a and

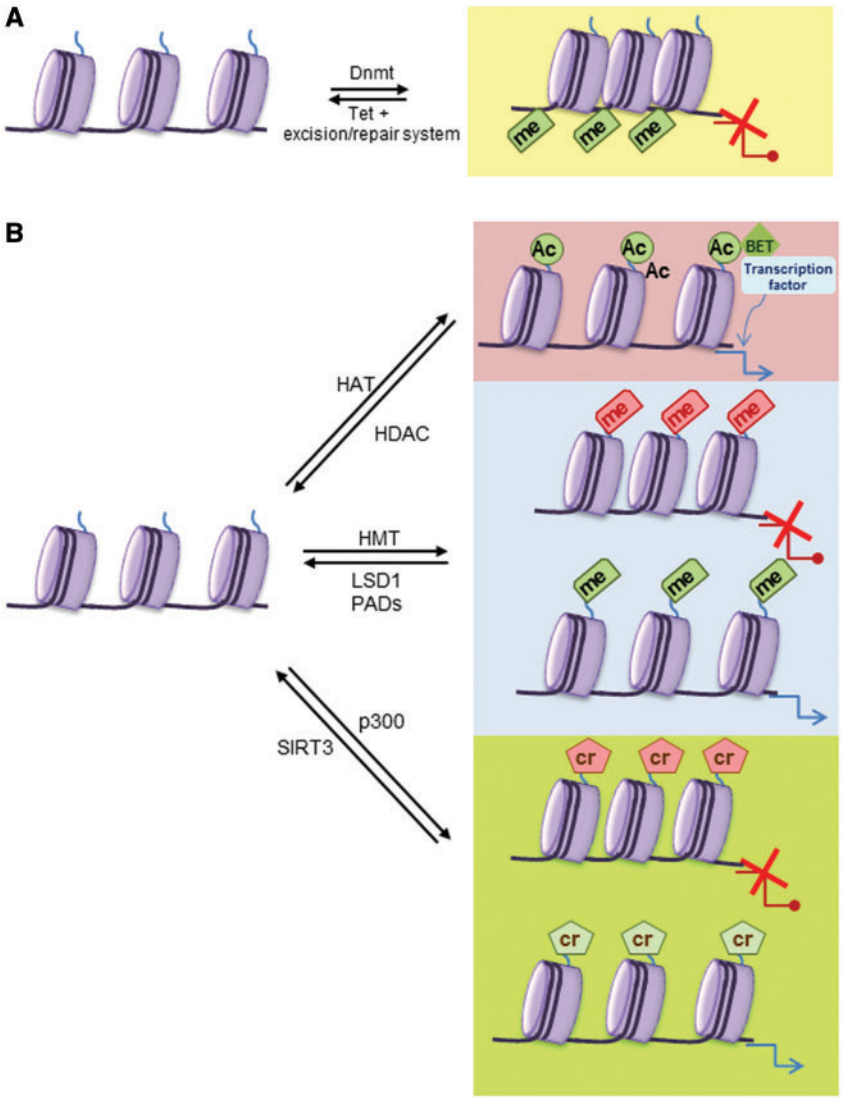

FIGURE 1: Enzymatic regulation of epigenetic modifications. **(A)** DNA methylation (me) is mediated by DNA-methyl transferases (DNMT) and represses gene expression. DNA demethylation is a passive process and it may be accelerated by Ten-eleven-translocation (TET) methylcytosine dioxygenases. **(B)** Histone modifications: acetylation (Ac), methylation and crotonylation (cr). Acetylation relaxes the chromatin and activates gene expression. BET are histone acetylation readers that bind to acetylated histones and favour assembly of transcriptional regulator complexes. Lysine or arginine methylation may repress or activate gene expression depending on the context. It is as yet unclear whether crotonylation activates and/or represses transcription.

Dnmt3b can methylate cytosines *de novo*, that is, at sites not previously methylated. 5-cytosine methylation (5mC) may be reversed passively or by the action of Ten-eleven-translocation (TET) methylcytosine dioxygenases Tet1, Tet2 and Tet3 that generate sequential DNA methylation cytosine changes, such as 5-hydroxymethyl cytosine (5hmC), culminating in excision by base excision repair glycosylases and replacement by non-methylated cytosines [14].

Histone modifications

More than 60 different histone residues may be post-translationally modified [15]. Different combinations of histone modifications regulate chromatin structure and transcriptional status [16, 17]. Methylation and acetylation of lysine residues on the H3 histone are the best characterized modifications. Modifications are identified by the specific lysine and histone modified, e.g. lysine 4 of histone H3 is H3K4.

Histone methylation. Histone methylation involves the transfer of methyl groups (CH_3) from S-adenosyl-L-methionine to histone lysine or arginine residues by histone methyltransferases (HMTs), such as protein arginine methyltransferases (PRMTs) and lysine methyltransferases (KMTs), including the Su(var)3-9, Enhancer of Zeste and Trithorax (SET) domain KMTs. Transferring up to three methyl groups results in mono-, di- or trimethylated lysine (e.g. H3K4me2 and H3K4me3) or in mono- or di- (asymmetric or symmetric) methylated arginine. Methyl groups are removed by histone demethylases such as amine oxidase lysine-specific demethylase (LSD1, KDM1A) [18]. Arginine and mono-methylated arginine can be converted to citrulline by protein-arginine deiminases (PADs or PADIs) [19]. Methylation generates a platform for transcription factor binding that may favour or suppress gene expression, depending on the context, lysine residue and extent of methylation [20].

Histone acetylation. Histone acetylation involves histone acetyltranferases (HATs) transferring an acetyl group ($COCH_3$) from acetyl-coenzyme A (acetyl-CoA) to lysine residues. Families of HATs include Gcn5-related N-acetyltransferases (GNATs: GCN5, PCAF), MYST (MOZ, Ybf2/Sas3), Sas2 and Tip60, coactivator p300/CREB-binding protein (CBP), nuclear receptor coactivators (e.g. ACTR/SRC-1) and other HATs (TAFII250, TFIIIC, Rtt109, CLOCK). Four classes of histone deacetylases (HDAC) remove acetyl groups. Class I HDAC are expressed ubiquitously in nuclei and regulate cell survival and proliferation, whereas Class II HDACs may have tissue-specific roles and are localized in nuclei and cytosol [21]. Class III HDACs are the sirtuins, and there is only one Class IV HDAC, HDAC11. Histone acetylation may also be regulated metabolically by the concentration of acetyl-CoA. Histone acetylation relaxes the chromatin, facilitating transcription factor recruitment and transcription [22].

The bromodomain is a highly conserved motif of 110 amino acids with protein interaction functions that recognizes and binds to acetylated lysines. Bromodomain proteins functioning as epigenetic readers of histone acetylation are involved in chromatin remodelling and transcriptional regulation by facilitating acetylation-dependent assembly of transcriptional regulator complexes [23, 24]. The human proteome comprises 61 bromodomains and 46 bromodomain-containing proteins, including transcription factors, histone acetylases and nucleosome remodelling complexes. Proteins containing two bromodomains and an extra-terminal domain belong to bromodomain and extra-terminal (BET) family, including BRD2, BRD3, BRD4 and BRDT [25].

Histone crotonylation. Histone crotonylation involves histone crotonylases transferring a crotonyl group [$CH_3CH{=}CHCO_2$, (E)-2-butenoyl] from crotonyl-coenzyme A (crotonyl-CoA) to lysine residues. Lysine crotonylation (Kcr) is a recently described evolutionarily conserved histone post-translational modification present in somatic tissues, including the kidney [11]. A potential role has been suggested during spermatogenesis and more recently in AKI [11, 26]. Histone crotonylation shares enzyme regulators with histone acetylation. Thus, coactivator p300 has histone crotonylase activity and Sirt3 has decrotonylase activity [27, 28]. However, the genomic pattern of histone crotonylation differs from histone acetylation [11]. As is the case for acetyl-CoA and histone acetylation, the concentration of crotonate and crotonyl-CoA can also regulate histone crotonylation [11, 26]. Histone crotonylation may activate or repress transcription in a gene- and/or environment-dependent manner [26, 27]. Thus, p300-catalyzed histone crotonylation directly stimulates transcription to a greater degree than histone acetylation and increasing or decreasing the cellular concentration of crotonyl-CoA led to enhanced or diminished gene expression, respectively, which correlated with the levels of histone crotonylation [27]. *De novo* crotonylation following bacterial lipopolysaccharide (LPS) administration was reported to activate the expression of inflammatory genes [27]. By contrast, in cultured kidney cells, crotonate availability was associated with increased or decreased gene expression, suggesting that the effect of crotonylation on gene expression could be gene- and environment-dependent or that at least, increased crotonylation may promote gene expression changes that result in repression of the expression of certain genes [26].

CHANGES IN OVERALL PATTERN OF DNA OR HISTONE MODIFICATIONS DURING KIDNEY DISEASE

The kidney is a complex tissue that contains multiple different cell types, despite sharing the same DNA, as a result of differential epigenetic modulation that determines the characteristics of each cell type. Furthermore, the environment may drive additional epigenetic modifications for specific genes. Both global changes and gene-specific changes in epigenetic modifications have been observed in kidney disease [29, 30] (Table 1). AKI results in an acute and usually transient decrease in renal function, and several cellular mechanisms, including cell death, inflammation and fibrosis, are associated with AKI. Epigenetic modifications may have a role in AKI, indeed, expression of pro-inflammatory and pro-fibrotic genes are regulated by histone and DNA modifications.

Table 1. Examples of changes in the overall pattern of DNA methylation or histone modifications during kidney disease

		Change in kidney injury	Model[a]	Sample	Ref.
(A) DNA methylation					
5hmC		Decreased at 24 h	Mouse IRI AKI	Kidney	[31]
5mC		Unchanged at 24 h	Mouse IRI AKI	Kidney	[31]
5hmC+5mC		Decreased at 24 h and 7 days	Mouse IRI AKI	Kidney	[32]
Microarray methylation assay		Decreased in 70% of differentially methylated regions	CKD patients	Human kidney tubules	[30]
Luminometric methylation assay		Unchanged in non-inflamed CKD G2–G5, decreased in inflamed G5	CKD patients	PBMCs	[33]
(B) Kidney histone modifications					
Methylation	H3K9me3	Increased	UUO (renal fibrosis)	Kidney	[34]
	H3K27me3	Increased	UUO (renal fibrosis) CKD (humans)	Kidney	[35]
	H3K4me2	Increased	Diabetic nephropathy in uninephrectomy db/db mice	Kidney	[36]
	H3K4me2	Decreased	db/db mice	Kidney	[36]
	H3K4me2	Decreased	Uninephrectomy C57BL/6 mice	Kidney	[36]
Acetylation	H3K9Ac	Transient (<24 h) decrease	AKI (IRI)	Kidney	[37]
	H3Ac	Increased progressively up to 3 weeks	AKI (IRI)	Kidney	[38]
	H3K9Ac	Increased	UUO (renal fibrosis)	Kidney	[34]
	H3K9Ac/H3K23Ac	Increased	Diabetic nephropathy in uninephrectomy db/db mice	Kidney	[36]
	H3K23Ac	Decreased	db/db mice	Kidney	[36]
	H3K23Ac	Decreased	Uninephrectomy C57BL/6 mice	Kidney	[36]
Crotonylation	Lysine crotonylation (Kcr)	Increased	AKI (folic acid, cisplatin)	Kidney	[26]

PBMCs, peripheral blood mononuclear cells.
[a]Mouse except if otherwise specified.
H3K9, histone 3 lysine 9; H3K23, histone 3 lysine 23; H3K4me3, histone 3 lysine 4 trimethylation; H3K27me3, histone 3 lysine 27 trimethylation; H3K9Ac, histone 3 lysine 9 acetylation; Kcr, histone crotonylation.

DNA methylation

Abnormal DNA methylation has been observed at the whole-genome level and in specific genes during AKI and CKD both in the kidney and in peripheral blood leucocytes.

In mouse ischaemia-reperfusion renal injury (IRI), global kidney cytosine hydroxymethylation (5hmC) was reduced while cytosine methylation (5mC) was unchanged, and this was associated with downregulation of *Tet1* and *Tet2*, but not of *Tet3* gene expression [31]. Decreased 5hmC enrichment was observed at promoter regions of the pro-inflammatory genes *Cxcl10* and *Ifngr2*, which was associated with their increased expression [31]. Decreased genome-wide methylation and CpG methylation persisted for up to 7 days and was associated with downregulation of gene expression for 18 methylated genes, suggesting that promoter methylation contributes to persistent alteration of gene expression [32]. In tubules from human CKD kidneys, most of the differentially methylated regions reflected decreased methylation in CKD [30]. Differentially methylated regions mostly overlapped with putative enhancer regions enriched in consensus binding sequences for transcription factors. Regions that regulate the expression of genes related to kidney fibrosis, such as those in the TGFβ pathway, showed cytosine methylation changes that correlated with transcript levels [30]. In cisplatin-induced AKI mice, 215 differentially methylated DNA regions were found, including the promoter or promoter-regulatory regions of 15 protein-coding genes [39].

Abnormal DNA methylation patterns have also been reported in peripheral blood leucocytes from CKD patients. Global DNA hypermethylation was observed in peripheral blood leucocytes from inflamed CKD grade 5 (G5) patients, but not from non-inflamed G3–G5 patients [33]. In this regard, uraemia induced dysregulation of DNA methylation in cultured differentiating monocytes [40].

Aberrant methylation of specific genes has been observed in kidney, urine or blood of kidney disease patients or animals [41–48] (Supplementary data, Table S1). In rat IRI, the promoter of the *C3* gene was strongly demethylated [43]. In addition, increased expression of Dnmt1, Dnmt3a and Dnmt3b, and aberrant methylation or demethylation of genes involved in progression of CKD, such as the anti-aging gene *Klotho*, *erythropoietin*, *podocyte nephrin* and *fibrosis-related RASAL1*, has been observed in experimental kidney disease [44–48]. In kidney tissue and peripheral blood leucocytes from CKD patients, Klotho expression negatively correlated with methylation of its promoter [49].

Histone methylation

The overall pattern of histone methylation has not been analysed during kidney injury, although there is information on specific markers. In kidney fibrosis, 10 days after unilateral ureteral obstruction (UUO), global kidney H3K9 trimethylation (H3K9me3) was increased [34]. Expression of enhancer of zeste homologue 2 (EZH2), a methyltransferase that induces histone

H3 lysine 27 trimethylation (H3K27me3), as well as H3K27me3 itself are increased in fibrotic kidneys from mice with UUO and humans with CKD [35]. In experimental diabetic nephropathy, histone methylation was associated with progressive glomerulosclerosis and it was reverted with an anti-CCL2 antibody, suggesting a role for CCL2 or inflammation in epigenetic regulation [36].

In addition, there has been a flurry of reports on altered histone methylation at specific genes. As examples, increased H3K4me3 histone methylation at inflammatory (*TNFα*, *CCL2*), pro-fibrotic (*TGFβ1*, *type III collagen*) and cholesterol regulatory genes (*HMGRC*) was associated with increased gene expression in LPS-AKI and/or IRI [50–53].

Histone acetylation

Histone acetylation has been extensively studied in AKI and renal fibrosis. In murine IRI, a global reduction of histone acetylation during ischaemia had recovered after 24 h of reperfusion. This was mediated, at least in part, by decreased HAT activity during ischaemia and by HDAC downregulation during the recovery phase [37]. However, the degree of H3 histone acetylation may progressively increase over baseline concomitantly with the expression of inflammatory and pro-fibrotic genes, coinciding with the AKI-to-CKD transition [38]. Ten days after UUO, global kidney H3K9 acetylation (H3K9Ac) was increased [34]. By contrast, the nephroprotective genes *Klotho* and *PGC1a* are downregulated in AKI and HDAC inhibitors prevented their downregulation in cultured tubular cells exposed to inflammatory cytokines [54, 55]. In this regard, the modification of histone acetylation during AKI is heterogeneous and it could be injury-, time- and gene-specific [56].

In experimental diabetic nephropathy, histone acetylation was associated with progressive glomerulosclerosis and it was reverted with an anti-CCL2 antibody, suggesting again a role for inflammation in epigenetic regulation [36].

In some cases, a similar pattern is observed for histone acetylation and methylation, being difficult to discern the specific contribution of each histone modification to gene expression differences [36, 52].

Histone crotonylation

Global kidney histone crotonylation was increased during experimental nephrotoxic AKI [26]. Histone crotonylation localized to tubular cell nuclei in human and murine AKI. A driver of histone crotonylation during AKI may be inflammation, since the inflammatory cytokine TWEAK, which is causally involved in kidney injury [57], increased histone crotonylation in cultured tubular cells [26].

PRECLINICAL THERAPEUTIC TARGETING OF DNA AND HISTONE MODIFICATIONS

There is functional evidence supporting the contribution of epigenetic changes to kidney injury, obtained from preclinical animal models through the use of inhibitors or promoters of DNA and histone modifications (Table 2) (Figure 2).

DNA methylation

Inhibitors of DNMTs, such as 5-azacytidine (5-aza) and 5-aza-2′-deoxycytidine (5-aza-2de, decitabine) induce DNA hypomethylation [86, 87]. There is preclinical evidence that DNMT inhibitors may be beneficial in renal diseases by restoring the expression of downregulated genes responsible for CKD manifestations, such as *Klotho*, *RASAL1* and *erythropoietin* [44, 45, 48], thus preventing the AKI-to-CKD transition [39, 43, 58].

Low-dose 5-aza prevented TGF-β1-driven differentiation of erythropoietin-secreting pericytes into myofibroblasts and *erythropoietin* gene hypermethylation, increasing erythropoietin and Klotho production, improving anaemia and protecting from kidney dysfunction in experimental kidney fibrosis [47, 48]. TGF-β1 also induced delayed (5 days) hypermethylation of *RASAL1* through an increased expression of Dnmt1 in kidney fibroblasts, leading to decreased *RASAL1* expression, Ras hyperactivity and proliferation. *RASAL1* hypermethylation was also observed in experimental kidney fibrosis and Dnmt1$^{+/-}$ mice or mice treated with decitabine were protected from renal fibrosis [44].

Hydralazine exhibits demethylating activity, probably by increasing TET3 expression. In preclinical models, hydralazine led to *RASAL1* promoter demethylation, attenuated renal fibrosis and preserved renal function independently from its blood pressure-lowering effects [59].

There are controversial data regarding the role of DNA methylation in AKI. Decitabine prevented cisplatin-induced nephrotoxicity in rats while potentiating the anticancer activity [58]. By contrast, decitabine increased cisplatin-induced apoptosis in cultured proximal tubular cells and kidney proximal tubule-specific DNMT1 knockout mice had more severe cisplatin-induced AKI [39].

Histone methylation

Diverse HMTs have been successfully targeted to prevent renal fibrosis and cyst growth. Kidney fibrosis induced by UUO is associated with increased H3K4 methyltransferase SET7/9 and H3K9 methyltransferase G9a activity [60, 61]. Genetic or chemical (SET7/9 inhibitor sinefungin or G9a inhibitor BIX01294I) inhibition of these methyltransferases resulted in decreased fibrosis and decreased levels of H3K4 (H3K4me1) or H3K9 (H3K9me1) monomethylation, respectively, in kidneys from UUO mice [60, 61]. Additionally, G9a targeting increased Klotho [61]. EZH2 catalyzes the formation of H3K9me and H3K27me. Its inhibitor 3-deazaneplanocin A (3-DZNeP) decreased fibrosis in the UUO model and decreased signalling from several receptors [TGFβ receptor 1, epidermal growth factor receptor (EGFR) and platelet-derived growth factor β receptor (PDGFβR)] and increased phosphatase and tensin homologue (PTEN); events thought to contribute to the therapeutic effect [35].

In preclinical and clinical polycystic kidney disease, the expression of the lysine methyltransferase SMYD2 was upregulated and SMYD2 genetic targeting or its inhibitor AZ505 delayed cyst growth in mice. Inhibition of SMYD2 decreased the mono-, di- and trimethylation of H3K4 and H3K36, but

Table 2. Examples of tools to modulate epigenetic modifications during experimental kidney disease. Focus on DNA and histone modifications and epigenetic readers

Tool	Target	Preclinical kidney disease		Ref.
		Model	Effect	
DNA methylation				
Dnmt1$^{+/-}$ mice	DNMT1	Toxic (folic acid)	↓ Fibrosis	[44]
Proximal tubule Dnmt1$^{+/-}$ mice	DNMT1	Toxic (cisplatin)	↑ AKI severity	[39]
5′-azacytidine[a]	DNMT inhibitor	Toxic (cisplatin, adenine, folic acid)	↓ Nephrotoxicity ↑ Klotho, ↑ EPO, ↓ anaemia	[48, 58] [47]
		UUO	↓ Fibrosis	
Decitabine[a]	DNMT inhibitor	UUO	↓ Fibrosis	[44]
Hydralazine[a]	Demethylating activity: induction of TET3	IRI	↓ Fibrosis ↑ Renal function	[59]
Histone methylation				
Sinefungin	HMT SET7/9 inhibitor	UUO	↓ Fibrosis	[60]
BIX01294	HMT G9a inhibitor	UUO	↓ Fibrosis ↑ Klotho	[61]
3-DZNeP	HMT EZH2 inhibitor	UUO	↓ Fibrosis	[35]
AZ505	HMT SMYD2 inhibitor	Polycystic kidney disease	↓ Cyst growth	[62]
Histone acetylation				
TSA	Class I and II HDAC inhibitor	Toxic (cisplatin)	↓ Renal injury	[63]
		IRI and transplant	↓ Fibrosis	[64]
		UUO	↓ Fibrosis	[65]
		Immune (lupus nephritis, NTN)	↑ Renal function	[66, 68]
		DN	↓ Fibrosis	[67]
		PKD	↓ Cyst growth	[69]
Vorinostat[a]	Class I and II HDAC inhibitor	DN	↑ Renal function ↓ Oxidative stress, ↓ renal hypertrophy	[70, 71]
FR276457	Class I and II HDAC inhibitor	UUO	↓ Fibrosis ↓ Inflammation	[72]
HDAC1$^{-/-}$ mice	HDAC1	PKD	↓ Cyst growth	[69]
Valproic acid	Class I HDAC inhibitor	DN	↓ Renal injury, ↓ proteinuria	[73, 74]
		IRI	↓ Inflammation, ↓ fibrosis	[75, 76]
		Podocyte toxic (adriamycin)	↑ Renal function	[68, 77]
		PKD	↓ Cyst growth	[69]
Entinostat[a]	Class I HDAC inhibitor	IRI	↑ Renal function	[64, 78]
		UUO	↓ Fibrosis	
		Toxic (folic acid) and Rhabdomyolysis	↑ Renal injury ↓ Regeneration	[79]
M4-PTB	Class I HDAC inhibitor	Toxic (aristolochic acid)	↑ Proliferation	[80, 81]
		IRI	↓ Inflammation, ↓ fibrosis	
Histone crotonylation				
Crotonate	↑ Histone crotonylation	Toxic (folic acid AKI)	↓ Inflammation ↑ Renal function	[26]
Histone modification readers				
JQ1	BET inhibitor	PKD	↓ Inflammation	[82]
		UUO	↑ Renal function	[83]
		NTN	↓ Cyst growth	
		Angiotensin II infusion		
MS417	BET inhibitor	DN	↓ Renal injury, proteinuria	[84]

[a]Drugs under clinical development or clinically available (see Table 3). HDAC inhibitor specificity according to [85].
DN, diabetic nephropathy; NTN, nephrotoxic serum nephritis (anti-glomerular basement membrane); PKD, polycystic kidney disease; Dnmt1, DNA methyl transferase; 3-DZNeP, 3-deazaneplanocin A; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition.

SMYD2 methylated a wide range of proteins, ranging from transcription factors (STAT3, NF-κB, p53) to histones [62].

Histone acetylation

The therapeutic effect of histone acetylation modulation appears to depend on the specific molecule and its tested dosage, the timing of administration and the cause of kidney injury. In this regard, Class I and II HDAC inhibitors were protective in experimental kidney injury, while mixed results were reported for Class I HDAC inhibitors [88].

Class I and II HDAC inhibitors. Trichostatin A (TSA) increased the renal expression of protective genes such as *Klotho*, *PGC-1α* and *Bmp7* in cultured cells and in cisplatin-

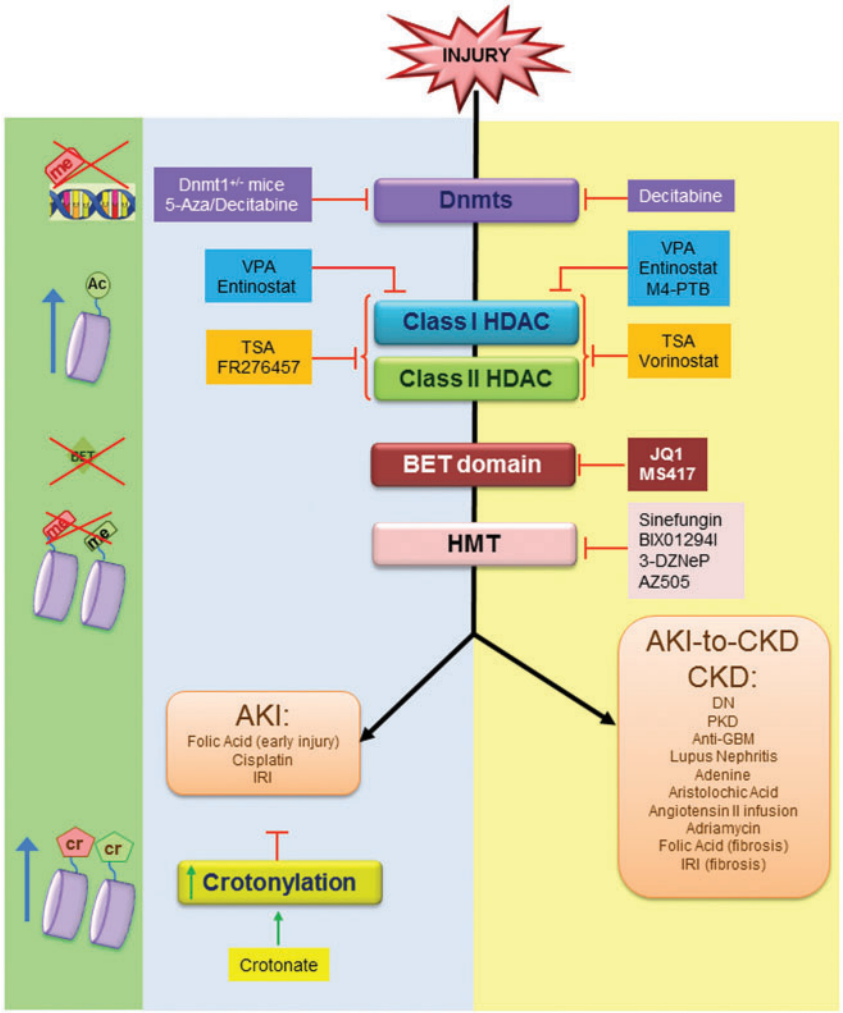

FIGURE 2: Summary of therapeutic intervention on epigenetic modifications with evidence of benefit in preclinical renal injury models. Various strategies targeting epigenetic modifications have been found to attenuate renal injury in different experimental models of AKI and CKD. The figure presents a list of compounds that have been shown to be beneficial in at least one of the preclinical models shown in the figure. In addition, impaired kidney outcomes have been reported for entinostat and folic acid or rhabdomyolysis-induced AKI (not shown in the figure). me, methylation; Ac, acetylation; cr, crotonylation; VPA, valproic acid; DN, diabetic nephropathy; PKD, polycystic kidney disease; GBM, glomerular basement membrane.

induced nephrotoxicity, and of miRNA-21 in experimental IRI [54, 55, 63, 64]. TSA improved renal function and reduced fibrosis in IRI and cold renal ischaemia transplantation, reduced fibrosis following UUO and improved renal function in cisplatin nephrotoxicity [63–65, 89]. Furthermore, TSA also protected from glomerular injury. It decreased extracellular matrix accumulation and epithelial–mesenchymal transition in diabetic nephropathy [67], progression of renal injury and fibrosis in nephrotoxic serum nephritis and inflammation and proteinuria in experimental MRL$^{lpr/lpr}$ lupus nephritis [66, 68]. FR276457 also reduced interstitial fibrosis following UUO [72].

Vorinostat (suberanilohydroxamic acid, SAHA) decreased albuminuria and fibrosis in diabetic mice, although the effect was not observed in endothelial nitric oxide synthase (eNOS)-deficient mice, suggesting that eNOS could be a key HDAC target in diabetic nephropathy [70, 90]. Vorinostat also reduced the expression of inflammatory cytokines in splenocytes from MRL$^{lpr/lpr}$ mice, but the effect on kidney injury was not tested [66].

Class I HDAC inhibitors. Valproic acid is in clinical use to treat epilepsy and it is also a HDAC inhibitor. Valproic acid prevented the decrease in histone acetylation and reduced renal injury and the expression of pro-fibrotic genes in rats with streptozotocin-induced diabetes and prevented proteinuria and the onset of glomerulosclerosis in adriamycin nephropathy [73, 74, 77]. Furthermore, valproic acid improved renal function and reduced renal injury, cell death and inflammation in experimental IRI and prevented TWEAK-induced downregulation of Klotho expression in tubular cells [55, 75, 76]. Genetic HDAC1 deficiency, TSA and valproic acid decreased kidney cyst growth in Pkd2-deficient mice [69].

Short-term administration of the Class I HDAC inhibitor entinostat (MS-275, SDNX-275) before the procedure (16 h and just prior to the procedure) protected from IRI, decreasing

Table 3. Clinical experience with epigenetic modulators. Focus on DNA and histone modifications and epigenetic readers.

Target	Drug	Clinical development stage	Indications	Data on preclinical kidney disease[a]	Clinical kidney disease
DNA methylation					
DNMT inhibitor	5′-Azacytidine	In clinical use	MDS	Yes	AE: increased sCr
	Decitabine	In clinical use	AML	Yes	AE: uncommon increased sCr
TET3 demethylase activator	Hydralazine	In clinical use	Hypertension	Yes	Slows progression of CKD as part of anti-hypertensive regimens
Histone methylation or demethylation inhibitors					
HMT KMT4 DOT1L (H3K79)	Pinometostat (EPZ5676)	Phase I	Leukaemia	No	ND
HMT KMT6 EZH2 (H3K27)	Tazemetostat	Phase II	Malignancy	No	ND
Demethylase KDM1A LSD1 H3K4	Tranylcypromine and IMG-7289	Phase II	AML	No	ND
Histone acetylation inhibitor					
Class I+II+IV HDAC	Panobinostat	In clinical use	Myeloma	No	AE: increased sCr
Class I+II HDAC	Vorinostat	In clinical use	Cutaneous T cell lymphoma	Yes	AE: increased sCr
Class I HDAC	Valproic acid	In clinical use	Epilepsy	Yes	AE: occasional Fanconi syndrome
	Entinostat	Phase III	Breast cancer	Yes	ND
Epigenetic reader blocker					
BRD2-4 and BRDT	Apabetalone	Phase III	Atherosclerosis	No	Potentially beneficial in *post hoc* analysis. Secondary endpoint in ongoing phase III RCT
Histone crotonylation					
Crotonate	ND	ND	ND	Yes	ND

HDAC inhibitor specificity according to [85].
[a]For details see Table 2.
AE, adverse effects; AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome, RCT, randomized clinical trial; ND, no data; BRD, bromodomain; HDAC, histone deacetylase; DOT1L, isruptor of telomeric silencing 1-like; EZH2, enhancer of zeste homologue 2; LSD1, lysine demethylase 1A; DNMT, DNA-methyl transferases; TET3, ten-eleven-translocation; sCr, serum creatinine.

BUN for up to 96 h and residual fibrosis at 30 days, although to a lesser extent than TSA [64]. By contrast, mice deficient of HDAC6, a Class II HDAC, were not protected [64]. However, entinostat increased renal injury in AKI induced by folic acid or rhabdomyolysis, as indicated by worsening renal dysfunction, increased neutrophil gelatinase-associated lipocalin expression and apoptosis [79]. The explanation for the discrepancy between protection in IRI and increased injury in nephrotoxic AKI is unclear. Genuine differences may exist between the role of Class I HDAC inhibitors for different causes of AKI. Despite the use of a >3-fold higher entinostat dose in nephrotoxic AKI studies, a direct toxic effect of higher doses is unlikely, since no adverse effects were observed in sham control mice. However, the degree of HDAC inhibition may be critical for the outcome. Administration of entinostat after induction of injury in the nephrotoxicity model may have limited any putative beneficial effect over the very early stages of injury. Finally, continuous administration of entinostat in the nephrotoxicity studies until sacrifice at 48 h may have interfered with kidney regeneration, as supported by lower EGFR expression and phosphorylation, lower PAX2 expression and lower Proliferating cell nuclear antigen (PCNA)-positive cell numbers [79, 91].

Entinostat and M4PTB prevented renal fibrosis, induced by ureteral obstruction or aristolochic acid, respectively [78, 80]. In UUO, entinostat was also initiated after induction of injury and administered at high dose until sacrifice, but on every other day [78], as opposed to daily in nephrotoxicity studies [79]. In the UUO model, decreased EGFR activation was thought to contribute to the benefit observed. These studies illustrate the potential for benefit and harm from therapeutic epigenetic modulation, the potential influence of aetiology and the importance of precise dosing and timing of therapeutic intervention.

Class IV HDACs. HDAC11 is highly expressed in the kidney and together with HDAC9 is downregulated in IRI in an androgen-dependent manner [92]. Based on cell culture experiments, it has been proposed to contribute to male gender sensitivity to experimental IRI [92], but there are no functional studies *in vivo* targeting HDAC11 in kidney injury.

HATs. Inhibition of HATs has been barely explored in kidney injury. Curcumin inhibits the HAT p300/CBP and was protective in experimental cisplatin nephrotoxicity, reducing inflammation and oxidative stress [7]. However, curcumin has a variety of other targets, including HDACs, and whether a beneficial effect depends on HAT inhibition is questionable [93, 94]. Silencing the p300 gene in rat kidney reduced the production of IL-6 and TGF-β1 and renal lesions in rat anti-Thy glomerulonephritis and this was related to sublytic C5b-9 activation leading to up-regulation of p300 and p300-mediated C/EBPβ acetylation [95].

BET inhibitors. An interesting line of research is the use of epigenetic reader modifiers, such as BET inhibitors [96]. MS417 attenuated experimental diabetic proteinuria and kidney injury [84], while JQ1 prevented the association of BRD4 with acetylated histone-packaged promoters, reducing NF-κB activation, the transcription of proinflammatory genes and kidney inflammation and/or preserved renal function in experimental polycystic kidney disease, UUO, anti-glomerular basement membrane glomerulonephritis and angiotensin II infusion-induced kidney injury [82, 83].

Histone crotonylation

Exogenous crotonate increases histone crotonylation in murine cultured tubular cells and in kidneys *in vivo*, demonstrating that modulation of metabolite availability may be used to therapeutically target crotonylation [26]. Crotonate elicited similar biological responses in cultured tubular cells and in the whole kidney in healthy mice *in vivo*, upregulating the expression of some protective genes such as *PGC-1α* and downregulated genes involved in tissue injury such as *CCL2*, which encodes the MCP-1 chemokine. Crotonate also increases the expression of the SIRT3 decrotonylase, suggesting the triggering of negative feedback loop.

Furthermore, parenteral crotonate increased kidney histone crotonylation and protected from experimental AKI induced by a folic acid overdose, decreasing inflammation, mitochondrial stress and markers of renal dysfunction and kidney injury. Crotonate also prevented the decrease in kidney *PGC-1α* and *SIRT3* levels in AKI as well as the increase in *CCL2* mRNA expression [26].

HISTONES AS CYTOTOXIC MOLECULES: A ROLE BEYOND REGULATION OF GENE EXPRESSION

Nuclear histones are inert, but they could be released into the extracellular space during necrosis (e.g. necroptosis, NETosis or pyroptosis) and induce inflammation and cytotoxicity [97, 98]. Neutrophil extracellular traps (NETs) are chromatin structures composed mainly of histones, and citrullinated histones (CitH) are key in NETs formation [99]. Histone citrullination is another post-translational histone modification catalyzed by peptidylarginine deiminase 4 (PAD4). In this regard, the roles of other histone modifications beyond gene regulation are incompletely characterized. NETs were described as structures with antimicrobial function released by viable neutrophils. However, NETs formation is also associated with neutrophil cell death by NETosis and has been also observed during sterile inflammation in different tissues, including the kidney [100, 101]. Recently, NETs formation and CitH were detected in renal human biopsies and from mice with AKI. In addition, histones released by dying tubular cells behave as danger-associated molecular patterns (DAMPs), promoting NETs formation and leading to necroinflammation, and anti-histone IgG reduces renal injury in experimental AKI. Moreover, circulating NETs and histones may induce remote organ injury associated with AKI [102]. Altogether, these results suggest that histone post-translational modifications may contribute to AKI beyond regulation of gene expression.

CLINICAL EXPERIENCE WITH EPIGENETIC MODIFIERS

There is clinical experience with a number of epigenetic modifiers, although only anecdotal or *post hoc* data in the field of kidney disease (Table 3). Some drugs, such as valproic acid, have long been used for other purposes, based on additional properties of the drug. Nephrologists may be more familiar with the antihypertensive drug hydralazine, which has optimum DNA demethylating activity at concentrations below blood pressure-lowering doses [59]. For drugs that specifically interfere with epigenetic mechanisms or that have been specifically developed for that purpose, clinical experience was mostly acquired in the oncology field.

Decitabine and 5′-azacytidine are approved to treat acute myeloid leukaemia and myelodysplastic syndrome, respectively. Renal failure is a recognized complication of 5′-azacytidine and has been observed with decitabine, although these drugs are frequently used associated with additional drugs in complex patients and direct renal toxicity is difficult to prove [103–105]. These reports are in line with some preclinical data [39].

Histone methylation and demethylation inhibitors are undergoing clinical trials for malignant haematologic disease. However, there is no information on the drugs being tested in the clinic and their effects on experimental or clinical kidney injury.

HDAC inhibitors are used to treat malignancy. Panobinostat and vorinostat are indicated for myeloma and cutaneous T cell lymphoma, respectively [88, 106]. However, increased serum creatinine and proteinuria were observed in ∼50% of vorinostat-treated patients [106]. Increased serum creatinine occurred in >40% of patients on panobinostat, which has not been tested in preclinical nephropathies [107].

BET inhibitors are in clinical trials for haematological malignancies, solid tumours and cardiovascular disease [96]. Of specific interest in the nephrology context, apabetalone (RVX-208, RVX000222) is a BET inhibitor with specificity for BRD2-4 and BRDT with selectivity for the second bromodomain (BD2) [108]. A Phase 3 clinical trial (BETonMACE, NCT02586155) is assessing the effect of apabetalone on cardiovascular events in high-risk diabetic patients with coronary artery disease [109]. Change in kidney function is a secondary outcome measure. In this regard, a significant increase in estimated glomerular filtration rate was observed in diabetic patients in a *post hoc* analysis of Phase 2 results [110]. Among the pleiotropic actions of apabetalone of potential interest in kidney disease, we find downregulation of inflammatory mediators and circulating activated fragments C5a, C3b and C5b-C6 [111], and reduced oral glucose absorption and endogenous glucose production [112].

SUMMARY AND FUTURE PERSPECTIVES

There is an increasing interest in epigenetic regulation of kidney injury, especially from the chronicity and aging point of view [113]. In this regard, epigenetics has the potential to transform a transient environmental factor or event into a long-time driver of pathogenic changes. The terms hypoxic memory or metabolic memory illustrate this fact [114]. It has also the

potential to contribute to the cross-talk between the microbiota and kidney injury. Thus, the composition of the microbiota may modulate the availability of short-chain fatty acids, a determinant of epigenetic regulation, to modulate kidney disease through availability of substrate or inhibition of HDACs, such as butyrate [12, 85, 115]. In this context, preclinical data in general support the therapeutic potential of diverse interventions on DNA methylation or histone modifications. This may be surprising, since it is likely that during kidney injury some genes are upregulated and some genes are downregulated through epigenetic mechanisms and any sweeping epigenetic intervention will have opposing effects on certain genes. However, global epigenetic pattern changes have been observed, suggesting a predominance of certain epigenetic modifications in the course of kidney injury. It is encouraging that some epigenetic modifier drugs are already in clinical use of undergoing clinical trials and even, as is the case for apabetalone, reporting promising *post hoc* results. However, most of our understanding of epigenetic modifiers and the kidney in the clinic is derived for the adverse effects of drugs already in clinical use for non-renal indications. This may provide a biased assessment of the drug in the clinical context. However, some drugs reporting beneficial effects in preclinical studies are known to have nephrotoxic potential in the clinical context (e.g. DNMT and HDAC inhibitors). This does not preclude benefit to the kidney, as cyclosporine A exemplifies, but adds a note of caution, especially given the conflicting evidence on these same drug families for preclinical AKI. Furthermore, the potential for intergenerational and transgenerational effects is not well characterized [113]. Finally, an incomplete current understanding of the process will need further refinement in the interpretation of results. Thus, crotonylation is likely to mediate part of the results obtained by targeting p300/CBP or Sirt3, previously attributed to changes in histone acetylation. In any case, this promising field merits further research, which should focus on drugs already in clinical use or undergoing clinical trials, since they are likely to eventually translate these advances to the clinic in a reasonable time than completely new molecular entities.

FUNDING

Grants FIS PI15/00298, PI16/02057, PI16/01900, CP14/00133, CP12/03262, ISCIII-RETIC REDinREN RD016/0009 Fondos FEDER, Spanish Society of Nephrology, FRIAT-IRSIN, CYTED IBERERC, Programa Intensificación Actividad Investigadora (ISCIII) to A.O., Miguel Servet to M.D.S.-N. and A.B.S., Fundación Conchita Rabago to D.M.-S. and Consejería de Educación, Juventud y Deporte (CAM and FSE) to M.F.-B.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

CONFLICT OF INTEREST STATEMENT

None declared.

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Received: 5.9.2017; Editorial decision: 26.12.2017