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Epigenetic regulation in AKI and kidney repair: mechanisms and therapeutic implications

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Abstract

Acute kidney injury (AKI) is a major public health concern associated with high morbidity and mortality. Despite decades of research, the pathogenesis of AKI remains incompletely understood and effective therapies are lacking. An increasing body of evidence suggests a role for epigenetic regulation in the process of AKI and kidney repair, involving remarkable changes in histone modifications, DNA methylation and the expression of various non-coding RNAs. For instance, increases in levels of histone acetylation seem to protect kidneys from AKI and promote kidney repair. AKI is also associated with changes in genome-wide and gene-specific DNA methylation; however, the role and regulation of DNA methylation in kidney injury and repair remains largely elusive. MicroRNAs have been studied quite extensively in AKI, and a plethora of specific microRNAs have been implicated in the pathogenesis of AKI. Emerging research suggests potential for microRNAs as novel diagnostic biomarkers of AKI. Further investigation into these epigenetic mechanisms will not only generate novel insights into the mechanisms of AKI and kidney repair but also might lead to new strategies for the diagnosis and therapy of this disease.

Acute kidney injury (AKI), characterized by a rapid decline in kidney function, is a major public health problem. It is responsible for approximately 1.7 million deaths per year worldwide and is associated with increased length of hospital stay among hospitalized patients as well as high costs[1–3]. The pathophysiology of AKI is incompletely understood but involves the injury and death of renal tubular cells, particularly of cells in the proximal

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tubule[4,5]. Following injury, a repair response is activated, which involves epithelial cell de-differentiation, proliferation and re-differentiation. However, severe or sustained injury often results in maladaptive and incomplete repair, leading to tubular degeneration, inflammation, renal fibrosis and ultimately progression to chronic kidney disease (CKD) or end-stage renal disease [6–10] (FIG. 1). Currently, other than supportive care in the form of dialysis, no effective treatments for AKI are available.

Epigenetics is the study of the heritable mechanisms that control gene expression without changing the primary nucleotide sequence. Epigenetic mechanisms, including histone modifications, DNA methylation and non-coding RNAs, can induce changes to a phenotype but cannot change a genotype. In general, epigenetic modifications are considered to be stable and heritable during cell divisions; however, they are potentially reversible and can be affected by environmental factors, age and disease state. Emerging evidence suggests that epigenetic regulation contributes to various kidney diseases, including diabetic kidney disease, CKD and renal cell carcinoma. For example, epigenetic alterations commonly occur in CKD, associated with genes involved in fibrosis, inflammation and epithelial-to-mesenchymal transition[11,12]. In particular, global targeting or gene-specific targeting of DNA methylation has been reported to effectively inhibit CKD progression in animal models of disease, suggesting that targeting DNA methylation could be a new therapeutic approach for the treatment of CKD[13–17]. An accumulating body of evidence also suggests a crucial role for epigenetic mechanisms in AKI and repair. In this Review, we provide a general overview of the main epigenetic mechanisms that have been linked to AKI, describe available evidence linking epigenetic changes to kidney injury and repair in AKI and discuss the challenges and therapeutic implications of these findings.

Epigenetic mechanisms

Epigenetic regulation involves the covalent modification of DNA or histone proteins, or RNA interference by non-coding RNAs, to modulate gene expression. Modification of DNA or histone proteins is achieved through DNA methylation or a number of histone modifications in processes that are catalysed by specific enzymes called epigenetic writers. These modifications are then recognized by epigenetic readers and can be removed by epigenetic erasers.

Histone modifications

Histones, including core histones (H2A, H2B, H3 and H4) and linker histones (H1 and H5), are highly conserved, basic or positively charged proteins that are involved in DNA packaging. They can associate with negatively charged DNA through electrostatic interactions and package it into highly condensed and ordered chromatin structure units called nucleosomes[18]. Each nucleosome consists of a segment of DNA wrapped around a core histone octamer, which contains two copies of each core histone protein (that is, two H2A–H2B dimers and one H3–H4 tetramer)[19,20]. Histone modifications involve the covalent, post-translational modifications of core histone proteins and include, but are not limited to, acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, citrullination, biotinylation, crotonylation and ADP ribosylation. These modifications occur

predominantly, but not exclusively, on the amino-terminal tails of the histones and are thought to change the structure of chromatin or provide docking sites for transcriptional regulators to either positively or negatively regulate gene expression[19,20]. Acetylation, methylation and phosphorylation are the major forms of histone modification (FIG. 2).

Histone acetylation

Histone acetylation involves the addition of a negatively charged acetyl group to the lysine of core histones by histone acetyltransferases (HATs) and is one of the most extensively studied forms of histone modification. Histone acetylation neutralizes the positive charge of lysines, promoting an open chromatin configuration and facilitating access of transcription factors, leading to permissive transcription. Moreover, acetylated histone residues serve as docking sites for many transcriptional co-activators that promote transcription[21]. Histone acetylation is reversible and deacetylation is catalysed by histone deacetylases (HDACs). To date, 18 mammalian HDAC proteins have been identified, which, on the basis of their domain organization and sequence homology to yeast orthologues, are divided into four classes: class I (HDACs 1, 2, 3 and 8), class II (HDACs 4, 5, 6, 7, 9 and 10), class III (sirtuin (SIRT) 1–7) and class IV (HDAC11). Classes I, II and IV require zinc for their catalytic activity and are considered to be the ‘classical’ HDACs, whereas class III HDACs depend on NAD^+ for their catalytic activity[22].

Histone methylation

Histone methylation involves the addition of a methyl group to a basic amino acid on core histones in a process mediated by histone methyltransferases (HMTs). Any of the three basic amino acids can be methylated, but it most commonly occurs on lysine and arginine residues and rarely at histidine residues. Lysine and arginine can be subjected to multiple methylations on their side chains. Specifically, lysine can be monomethylated, dimethylated or trimethylated on its ε-amine group and arginine can be monomethylated, or symmetrically or asymmetrically dimethylated, on its guanidyl group[23]. Unlike histone acetylation, histone methylation does not change the charge of the histone protein but rather provides sites for the docking of transcription regulators. Histone methylation is associated with either active or repressive transcription, depending on the amino acids methylated and the number of methyl groups attached. Generally, histone methylation at H3 lysine 4 (H3K4), H3K36 and H3K79 activates gene transcription, whereas methylation at H3K9, H3K27 and H4K20 is associated with repression of transcription[24]. Histone methylation was once thought to be permanent; however, the identification of novel histone demethylases, such as lysine-specific demethylases (LSD1 and LSD2) and Jumonji (JmjC)-domain containing histone demethylases, has challenged this view[25].

Histone phosphorylation

Histone phosphorylation is another major form of histone modification that changes the chromatin structure. It can take place on all four core histones, mainly on serine, threonine and tyrosine residues. Phosphorylation of different core histones requires distinct protein kinases, such as MEC1 for the phosphorylation of H2A in yeast, MST1 (mammalian sterile-20-like kinase) for the phosphorylation of H2B in mammals, the Aurora kinases for the phosphorylation of H3 and SPS1 (a sporulation-specific kinase) for the phosphorylation

of H4 in yeast[20]. Phosphorylation of histones is often associated with transcriptional activation (for example, phosphorylation of H3 at serine 10 (H3S10ph), threonine 11 (H3T11ph), serine 28 (H3S28ph) and tyrosine 41 (H3Y41ph) and phosphorylation of H2B at serine 32 (H2BS32ph)); however, it can sometimes be associated with transcriptional repression (for example, phosphorylation of H2A at serine 1 (H2AS1ph), phosphorylation of H2B at tyrosine 37 (H2BY37ph) and phosphorylation of H4 at serine 1 (H4S1ph))[26–28]. Increasing evidence indicates that histone phosphorylation not only is involved in the regulation of gene transcription but also is involved in the DNA damage repair response and chromatin condensation during mitosis and meiosis[26].

Histone modifications in AKI and repair

Histone modifications, particularly histone acetylation, are among the most studied epigenetic mechanisms in AKI and repair. Improved understanding of the contribution of these modifications to the process of injury and repair might open new therapeutic avenues to prevent injury or promote repair.

Histone acetylation in AKI and repair

An accumulating body of evidence suggests that AKI is associated with changes in histone acetylation, especially in the context of ischaemic AKI. A 2008 study showed dynamic changes in histone acetylation in AKI induced by renal ischaemia–reperfusion injury (IRI)[29]. In this model, severe unilateral ischaemia induced a transient reduction in H3 acetylation in proximal tubular cells, probably resulting from a decrease in HAT activity. During reperfusion, however, H3 acetylation was restored and expression of bone morphogenetic protein 7 (BMP7) — a key regulator of renal repair — was induced, at least in part owing to selective downregulation of HDAC5. In contrast to the findings from this study, another study using a model of moderate unilateral IRI reported an increase in H3 acetylation after 1 day of reperfusion that persisted for 3 weeks[30]. Moreover, another study in a mouse model of bilateral IRI reported an initial increase in acetylation of H3K14 (H3K14Ac) followed by a decrease to basal levels[31]. By contrast, H4K5Ac and H4K12Ac were reduced and then restored. Dynamic regulation of the HBO1 (also known as KAT7, an H4-specific HAT)–JADE1 complex, was found to contribute to changes in histone H4 acetylation[31]. Although these studies demonstrate that dynamic alterations in histone acetylation occur in the context of AKI, inconsistencies in the direction of change require clarification. Several possible reasons exist for the differences in histone acetylation observed between the different studies, including differences in the severity of injury and mouse strain and the use of different time points and methods for the analysis of histone acetylation.

Several other studies have examined the role of histone acetylation on the expression of genes involved in ischaemic AKI. One study demonstrated that upregulation of the rate-limiting enzyme in cholesterol synthesis, high mobility group CoA reductase (HMG-CoA reductase; encoded by *HMGCR*), contributed to cholesterol loading and cytoprotective effects in ischaemic AKI[32]. Interestingly, the histone variant H2A.Z and histone modifications (trimethylation at H3K4 (H3K4m3) and H3K19Ac) in the *Hmgcr* promoter

and along the gene were increased and likely contributed to the upregulation of HMG-CoA reductase expression[32]. This hypothesis is supported by the finding that compared with patients without AKI, patients with AKI had increased HMG-CoA reductase activity associated with increased H3K4m3 at *HMGCR*[33]. The transcriptional repressor ATF3 (activating transcription factor 3) has renoprotective effects in ischaemic AKI by down-regulating the expression of inflammatory cytokines, such as IL-6 and IL-12B[34]. Induction of ATF3 in a mouse model of ischaemic AKI led to the recruitment of HDAC1 to the promoter regions of *Il6* and *Il12b*, resulting in the suppression of *Il6* and *Il12b* expression and inhibiting the inflammatory response. Silencing of HDAC1 exacerbated AKI, associated with higher expression of IL-6 and IL-12B. In another study, upregulation of plasminogen activator inhibitor type 1 (PAI1; in mice encoded by the *Serpine1* gene (serine (cysteine) pepti-dase inhibitor, clade E, member 1)), which contributes to the susceptibility of male mice to ischaemic AKI, was associated with a decrease in HDAC11 expression and increased acetylation of H3 in the *Serpine1* promoter[35].

In addition to renal IRI, histone acetylation is involved in other forms of AKI. For example, decreased H3 acetylation has been reported in both in vivo and in vitro experimental models of septic AKI, and administration of the α_2-adrenoceptor agonist dexmedetomidine protected mice against septic AKI by inhibiting HDAC2 and HDAC5 to induce H3 acetylation and the expression of BMP7 (REF.[36]). In a mouse model of lipopolysaccharide (LPS)-induced septic AKI, upregulation of p300/CBP-associated factor (PCAF; a HAT) was associated with increased acetylation of histones, such as H3K18Ac, and increased expression of inflammatory genes[37]. Silencing of PCAF in cultured renal tubular cells significantly reduced H3K18Ac, resulting in decreased expression of inflammatory factors, such as vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule 1 (ICAM1) and monocyte chemoattractant 1 (MCP1; also known as CCL2), suggesting that induction of H3K18Ac contributes to the upregulation of inflammatory genes in septic AKI. Peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α; in mice encoded by the *Ppargc1a* gene) is a regulator of mitochondrial biogenesis that is protective in AKI and is downregulated in several experimental models of AKI[38–40]. A 2016 study showed that suppression of PGC1α in mice with folic-acid-induced AKI was mediated through the actions of a cytokine called TWEAK (TNF-related weak inducer of apoptosis), which promoted H3 deacetylation at the *Ppargc1a* promoter, leading to chromatin condensation and repression of PGC1α expression[41]. Moreover, downregulation of another renoprotective factor, Klotho (in mice encoded by *Kl*), in folic acid-induced AKI was also shown to occur via TWEAK-mediated deacetylation of the *Kl* gene promoter[42]. In rats with cisplatin-induced AKI, induction of the class III HDAC protein SIRT1 occurred as early as 6 h after cisplatin administration and was associated with deacetylation of histone H3, although expression of HDAC5 and HDAC3 were not induced until days 2 and 3, respectively[43]. Overexpression of SIRT1 protects against cisplatin-induced AKI by retaining peroxisome function[44]. The protective effects of SIRT1 are also supported by the finding that suppression of SIRT1 reduced resistance of cultured renal medullary interstitial cells to oxidative stress and that SIRT1 deficiency aggravated renal apoptosis and fibrosis in mice with unilateral ureteral obstruction (UUO)[45]. Taken together, these studies support an important role of histone acetylation in various forms of AKI.

Histone acetylation-based drugs in AKI and repair.

As described above, histone acetylation, which is regulated by the balance between HATs and HDACs, has a key role in chromatin remodelling and therefore in the regulation of gene expression. The involvement of histone acetylation in AKI and repair suggests that HDAC inhibitors (HDACis), HDAC activators or HAT inhibitors might have therapeutic value (TABLE 1).

The effects of HDACis on AKI were first investigated in the experimental models of cisplatin-induced nephrotoxicity, with conflicting findings[46,47]. We showed that the HDACis trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) were toxic to renal tubular cells[46], whereas other researchers found TSA to exert cytoprotective effects against cisplatin-induced apoptosis in renal tubular cells[47]. Our follow-up study showed that the effects of both TSA and SAHA on renal tubular cells are dose-dependent: they are toxic at high-micromolar concentrations, but at concentrations <1 μmol/l, they can protect against cisplatin-induced cell death[48]. In mice with cisplatin-induced AKI, the renoprotective effects of low-dose TSA involve upregulation of an anti-inflammatory protein called activated microglia/macrophage WAP domain protein (AMWAP)[49]. Our most recent work showed that the protective effect of TSA in cisplatin-induced AKI is diminished by blocking renal tubular autophagy[50], suggesting a critical role for autophagy in mediating the protective effect of this HDACi and supporting previous work showing that tubular cell autophagy is protective in AKI[51,52]. TSA has also been investigated in other models of AKI. A 2009 study demonstrated that TSA ameliorated renal tubular cell apoptosis, caspase 3 activation and renal fibrosis in a mouse model of UUO[53]. Similarly, TSA protected against ischaemic AKI and consequent interstitial fibrosis in a mouse model of unilateral renal IRI[54]. Moreover, the HDACi SAHA protected kidney cells from apoptosis induced by lethal haemorrhagic shock in rats, associated with increased H3K9 acetylation[55]. Valproic acid (VPA), a compound that has HDACi properties, attenuated proteinuria and kidney injury in models of adriamycin-induced nephropathy and IRI, respectively[56,57]. Similar to the effects of TSA, MS-275, an inhibitor of HDAC1 and HDAC3, attenuated kidney injury and the development of renal fibrosis in models of cisplatin-induced AKI and ischaemic AKI[49,54]. In models of folic acid and rhabdomyolysis-induced AKI, however, MS-275 exacerbated kidney injury and impaired renal regeneration[58]. Studies have also assessed the effects of the HDACi 4-methyl-thiobutanate (m4PTB), an analogue of 4-(phenylthio) butanoic acid. Studies of zebrafish and mouse models of AKI suggest that administration of m4PTB promotes recovery following injury[59,60]. In mouse models of IRI and aristolochic-acid-induced AKI, treatment with m4PTB enhanced proliferation and reduced G2/M arrest of renal tubular epithelial cells and prevented the development of interstitial fibrosis[59,60]. In addition, inhibition of HDAC6 by tubastatin A enhanced H3 acetylation, suppressed inflammation and oxidative stress and prevented the development of rhabdomyolysis-induced and cisplatin-induced AKI[61,62].

In addition to HDACis, the effects of HDAC activators — particularly the SIRT1 activators, resveratrol, SRT-2183 and SRT-1720 — on AKI and subsequent repair have been investigated. A 2010 study reported that resveratrol and SRT-2183 enhanced resistance of primary cultured mouse renal medullary interstitial cells to oxidative stress-induced

apoptosis and that administration of SRT-1720 attenuated apoptosis and fibrosis in mice with UUO[45]. The renoprotective effects of resveratrol have also been demonstrated in experimental models of cisplatin-induced nephrotoxicity and septic AKI[63,64]. Administration of SRT-1720 to mice with IRI induced proliferation of renal tubular cells and attenuated renal injury, whereas SIRT1 deficiency had the opposite effects, further supporting a role for SIRT1 in the regulation of kidney injury and repair[65].

Several studies have examined the role of curcumin, an inhibitor of the HAT PCAF in AKI. In rats, administration of curcumin showed renoprotective effects in various AKI models, including cisplatin-induced nephrotoxicity, IRI and LPS-induced AKI[66–68].

Together, these studies support the functional relevance of histone acetylation in AKI and repair. In general, pharmacological inhibition of HDACs seems to protect the kidney against multiple forms of AKI and promotes kidney repair. However, the role of histone acetylation in AKI is controversial (TABLE 1). As described above, HDAC activators, particularly activators of SIRT1, and the HAT inhibitor curcumin also have renoprotective effects, complicating interpretation of the role of histone acetylation in kidney injury and repair. Specific HATs and HDACs might have distinct roles in the pathogenesis of AKI. To date, pan-HDACis and class-specific HDACis have mainly been used to study the role of histone acetylation in AKI and repair. Future studies will need to elucidate the function of specific HDAC isoforms in AKI and repair and develop isoform-selective HDACis for research and therapeutic use. Of note, in addition to changing the acetylation status of histones, HATs and HDACs can acetylate and deacetylate non-histone proteins. For example, p53, a tumour suppressor protein that regulates the cell cycle and has many functions, including a pathogenic role in AKI, is a target of both the HAT PCAF and the HDACs HDCA1 and SIRT1 (REF[69]). In a model of ischaemic AKI, SIRT1 deficiency increased acetylation and expression of p53, which contributed to the development of kidney injury[65]. Inhibition of PCAF or activation of SIRT1 might result in the deacetylation and inactivation of p53, suggesting that the renoprotective effects of HDACis, HDAC activators or HAT inhibitors in AKI might involve alterations in the acetylation status of non-histone proteins. However, this proposal requires verification. Identification of the targets of specific HDACs of interest is important to delineate the underlying mechanisms of their roles in the pathogenesis of AKI and could lead to the discovery of novel strategies for the prevention and treatment of AKI.

Other histone modifications

Compared with histone acetylation, the role of histone methylation in AKI and kidney repair has been much less studied. However, induction of inflammatory factors (TNF and MCP1) and HMG-CoA reductase is associated with increased levels of H3K4m3 in various mouse models of AKI[32,70,71]. Interestingly, urinary levels of MCP1 and H3K4m3 specifically associated with *MCP1* and *HMGCR* were elevated in patients with AKI, suggesting these methylation changes might have potential as biomarkers of AKI[33,71]. Upregulation of enhancer of zeste homologue 2 (EZH2) — a histone methyltransferase that specifically mediates trimethylation of lysine 27 on histone H3 (H3K27m3) — has been documented in fibrotic kidneys from mice with UUO and in patients with CKD, suggesting that this methyltransferase has profibrotic functions[72,73]. Pharmacological inhibition of EZH2

decreased H3K27m3 and attenuated renal fibrosis in mice with IRI by suppressing the expression of genes encoding profibrotic proteins, such as collagen type 3a1 (COL3A1) and tissue inhibitor of metalloproteinase 2 (TIMP2)[74].

Knowledge of the role of histone phosphorylation in AKI is also very limited. A 2001 study demonstrated that histone H3 phosphorylation induced chromatin condensation and promoted the damage and death of renal proximal tubular cells in response to oxidative stress[75]. Prevention of H3 phosphorylation by inhibiting MAPK kinase (with the selective MAPK inhibitor PD98059) or by inhibiting poly(ADP-ribose) polymerase (PARP) (with 3-aminobenzamide) promoted renal tubular cell survival[75]. Phosphorylation of the histone H2A variant H2AX at serine 139, indicative of double-strand DNA damage, has been linked to AKI in various models, including cisplatin-induced AKI and renal IRI[76–79]. No information is available to date regarding the potential role of histone phosphorylation in kidney repair.

Histone crotonylation is another histone modification that involves the addition of a crotonyl group to lysine residues on core histones by the actions of histone crotonyltransferases. Similar to histone acetylation, histone crotonylation neutralizes the positive charge of lysine residues and stimulates transcription; however, the mechanisms governing crotonylation versus acetylation remain largely unclear[80]. A 2016 study demonstrated an increase in histone crotonylation in kidney tissues in models of AKI induced by folic acid and cisplatin[81]. Further analysis indicated that histone crotonylation increased the expression of PGC1α and SIRT3, promoting protective pathways.

Obviously, multiple forms of histone modifications regulate gene expression under pathophysiological conditions, including AKI. A 2015 study mapped patterns of histone modifications in relation to changes in the expression of several genes induced by three models of AKI: unilateral renal IRI, LPS-induced AKI and combined IRI and LPS-induced AKI[82]. Using matrix chromatin immunoprecipitation, the researchers determined levels of key permissive histone marks (that is, H3K9Ac, H3K18Ac, H3K27Ac, H4K5/8/12/16Ac, H3K4m3, H3K4m2 and H3S10ph), elongation marks (H3K36m3, H3K36m2 and H3K79m2) and repressive histone marks (H3K27m3, M3K9m2, macroH2A, H2AK119ub1, H2BK120ub1 and H4S1ph) at genes that encode the injury markers NGAL and KIM1 and the inflammatory factors TNF and ICAM1. In general, the permissive histone marks were increased and the repressive marks were decreased in these AKI models, indicating that these epigenetic modifications might be responsible for the increased expression of these genes in the context of kidney injury. Unexpectedly, however, histone modifications exhibited striking heterogeneity at different histone marks, in different genes and in different AKI models, and exhibited time-dependent alterations. These findings highlight the complex regulation of histone modifications in AKI and the need to better understand the mechanisms of epigenetic regulation in this disease.

DNA methylation

DNA methylation is the typical epigenetic modification that affects DNA. In eukaryotes, DNA methylation occurs only at cytosine residues and involves the covalent addition of a

methyl group (CH_3), which is commonly donated by *S*-adenosyl-L-methionine (SAM), to the 5-carbon position of the cytosine, creating 5-methylcytosine (5mC) via DNA methyltransferases (DNMTs)[83,84]. DNA methylation occurs predominantly in CpG dinucleotides; however, DNA methylation can also occur at low frequency in non-CpG sites, particularly in embryonic stem cells[85,86], oocytes[87] and brain tissues[88,89]. In the mammalian genome, the majority (70–80%) of CpG sites are methylated. CpG islands within promoter regions of genes are usually unmethylated, enabling transcription. In mammals, DNA methylation is critical for the regulation of many biological processes, including embryonic development, genomic integrity, X chromosome inactivation (in females) and genomic imprinting; DNA methylation has also been linked to complex human diseases, including cancer[90–92].

The machinery involved in mammalian DNA methylation consists of three parts: DNA methylation writers, which establish and maintain the methylation marks in the genome; DNA methylation readers, which read or interpret the methylation marks; and DNA methylation erasers, which mediate DNA demethylation.

The most notable function of DNA methylation is transcriptional regulation (FIG. 3a). Generally, low levels of DNA methylation (hypomethylation) at a gene promoter correlate with transactivation of that gene through increased binding of transcription factors. Conversely, elevated DNA methylation (hypermethylation) in a gene promoter region typically leads to loss of gene expression[93–95]. DNA methylation can suppress gene expression by either directly preventing transcription factors from binding the promoter or indirectly through the actions of DNA methylation readers, which bind to methylated CpGs and stimulate the recruitment of repressor complexes.

DNA methylation writers

The DNMT family includes DNMT1, DNMT2 and DNMT3 (DNMT3a, DNMT3b and DNMT3-like (DNMT3L)). DNMT1, DNMT3a and DNMT3b are the major DNMTs that are required for DNA methylation and are conserved in mammals and plants (FIG. 3a). DNMT1 is the maintenance DNMT and has a preference for hemimethylated DNA. During cell division, the newly synthesized daughter DNA strands are recognized by DNMT1 and are methylated faithfully according to the parental DNA strands. DNMT1 is the most abundant DNMT in most cell types, especially in proliferating cells. By contrast, DNMT3a and DNMT3b are de novo DNMTs, which establish the initial DNA methylation patterns. DNMT3a and DNMT3b are predominantly expressed in embryonic tissues with low expression in somatic tissues[96]. DNMT3L is not an active enzyme but can facilitate the catalytic activity of DNMT3a and DNMT3b by enhancing the binding of the methyl donor, SAM[97]. DNMT2, also known as transfer RNA aspartic acid methyltransferase 1 (TRDMT1), has barely detectable methylation activity on DNA but has a robust methylation activity on tRNAs, especially at the 38th cytosine residue in the anticodon loop of aspartic acid, glycine and valine tRNAs[98,99]. Methylation of tRNAs enhances their stability and protects them from stress-induced cleavage[99]. Deficiency of DNMT1 or DNMT3b is embryonic lethal in mice, whereas mice that are deficient in DNMT3a die within a few weeks of birth, indicating that these DNMTs are essential for animal development[100,101].

DNA methylation readers

Three families of DNA methylation readers exist: the methyl-CpG-binding domain (MBD) protein family, the zinc-finger proteins and the ubiquitin-like with PHD and ring finger domain-containing (UHRF) protein family. The MBD family consists of five members: methyl-CpG-binding protein 2 (MeCP2) and MBD1–MBD4 (REFS[102,103]). Except for MBD4, the MBDs bind to methylated cytosine and repress transcription by recruiting various repressor complexes through their transcriptional repression domain[103]. For example, MeCP2 binds a single symmetrically methylated CpG site and recruits the transcriptional co-repressors Sin3a (SIN3 transcription regulator family member A) and HDACs[104]; MBD2 binds to methylated DNA and interacts with MeCP1, a histone deacetylase complex, to inhibit transcription[105]. The zinc-finger proteins include ZBTB4, ZBTB38 and transcriptional regulator Kaiso (ZBTB33). ZBTB4 and ZBTB38 act as transcription repressors and repress transcription in a methyl-CpG-dependent manner. Kaiso recruits the repressor complexes and suppresses transcription[102,106]. The UHRF family consists of UHRF1 and UHRF2. Unlike most of the MBD family and the zinc-finger proteins, the UHRF family does not directly repress transcription but recruits DNMT1 to the hemimethylated DNA to maintain DNA methylation[103].

DNA methylation erasers

DNA methylation was traditionally considered to be a very stable modification inherited by daughter cells; however, studies over the past decade have demonstrated that DNA methylation is not permanent and can be erased by demethylation, which can occur via passive or active mechanisms (FIG. 3b). Passive DNA demethylation can occur during cell division if DNMT1 is missing or inhibited. Under these circumstances, the newly synthesized DNA strands remain unmethylated, leading to passive demethylation in the daughter cells. Conversely, active demethylation can be achieved in both dividing and non-dividing cells by DNA methylation erasers such as activation-induced cytidine deaminase (AID) and proteins of the ten-eleven translocation (TET) family[107]. AID removes the amino group from 5mC, generating a mismatch between thymine (T) and guanine (G) bases, which is then recognized and corrected by thymine DNA glycosylase (TDG) and other DNA repair mechanisms, ultimately resulting in loss of methylation[108]. Proteins of the TET family (TET1, TET2 and TET3) catalyse the oxidative conversion of 5mC to 5-hydroxymethylcytosine (5hmC), which can be then either further oxidized to form 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) or deaminated to form 5-hydroxymethyl-uracil (5hmU). These intermediates are then replaced by cytosine via TDG and base excision repair mechanisms[109–111]. Unlike the DNMT-deficient mice, mice deficient in AID, TET1 or TET2, or both TET1 and TET2, are viable[112–114], but deletion of *Tet3* is neonatal lethal[115], suggesting that TET3 is more important than other DNA methylation erasers in early development.

DNA methylation in AKI and repair

Although DNA methylation was the first epigenetic mark to be identified, the study of DNA methylation in kidney diseases is in its infancy. The involvement of DNA methylation changes has been implicated in chronic kidney disorders, including CKD[116–118] and diabetic

kidney disease[119,120]. By contrast, although evidence is available to support a role for DNA methylation in AKI and repair, research into this area is limited.

DNA methylation in AKI

A 2006 study reported demethylation of a cytosine in the IFNγ responsive element within the promoter region of complement factor *C3* in response to cold ischaemia and warm reperfusion in rat kidneys[121]. A follow-up study indicated that demethylation of the *C3* promoter not only occurred in the acute phase of injury but also persisted in the chronic injury phase, suggesting that the aberrant demethylation could contribute to the pathological upregulation of C3 in IRI[122]. In another study, hypomethylation of the promoter region of *Slc22a12*, which encodes the urate transporter 1 responsible for urate reabsorption, was observed in the plasma of mice with contact-freezing-induced AKI[123]. In humans, hypermethylation of the promoter region of *KLK1*, encoding renal kallikrein, was observed in blood DNA from patients with established AKI[124], and hypermethylation of the promoter of *CALCA*, encoding calcitonin, was observed in the urine from kidney transplant recipients[125]. These studies indicate that the status of DNA methylation is altered in the promoter regions of specific genes in AKI (TABLE 2), suggesting the potential use of methylation status as a biomarker for AKI.

Using dot blot and immunohistochemical analysis, Huang et al.[126] showed that the global level of 5hmC was decreased following ischaemic AKI in mice whereas the global level of 5mC was unchanged. Intriguingly, the decrease in 5hmC was associated with reduced expression of TET1 and TET2, the enzymes that oxidize 5mC to 5hmC in the process of DNA demethylation, suggesting a mechanism for the reduction in 5hmC. In addition, DNA hydroxymethylome profiling data showed that lower levels of 5hmC in the promoter regions of *Cxcl10* (encoding CXC-chemokine ligand 10) and *Ifngr2* (encoding IFNγ receptor 2) were associated with increased expression of these two genes after IRI. Subsequent work showed that genes associated with IRI exhibited significantly higher 5hmC enrichment in their gene body regions, leading the researchers to suggest that hyper-hydroxymethylation in the gene body alters the expression of these genes in AKI[127]. In contrast to the findings by Huang et al.[126], a more recent and more quantitative analysis of the genome-wide DNA methylation status of mouse renal tissues using reduced representation bisulfite sequencing (RRBS) found that both acute and chronic IRI (involving reperfusion for 24 h or 7 days, respectively) induced genome-wide DNA demethylation[128]. This demethylation status was apparent in analyses of overall DNA methylation status as well as in analyses of promoter, exon and intron regions. The discrepancy between these two studies might relate to the different degrees of injury induced and the different detection methods used. The latter study used RRBS with higher sensitivity. The researchers also identified 200 and 191 genes that had differentially methylated promoter regions in acute and chronic IRI, respectively. Of these genes, 79 were common to both conditions, and further investigation indicated that promoter methylation alterations contributed to differences in their expression.

Using RRBS, we performed genome-wide DNA methylation profiling and unveiled widespread DNA methylation alterations throughout the genome in kidney tissues from mice with cisplatin-induced AKI[129]. Treatment of cultured rat proximal tubular cells with the

DNA methylation inhibitor 5-aza-2′-deoxycytidine augmented cisplatin-induced apoptosis, and in line with these findings, genetic ablation of *Dnmt1* in proximal tubular cells exacerbated cisplatin-induced AKI in mice. Furthermore, hypomethylation and induction of interferon regulatory factor 8 (*Irf8*) by cisplatin treatment contributed to renal tubular cell apoptosis, demonstrating a pathological role of DNA methylation in the pathogenesis of AKI[129].

Clearly, the study of DNA methylation in AKI is an emerging area and further research is needed in a number of areas. First, genome-wide DNA methylation profiling of additional mouse models other than ischaemic and cisplatin-induced AKI is needed, as well as profiling of DNA methylation status in patients with AKI. The newly developed microarray- and deep-sequencing-based technologies will facilitate the quantitative analysis of DNA methylation sites at single-nucleotide resolution or even at single-cell resolution across the genome (BOX 1). These technological advances are particularly important as the DNA methylation profiles of specific cell types represent another area that requires further study. The kidney is a highly complex organ with many different cell types. Notably, different cell types can respond differently to kidney injury. Because whole-kidney DNA methylation profiling reveals the overall DNA methylation changes of various cell types, cell-type-specific DNA methylation profiling is needed to better understand DNA methylation changes and regulation in AKI. In addition, the role of DNA methylation in AKI requires better understanding; although available limited evidence supports a role of DNA methylation in the process of AKI, the exact role is unclear. The roles of DNA methylation writers, readers and erasers in AKI also remain unknown and require further study using pharmacological and genetic modulation of DNA methylation in models of AKI. Finally, the function of the 5mC derivative, 5hmC, remains largely unclear, although it is thought to have profound effects on gene regulation[130]. A moderate level (0.15–0.17%) of 5hmC was observed in the kidney[131]; however, as mentioned above, 5hmC levels decrease after ischaemic AKI, although the relevance of this decrease is unclear[126].

DNA methylation in kidney repair

Maladaptive or incomplete repair after AKI can lead to renal fibrosis. Although DNA methylation has been explored quite extensively in the context of renal fibrosis in CKD, only a few studies have investigated the role of DNA methylation in the context of AKI progression.

DNA methylation profiling has revealed that kidney samples from patients with CKD exhibit distinct DNA methylation profiles compared with samples from healthy controls[116–118]. A 2017 study identified methylation levels of 19 CpG sites in whole blood associated with estimated glomerular filtration rate and CKD, 5 of which showed consistent changes in biopsy samples from patients with CKD[132]. Other studies have shown that pharmacological inhibition of DNA methylation (using DNA methylation inhibitors, such as 5′-azacytidine and 5-aza-2′-deoxycytidine or a DNA demethylation activator, such as hydralazine) attenuates renal fibrosis in various mouse models of CKD; similarly, heterozygous *Dnmt1*-knockout mice are also protected from fibrosis in CKD models[13–16]. One study has examined the effects of inhibiting DNA methylation on AKI progression to CKD; in that

study, suppression of DNA methylation with low-dose hydralazine ameliorated renal fibrosis and prevented the progression of AKI to CKD in a model of IRI[133].

Expression of *RASALI*, which encodes the tumour suppressor RAS GTPase-activating-like protein 1, is suppressed in fibroblasts isolated from human fibrotic kidneys and in a number of mouse models of CKD, in association with hypermethylation of its promoter region[13–15]. Silencing of *Rasal1* by promoter hypermethylation leads to persistent activation of RAS, which contributes to the sustained activation of fibroblasts and renal fibrosis[13]. A 2017 study further showed that low-dose hydralazine induced TET3 expression and promoted demethylation of the *Rasal1* promoter, resulting in attenuation of renal fibrosis in a model of unilateral IRI, suggesting that hypermethylation of the *Rasal1* promoter is involved in maladaptive kidney repair after AKI[133].

The renoprotective protein Klotho is primarily expressed in tubular cells; its expression is reduced in patients and animal models of AKI and CKD, and it is being investigated as a potential therapeutic for AKI and CKD[134,135]. The suppression of Klotho in renal fibrosis is now understood to be regulated by DNA methylation. Hypermethylation of the *KL* promoter was demonstrated in kidney tissues and peripheral blood mononuclear cells from patients with CKD[136] as well as in the animal models of UUO, ciclosporin-A-induced nephropathy and adenine-induced CKD[16,137–139]. Use of the DNA methylation inhibitor 5-aza-2′-deoxycytidine or knockdown of *DNMT1* or *DNMT3A* inhibited hypermethylation of the *KL* promoter and preserved Klotho expression[16,137,139]. Although no direct evidence has shown that DNA methylation regulation of *KL* is involved in the progression of AKI to CKD, the above evidence suggests that this is a possibility.

Non-coding RNAs

Less than 2% of the human genome is transcribed into RNAs with protein-coding potential; the majority is transcribed into non-coding RNAs. Non-coding RNAs are classified according to their size as either small non-coding RNAs (<200 nucleotides) or long non-coding RNAs (lncRNAs) (>200 nucleotides). These RNAs cannot be translated into proteins but act as crucial epigenetic modulators of gene expression.

Of the endogenous small non-coding RNAs, microRNAs have been most extensively investigated. MicroRNAs usually contain 21–25 nucleotides and act as post-transcriptional inhibitory regulators of gene expression (FIG. 4a), predominantly by binding to the 3′ untranslated region of their target gene mRNAs, which either induces degradation of the mRNA or, more commonly, blocks translation of the mRNA into protein[140,141]. According to miRBase 22, approximately 2,600 mature microRNAs have been identified in humans, which are thought to regulate more than half of the human genome.

lncRNAs exist in both the cell nucleus and cytoplasm. Unlike microRNAs, which regulate gene expression exclusively at a post-transcriptional level, lncRNAs can function both transcriptionally and post-transcriptionally by interacting with DNA, RNA and protein (FIG. 4b). lncRNAs can be categorized according to their genomic location as sense, antisense, bidirectional, intronic, intergenic, enhancer or circular lncRNAs. They can also be classified

according to their molecular function at the transcriptional level as signal lncRNAs, which act as molecular signals of transcription activity in spatial-dependent and temporal-dependent manners; decoy lncRNAs, which inhibit transcription by binding and sequestering transcription factors and other proteins or regulatory RNAs away from chromatin; guide lncRNAs, which modulate chromatin states and gene expression by guiding chromatin-modifying enzymes to specific gene targets; and scaffold lncRNAs, which facilitate the assembly of transcription regulatory complexes that positively or negatively regulate gene expression[142]. In addition, lncRNAs can participate in post-transcriptional regulation. For example, lncRNAs can affect mRNA processing, translocation and stability by binding to the complementary RNAs and can also interact with splicing factors to modulate RNA splicing. lncRNAs can also recruit RNA-binding proteins to target mRNAs to promote or inhibit protein translation. Moreover, they can bind to microRNAs to titrate them away from their targets. Furthermore, some lncRNAs act as precursors for small regulatory RNAs that can be processed into small interfering RNAS, microRNAs or piwi-interacting RNAs, with effects on gene expression[143,144]. To date, approximately 30,000 lncRNAs have been identified in the human genome; the functions of about 300 of these are annotated in the Long Non-coding RNA database v2.0 (REF.[145]).

Non-coding RNAs in AKI and kidney repair

Non-coding RNAs, particularly microRNAs and lncRNAs, have emerged as important epigenetic regulators of AKI and kidney repair. In addition, circulating non-coding RNAS might represent potential biomarkers of AKI.

MicroRNAs in AKI

By regulating gene expression, microRNAs regulate diverse cellular and physiological processes, such as proliferation, differentiation, cell death and organ development[146–149]. MicroRNAs have also been implicated in the pathogenesis of various human diseases[149–153].

An accumulating body of evidence suggests that microRNAs are critical regulators of renal development and pathophysiology[154–160]. Given that the role of microRNAs in kidney diseases has been extensively reviewed else-where[156,157,161–167], we focus here on the recent research progress of microRNA in AKI and repair.

MicroRNAs in ischaemic AKI.—We demonstrated a role for microRNAs in AKI by generating conditional *Dicer1* (which encodes Dicer)-knockout mice, in which over 80% of microRNAs were depleted specifically in kidney proximal tubules[168]. These mice are remarkably resistant to ischaemic AKI, suggesting a pathogenic role of microRNAs in ischaemic AKI.

Microarray analyses by our group and others have demonstrated that the expression levels of microRNAs markedly alter in response to ischaemic AKI in mice[168–171]. Furthermore, a number of microRNAs have been identified to have a pathogenic or protective role in ischaemic AKI (TABLE 3). Some of these microRNAs, including miR-24 (REF. [172]), miR-150 (REF. [173]), miR-494 (REF. [174]) and miR-687 (REF. [175]), are pathogenic and contribute to kidney injury, whereas others, including miR-17–5p[176], miR-26a[177], miR-126

(REF. [178]), miR-127 (REF. [179]), miR-146a[169,180,181], miR-205 (REF. [182]) and miR-489 (REF. [183]) protect against ischaemic AKI. miR-21 has in general been shown to be protective in ischaemic AKI[169,184–187]; however, one study showed that overexpression of miR-21 increased apoptosis of cultured rat kidney epithelial cells in response to hypoxia–reoxygenation and that inhibition of miR-21 preserved expression of the intracellular membrane trafficking protein Rab11a and ameliorated autophagy activation, protecting against renal IRI in rats[188].

Our group has elucidated the roles of three specific microRNAs in ischaemic AKI, including miR-687, miR-489 and miR-17–5p[175,176,183]. All of these microRNAs are markedly induced in both in vivo and in vitro models of ischaemic AKI. The induction of miR-687 and miR-489 is mediated by hypoxia-inducible factor 1 (HIF1). Induction of miR-678 suppresses phosphatase and tensin homologue (PTEN) — a known inducer of G1 arrest — which in turn promotes cell cycle progression and apoptosis and exacerbates ischaemic AKI[175]. By contrast, miR-489 and miR-17–5p have a renoprotective role in ischaemic AKI. Overexpression of miR-489 in an in vitro model of ischaemic AKI attenuated cell apoptosis, whereas inhibition of miR-489 increased apoptosis in vitro and aggravated ischaemic AKI in mice. Moreover, *PARP1* — a known cell death mediator in ischaemic AKI — was identified as a target gene of miR-489, suggesting that miR-489 protects against ischaemic AKI by repressing *PARP1* (REF. [183]). Upregulation of miR-17–5p was induced by p53, which led to suppression of death receptor 6 (DR6; also known as TNFRSF21), resulting in inhibition of apoptosis in vitro and renoprotective effects in vivo[176].

MicroRNAs are also involved in the regulation of inflammation in ischaemic AKI. For instance, miR-146a regulates inflammation via its inhibitory effects on the Toll-like receptor (TLR)-NF-KB pathway[189,190]. Several studies have reported increased expression of miR-146a in mice with renal IRI and suggested that it has a renoprotective role in ischaemic AKI[169,180,181]. Deficiency of miR-146a aggravates tubular injury, increases inflammation and increases fibrosis in mice subjected to unilateral IRI[181]. Moreover, knockdown of miR-146a neutralized the protective effect of LPS pretreatment on renal IRI[180]. Mechanistically, activation of the TLR-NF-KB pathway after renal IRI initiates an acute inflammatory response but also upregulates miR-146a, which targets IL-1 receptor-associated kinase 1 (IRAKI), the upstream target of NF-KB, which in turn negatively regulates TLR-NF-KB signalling and suppresses inflammation. By contrast, miR-26a is downregulated in response to IRI in mice[177]. Overexpression of miR-26a by tail-vein injection of a recombinant lentiviral vector containing the miR-26a precursor, pre-mir-26a, protected against ischaemic AKI and promoted expansion of $FOXP3^{+}CD4^{+}$ regulatory T cells by repressing IL-6. Moreover, administration of miR-26a had renoprotective effects even when given after IRI, suggesting that this approach might have therapeutic potential.

Another microRNA, miR-126, regulates vascular regeneration by mobilizing haematopoietic stem and progenitor cells. miR-126 targets PI3KR2 (phosphoinositide 3-kinase regulatory subunit 2; also known as PI3-kinase subunit p85β), a key regulator of the PI3K–AKT (protein kinase B) signalling pathway, and affects the expression of genes that are essential for angiogenesis and inflammation. In ischaemic AKI, mice with overexpression of miR-126 in the haematopoietic compartment had better renal function and lower levels of tubular

damage, inflammation and fibrosis than control mice[178]. The beneficial effects of miR-126 were attributed to the preservation of microvascular integrity by enhancing vasculogenic progenitor cell mobilization after ischaemic AKI[178].

MicroRNAs in cisplatin-induced AKI and other forms of AKI.—A number of microRNAs have also been implicated in the pathogenesis of cisplatin-induced AKI[163,191–199] (TABLE 3). Our laboratory examined cisplatin-induced AKI in mice with proximal-tubule-specific deletion of *Dicer1*, which, to our surprise, developed a similar level of AKI in response to cisplatin treatment as wild-type mice, implying that overall depletion of microRNAs does not significantly affect cisplatin-induced AKI[198]. We also assessed changes in the expression of microRNAs using microarray analysis, leading to the identification of miR-375 as a pathogenic microRNA. This microRNA was significantly induced in response to NF-κB and p53 following cisplatin treatment, which in turn suppressed expression of the anti-apoptotic factor HNF1β (hepatocyte nuclear factor 1 homeobox B) and thereby induced renal tubular cell death. This study therefore uncovered a novel p53-NF-κB-miR-375-HNF1β signalling pathway that contributes to cisplatin-induced nephrotoxicity during chemotherapy. Another study showed that induction of miR-449 after cisplatin treatment in cultured renal tubular epithelial cells induced inhibition of SIRT1, increased acetylation of p53 and increased expression of the pro-apoptotic factor BAX (BCL-2 (B cell lymphoma 2)-associated X protein)[197]. Accordingly, blockade of miR-449 suppressed cell apoptosis induced by cisplatin in vitro via preservation of the SIRT1–p53–BAX signalling pathway. Moreover, miR-146b was found to be upregulated in cisplatin-treated renal tubular epithelial cells and cisplatin-treated rats[194]. The researchers further showed that miR-146b directly targets a regulator of cell proliferation and differentiation — erb-B2 receptor tyrosine kinase 4 (ErbB4). Suppression of miR-146b attenuated cisplatin nephrotoxicity in cultured tubular epithelial cells by inducing expression of ErbB4 and increasing cell proliferation. Finally, a 2018 study showed that miR-709, which is induced in mouse and human kidney samples following cisplatin treatment, targets the gene encoding mitochondrial transcriptional factor A (mtTFA) to induce mitochondrial dysfunction, thereby contributing to cisplatin-induced AKI[199].

In addition to ischaemic and cisplatin-induced AKI, microRNAs have been implicated in other types of AKI (TABLE 3). For example, miR-150 has been linked to myocardial-infarction-induced AKI; mice deficient in miR-150 were protected from inflammation, interstitial cell apoptosis and AKI following myocardial infarction[173]. In a mouse model of aristolochic-acid-induced nephropathy, induction of miR-192 in proximal tubular epithelial cells suppressed expression of MDM2 (murine double-minute 2), leading to activation of p53 and G2/M cell cycle arrest[200].

MicroRNAs as biomarkers for AKI

MicroRNAs are remarkably stable and can be detected and quantified in various body fluids, including plasma and urine[201]. The altered expression profile of specific microRNAs in AKI has led several groups to suggest that they could be used as biomarkers for the diagnosis of AKI[202–212] (TABLE 4). For example, miR-21 has been consistently reported to be highly elevated in urine and plasma samples of patients with AKI as well as in animal models of

IRI[204–206]. A 2011 study identified and validated the downregulation of miR-16 and miR-320 and upregulation of miR-210 in the plasma of patients with AKI (n = 77) compared with levels in healthy controls (n = 30)[202]. Another urinary microRNA profiling study revealed four microRNAs (miR-21, miR-200c, miR-423 and miR-4640) that were consistently changed in patients with AKI[206], whereas another identified a novel set of microRNAs, including miR-101-1-3p, miR-127-3p, miR-210-3p, miR-126-3p, miR-26b-5p, miR-29a-3p, miR-146a-5p, miR-27a-3p, miR-93-3p and miR-10a-5p, as downregulated in the serum of patients with AKI in an intensive care unit (n = 35) compared with levels in healthy controls (n = 20)[211]. This study also found that miR-26b-5p, miR-146a-5p, miR-93-3p and miR-127-3p had value as biomarkers to predict AKI predisposition in patients undergoing cardiac surgery as their levels were downregulated days before serum creatinine increased[211].

All the aforementioned studies demonstrate the potential of microRNAs as biomarkers for AKI; however, their specificity for AKI requires consideration. Some microRNAs are associated not only with AKI but also with other diseases, including other kidney diseases. For example, miR-21 is increased in AKI; however, serum miR-21 level is also significantly increased in patients with stroke or atherosclerosis[213], and urinary miR-21 levels are associated with CKD[214]. Moreover, microRNAs in different body fluids and blood fractions may have different biological relevance. For example, plasma levels of miR-210 were described by one study as an independent prognostic indicator of survival in AKI[202], but serum miR-210 levels were shown by another to be decreased in patients with AKI[211]. The involvement of many different microRNAs in AKI indicates that a panel of microRNAs would potentially be more useful than a single microRNA as a biomarker. Further research is needed in this area.

MicroRNAs in kidney repair

Despite intensive investigation of the role of microRNAs in AKI, a paucity of information exists regarding the role of microRNAs in kidney repair following AKI. A 2016 study used small RNA sequencing (RNA-seq) to generate temporal microRNA expression profiles, and 103 microRNAs were found to be differentially expressed during the injury and repair phase in a model of folic-acid-induced AKI[171]. Among these, three microRNAs were highly abundant and differentially expressed at different phases of injury: miR-18a-5p was induced during the acute injury phase, miR-132–3p was upregulated during the repair phase and miR-146b-5p was upregulated in the fibrotic phase. However, the function of these microRNAs was not investigated. Studies from the past few years have, however, indicated that some microRNAs that are induced during injury are possibly involved in kidney repair after AKI. For instance, induction of miR-687 during renal IRI inhibits PTEN to promote cell proliferation — an effect that might facilitate tissue repair or regeneration in ischaemic AKI[175]. On the other hand, miR-423–5p suppressed GSTM1 (glutathione-*S*-transferase Mu1) and inhibited cell proliferation, thereby preventing tissue repair following ischaemic AKI[215]. Similarly, miR-146b and miR-1247 limit kidney repair by inhibiting cell proliferation in cisplatin-induced or alcohol-induced AKI, respectively[194,216].

lncRNAs in AKI

Although less studied than microRNAs, some evidence exists to suggest a role for lncRNAs in AKI.

Using RNA-seq to perform whole-transcriptome profiling, one study identified a set of differentially expressed lncRNAs in renal proximal tubular epithelial cells under hypoxic and inflammatory conditions[217]. Among these, three lncRNAs (MIR210HG, long intergenic non-coding (linc)-ATP13A4–8 and linc-KIAA1737–2) were of particular note as they were most markedly induced by hypoxia and cytokines and were detectable in human kidney biopsy samples from kidney transplant patients, but the role of these lncRNAs in AKI and repair is unclear. Another study used RNA-seq and chromatin immunoprecipitation (ChIP) sequencing of HIF1 binding sites to identify a novel, hypoxia-inducible, HIF1-dependent lncRNA called DARS antisense RNA1 (DARS-AS1)[218]. Silencing of DARS-AS1 stimulated apoptosis of cultured renal tubular cells in response to hypoxia, indicating that DARS-AS1 protects against hypoxia-induced cell injury. Moreover, by quantitative-PCR-based disease-related lncRNA array, lncRNA-PRINS (psoriasis associated non-protein-coding RNA induced by stress) was identified as another hypoxia-induced, HIF1α-dependent lncRNA[219]. lncRNA-PRINS interacts with RANTES (regulated on activation, normal T cell expressed and secreted) — an important inflammatory mediator in ischaemic AKI — to regulate its production under hypoxic conditions, suggesting a role for lncRNA-PRINS in the pathogenesis of AKI[219].

Researchers have demonstrated a role for several disease-related lncRNAs, such as MALAT1 (metastasis-associated lung adenocarcinoma transcript 1; also known as NEAT2 (nuclear paraspeckle assembly transcript 2)) and GAS5 (growth arrest-specific 5), in ischaemic AKI. MALAT1, a large (6.5 kb), nuclear-enriched and highly conserved lncRNA, was one of the first lncRNAs to be associated with human disease; growing evidence indicates that MALAT1 participates in the development and progression of various cancers, including renal cell carcinoma. A 2015 study identified MALAT1 as the most strongly upregulated lncRNA in the kidneys — particularly proximal tubular cells — of mice subjected to inspiratory hypoxia[220]. A more recent study showed MALAT1 levels to be increased in biopsy samples from humans with ischaemic injury, plasma samples from patients with AKI, in kidneys of mice with IRI and in kidney cells cultured under hypoxia–reoxygenation conditions[221]. However, inhibition of MALAT1 by antisense oligonucleotides did not affect cell cycle progression, cell proliferation or apoptosis during hypoxia-reoxygenation injury in HK-2 (human kidney-2) cells. Moreover, genetic ablation of MALAT1 in mice did not affect kidney dysfunction or survival of mice following bilateral IRI or outer medullary injury, cell proliferation, interstitial fibrosis, inflammation or capillary rarefaction following unilateral IRI, indicating that MALAT1 is dispensable in the development of renal IRI and subsequent kidney repair[221]. A 2018 study identified GAS5 — a tumour suppressor lncRNA that regulates apoptosis and growth arrest — as a functional lncRNA in ischaemic AKI[222]. Similar to some of the other lncRNAs that have been linked to AKI, GAS5 is also hypoxia responsive, and its expression is increased in the kidneys of mice with renal IRI and in renal tubular epithelial cells that have undergone hypoxia–reoxygenation treatment. Knockdown of GAS5 reduced tubular cell death induced by

hypoxia–reoxygenation treatment, probably by regulating expression of thrombospondin 1 (REF.[222]).

In contrast to findings from MALAT1-knockdown studies in ischaemic AKI, knockdown of MALAT1 inhibited LPS-induced cell death in cultured renal tubular epithelial cells[223]. The detrimental effects of MALAT1 in LPS-induced tubular cell death might be mediated by inhibiting the suppressive effects of miR-146a on NF-KB signalling[223]. Several other lncRNAs are also upregulated in septic AKI and might contribute to disease pathogenesis. Similar to MALAT1, the lncRNA PVT1 (plasmacytoma variant translocation 1) is associated with diverse cancers. Expression of PVT1 is significantly upregulated by LPS treatment and might aggravate septic AKI by modulating TNF and JNK-NF-KB signalling pathways[224]. Similarly, the lncRNA NEAT1 is upregulated in patients with sepsis-induced AKI, acts to suppress miR-204 and activates NF-KB signalling, thereby promoting LPS-induced cell injury[225]. In addition, lncRNA MEG3 (maternally expressed gene 3) exacerbated LPS-induced kidney injury by modulating the miR-21-PDCD4 (programmed cell death protein 4) axis[226]. Moreover, inhibition of lncRNA HOTAIR (HOX transcript antisense RNA) attenuated sepsis-induced AKI in in vitro and in vivo models by regulating the miR-22–HMGB1 (high mobility group box 1) pathway[227]. These data indicate that lncRNAs might serve as novel therapeutic targets for ischaemic and septic AKI; however, information relating to the role of lncRNAs in other forms of AKI are lacking.

Circulating lncRNAs might also represent biomarkers for the diagnosis and prognosis of various diseases, including AKI. Several circulating lncRNAs — including MALAT1, NEAT1 and transcript predicting survival in AKI (TapSAKI; also known as MGAT3-AS1) — are upregulated in plasma and kidney biopsy samples of patients with AKI[221,225,228]. Importantly, levels of NEAT1 and TapSAKI are also associated with severity of AKI[225,228].

lncRNAs in kidney repair

Direct evidence linking lncRNAs to kidney repair following AKI is lacking; however, studies of lncRNAs in renal fibrosis provide some useful insights into the potential role of lncRNAs in kidney repair. One study used RNA-seq to identify a number of differentially expressed lncRNAs during the progression of renal fibrosis in mice with UUO[229]. Among these, 3110045C21Rik was found to regulate several fibrosis-related genes independent of TGFβ or IL-1β signalling pathways. Another two studies performed whole-transcriptome analyses in wild-type and *Smad3*-knockout mice with UUO, and in rats with UUO, to identify 151 SMAD3-dependent lncRNAs and 19 lncRNAs with SMAD3 binding motifs, respectively[230,231]. Further investigation revealed that TCONS_00088786, TCONS_01496394 and np_5318 (ErbB4-IR) contribute to TGFβ-SMAD3-mediated renal fibrosis in these models[231,232]. These findings suggest that lncRNAs might participate in the process of maladaptive repair after AKI, a proposal that requires further investigation.

Potential therapeutic applications

A number of epigenetic-modifying drugs, such as histone modifiers and DNA methylation inhibitors, are currently available and have been tested in preclinical models of AKI and CKD. Increasing histone acetylation with the use of HDACis typically protects kidneys from

AKI and promotes kidney repair. Similarly, inhibition of DNA methylation with the use of DNA methylation inhibitors (such as 5′-azacytidine and 5-aza-2′-deoxycytidine) or DNA demethylation activators (such as hydralazine) ameliorates renal fibrosis. Findings from preclinical studies suggest that targeting epigenetic mechanisms is a promising approach for the treatment of AKI and associated kidney diseases. Encouragingly, some of the epigenetic drugs are already in clinical use for other diseases, including the HDACis SAHA (vorinostat) for cutaneous T cell lymphoma and VPA for seizures and bipolar disorder, the DNA methylation inhibitors 5′-azacytidine (azacitidine) for myelodysplastic syndrome and 5-aza-2′-deoxycytidine (decitabine) for myelodysplastic syndrome and acute myeloid leukaemia and the DNA demethylation activator hydralazine for hypertension[233]. Of note, some of these drugs might be nephrotoxic, but none have been used in clinical trials for kidney diseases[233]. Preclinical studies have shown that some epigenetic drugs, such as HDACis, exert renoprotective effects at low doses but are nephrotoxic at higher doses[46,48]. Another concern about epigenetic drugs is that they are highly unspecific and induce global epigenetic changes that are not gene-specific or organ-specific. A 2017 description of a novel high-fidelity CRISPR–Cas9-based method to enable hydroxymethylation of specific genes is therefore of interest. Fusion of the catalytic domain of the DNA methylation eraser TET3 to an endonuclease deactivated high-fidelity Cas9 (dHFCas9) created a construct with which to demethylate genes that were specifically targeted by guiding RNAs[17]. Use of this method led to the successful reactivation of *Rasal1* and *Kl* (two anti-fibrotic genes that are hypermethylated and silenced in renal fibrosis), resulting in the attenuation of UUO-associated renal fibrosis[17]. This new technology could open up new avenues for potential therapeutic applications. Moreover, microRNA-based therapy has gained great interest in AKI and kidney repair, with the identification of a number of microRNAs linked to AKI pathogenesis (TABLE 3). Manipulation of specific microRNAs with the use of microRNA mimics or anti-microRNA oligonucleotides might attenuate AKI and/or promote kidney repair and recovery. However, no microRNAs are in clinical trials for AKI right now. A few microRNAs to date are already in clinical trials of other diseases[234]. In particular, an anti-miR-21 (RG-012) is now in a phase II clinical trial for Alport syndrome, a genetic disorder associated with kidney dysfunction.

Conclusions

AKI is now recognized as one of the most deadly diseases worldwide; however, the available treatment is mainly supportive. The past few years have witnessed rapid progress in our understanding of the contribution of epigenetic mechanisms to kidney function and disease, including AKI and associated pathologies, shedding light on potential new diagnostic and therapeutic strategies. These studies have also highlighted the complexity of epigenetic modifications and the network of interacting factors involved in processes such as histone modifications and DNA methylation, and in gene regulation by non-coding RNAs, providing better understanding of the fundamental elements of epigenetic regulation, specifically in AKI. Further research in this area will continue to identify new therapeutic targets as well as sensitive and specific diagnostic biomarkers for AKI and its progression to CKD.

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Box 1 | Tools for the analysis of DNA methylation

New microarray- and deep-sequencing-based technologies will facilitate the quantitative analysis of DNA methylation sites at single-nucleotide or single-cell resolution across the genome. The Illumina Infinium Methylation BeadChip array is a microarray-based method that is cost-effective and requires a small amount of input DNA, enabling >850,000 methylation sites to be interrogated at single-nucleotide resolution with exceptional high coverage of RefSeq genes (which define genomic sequences that can be used as reference standards for well-characterized genes) and CpG islands. The drawback of this method is that it is currently applicable only to human samples. Reduced representation bisulfite sequencing (RRBS) is a deep-sequencing technique that is as sensitive as whole-genome bisulfite sequencing but is more cost-effective as it involves sequencing of a representative sample of the whole genome, covering about 60% of promoters and 85% of CpG islands. The obvious disadvantage of RRBS is that it captures most, but not all, CpG sites in the genome. Genome-wide DNA methylation analysis not only reveals novel epigenetic changes that occur in AKI but also facilitates the identification of new therapeutic targets.

Epigenetic writers

Specific enzymes that add epigenetic marks on histone proteins or DNA.

Epigenetic readers

Effector proteins that recognize and bind epigenetic marks.

Epigenetic erasers

Specific enzymes that remove the epigenetic marks on histone proteins or DNA.

Core histones

Histones H2A, H2B, H3 and H4, which form the nucleosome core (also known as the histone octamer).

Linker histones

Histones that bind to internucleosomal DNA (also known as linker DNA), facilitating the formation of a compact chromatin structure.

Histone acetylation

Histone modification that involves the addition of an acetyl group to the ε-amine of lysine on all four core histones by histone acetyltransferases.

Histone methylation

Histone modification that involves the addition of a methyl group to a basic amino acid on core histones by histone methyltransferases

Histone crotonylation

Histone modification that adds a crotonyl group to lysine residues on the core histones by histone crotonyltransferases.

Matrix chromatin immunoprecipitation

High-throughput chromatin immunoprecipitation method in which antibodies are immobilized in a 96-well plate and all the procedures are done on the same plate without sample transfer.

Permissive histone marks

Histone modifications that promote gene transcription.

Elongation marks

Histone modifications that promote transcription elongation.

Repressive histone marks

Histone modifications that inhibit gene transcription.

CpG dinucleotides

Regions of DNA in which a cytosine nucleotide is followed by a guanine nucleotide, connected by a phosphodiester bond.

CpG islands

Regions of DNA >200 base pairs in length that have a CG content >50% and observed CpG (number of CpGs observed in a window):expected CpG (number of Cs × number of Gs/window length) ratio ≥0.6.

Genomic integrity

Integrity of the genome or genome stability.

X chromosome inactivation

Process that inactivates one of the two X chromosomes in female mammals.

Genomic imprinting

Biological process that epigenetically marks a gene, leading to gene expression in a parent-of-origin manner.

Hemimethylated DNA

DNA that has one strand methylated and another unmethylated.

tRNAs

Class of small RNA that carries a particular amino acid to the ribosome on the basis of the mRNA nucleotide sequences.

DNA hydroxymethylome profiling

Genome-wide analysis of DNA hydroxymethylation.

Hyper-hydroxymethylation

Increased DNA hydroxymethylation.

Reduced representation bisulfite sequencing

(RRBS). Genome-wide DNA methylation analysis method based on bisulfite sequencing that involves sequencing of a reduced, representative sample of the whole genome.

Small interfering RNAs

Class of small non-coding RNAs that bind to complementary mRNAs, leading to mRNA degradation and inhibition of protein translation.

Circulating non-coding RNAs

Non-coding RNAs that are present in the body fluid.

Dicer

Double-stranded RNA endoribonuclease that cleaves long or hairpin double-stranded RNA into small interfering RNA or precursor microRNA into microRNA.

Chromatin immunoprecipitation (ChIP)

Sequencing Method combining chromatin immunoprecipitation and nextgeneration sequencing that analyses the genome- wide DNA binding sites for transcription factors or other chromatin- associated proteins.

Inspiratory hypoxia

Hypoxia condition that animals are subjected to with a low inspiratory oxygen concentration (such as 8% of oxygen).

Key points

- Acute kidney injury (AKI) and subsequent kidney repair are associated with substantial epigenetic changes that have important roles in the pathogenesis and outcome of AKI.
- An overall increase in histone acetylation (for example, with the use of histone deacetylase inhibitors) might attenuate AKI and promote kidney repair, but the enzymes and downstream genes that mediate these effects remain elusive.
- DNA methylation might also affect AKI and kidney repair via modulation of downstream genes, but the nature of this regulation remains largely unknown.
- MicroRNAs are important factors in the regulation of AKI and kidney repair, but they can be pathogenic or protective depending on the specific microRNA species.
- Additional research into the epigenetic mechanisms underlying AKI may lead to the discovery of novel biomarkers and therapies for AKI.

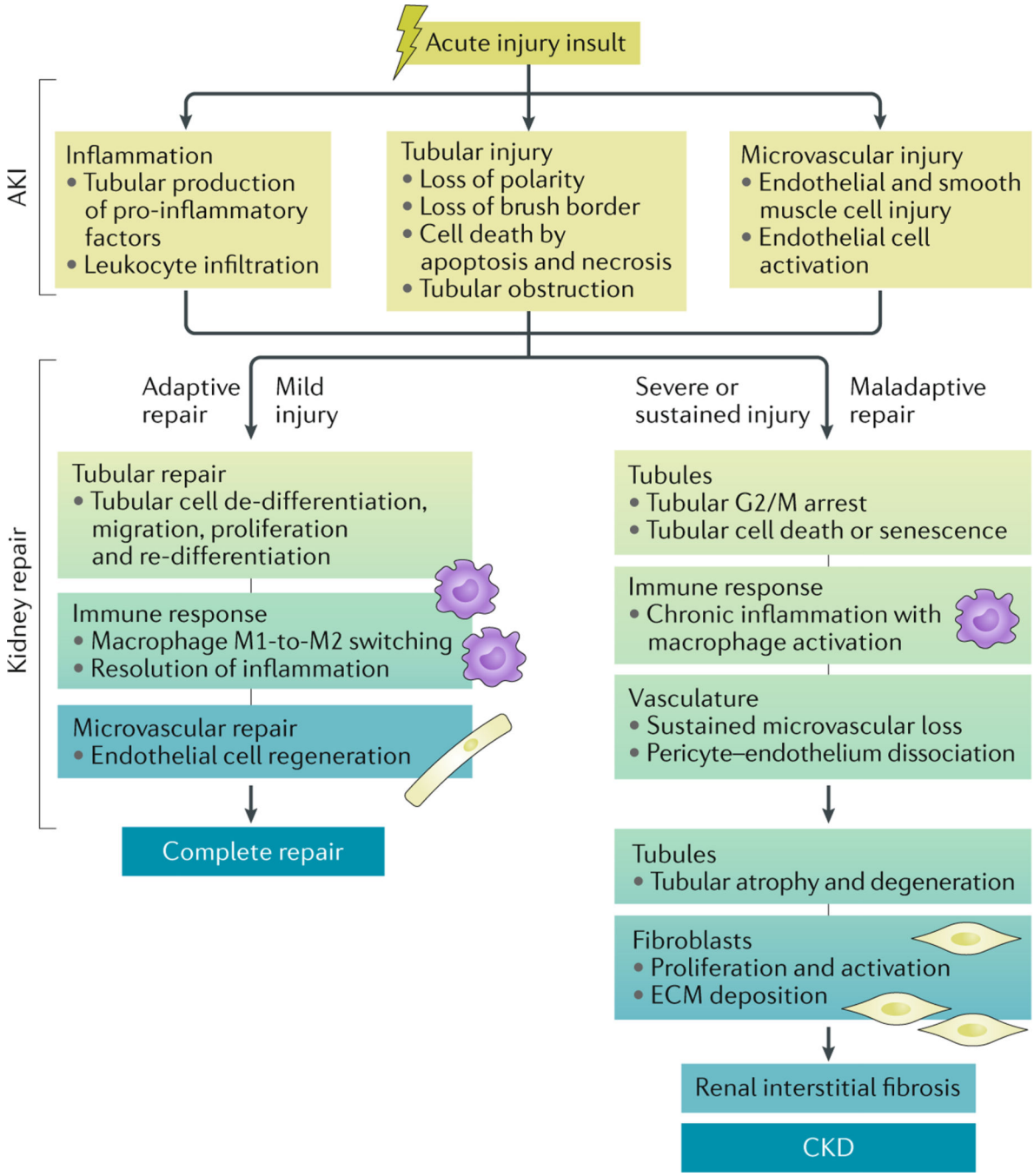

Fig. 1 |. Pathophysiology of AKI and repair.

The pathophysiology of acute kidney injury (AKI) is very complex, involving interplay between tubular, microvascular and inflammatory factors. Acute injury insults typically induce the injury and death of tubular epithelial cells, injury and activation of endothelial cells and leukocyte infiltration, culminating in renal dysfunction. In the presence of mild injury, adaptive repair mechanisms can restore epithelial integrity, suppress the immune response and re-establish healthy vasculature. By contrast, severe or persistent injury induces maladaptive repair. Tubular cells may undergo G2/M cell cycle arrest, senescence

and apoptosis or necrosis, leading to the release of profibrotic and pro-inflammatory factors. Tubular atrophy and degeneration, together with sustained inflammation and microvascular loss, result in renal interstitial fibrosis, characterized by the proliferation and activation of fibroblasts and deposition of extracellular matrix (ECM), ultimately leading to chronic kidney disease (CKD).

a

	Acetylation Ac	Methylation Me	Phosphorylation P
Writers	HATs	HMTs	Kinases
Readers	Bromodomains	Chromo, PHD, PWWP and tudor domains	SANT domain
Erasers	HDACs	HDMs	Phosphatases
Effects on transcription	Activation	Activation or repression	Activation or repression

b

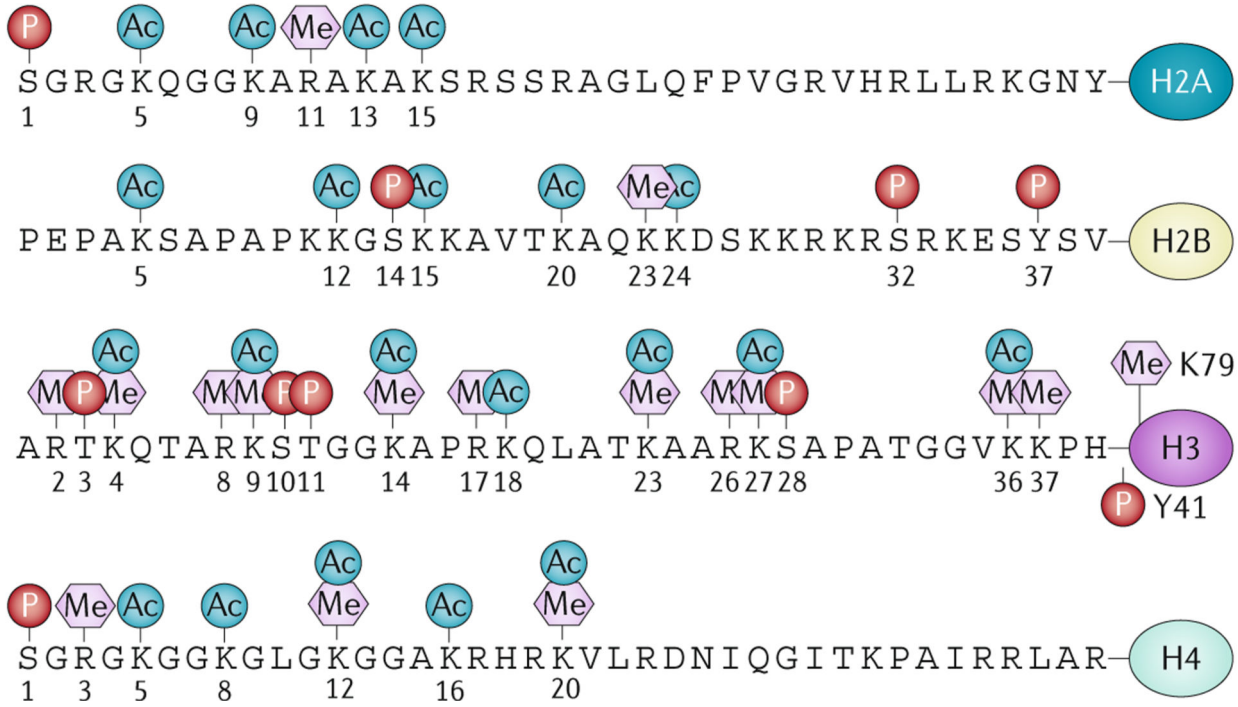

Fig. 2 |. Mechanisms and consequences of histone modifications.

a | Histone modifications (acetylation, methylation and phosphorylation) are catalysed by specific enzymes known as epigenetic writers, recognized by epigenetic readers and removed by epigenetic erasers. **b** | The major sites of histone acetylation, methylation and phosphorylation. Generally, histone acetylation is associated with permissive transcription, and histone methylation is associated with either active (H3 lysine 4 (H3K4), H3K36 and H3K79) or repressive (H3K9, H3K27 and H4K20) transcription. Phosphorylation of histones (for example, phosphorylation of H3 at serine 10 (H3S10ph), threonine 11 (H3T11ph), serine 28 (H3S28ph) and tyrosine 41 (H3Y41ph) and phosphorylation of H2B at serine 32 (H2BS32ph)) is often associated with transcriptional activation; however, histone phosphorylation (for example, of H2A at serine 1 (H2AS1ph), phosphorylation of H2B at tyrosine 37 (H2BY37ph) and phosphorylation of H4 at serine 1 (H4S1ph)) can be

associated with transcriptional repression. HATs, histone acetyltransferases; HDACs, histone deacetylases; HDMs, histone demethylases; HMTs, histone methyltransferases.

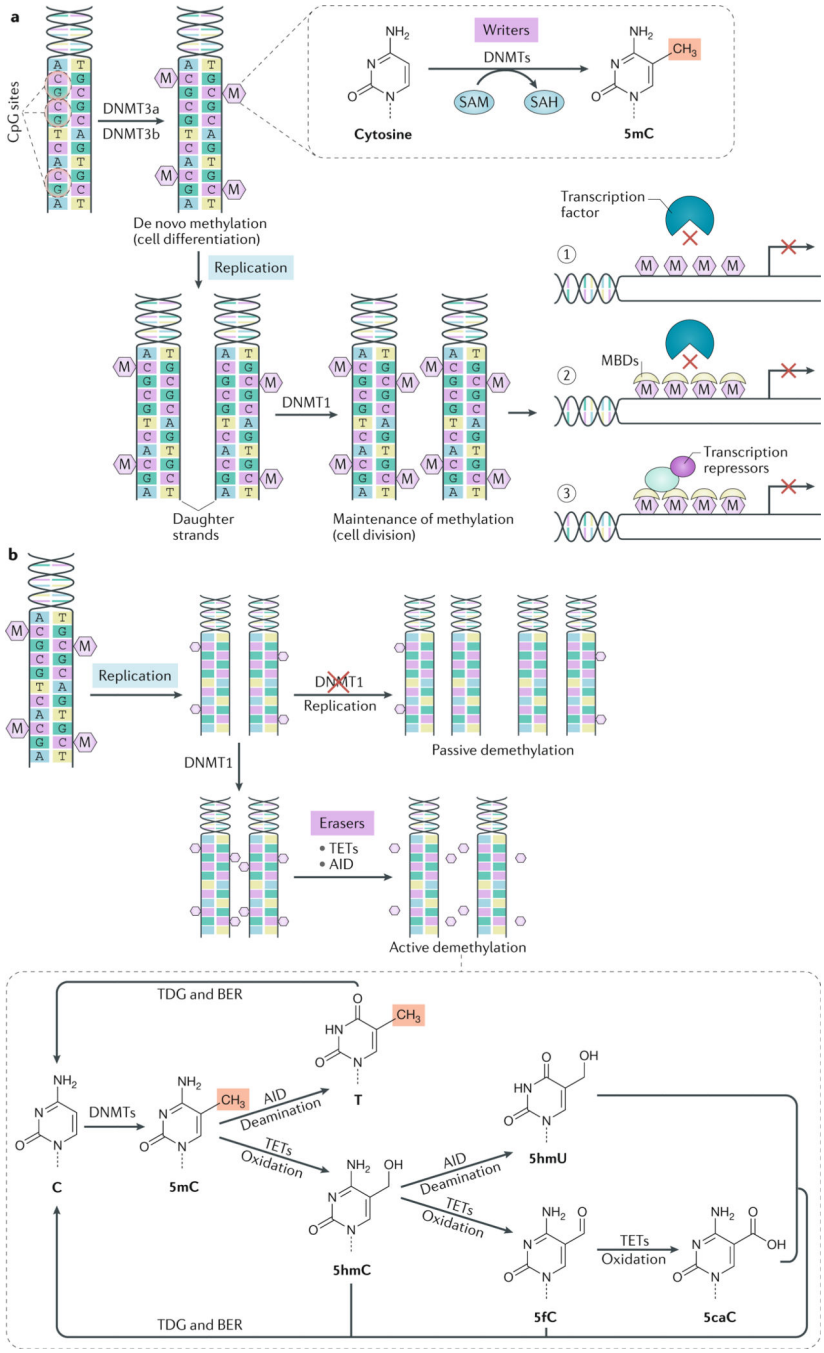

Fig. 3 |. Mechanisms of DNA methylation and demethylation.

a | DNA methylation involves the covalent addition of a methyl group (CH_3) to cytosine by DNA methyltransferases (DNMTs). Initial DNA methylation patterns are established by DNMT3a and DNMT3b. When DNA replication occurs, DNMT1 faithfully copies the DNA methylation patterns from parental strands to daughter strands. DNA methylation can inhibit gene expression directly by preventing transcription factor binding (1) or indirectly through the actions of DNA methylation readers, such as methyl-CpG-binding domain (MBD) proteins, which can either inhibit the binding of transcription factors (2) or recruit

transcription repressors (3), leading to gene suppression. **b** | The process of DNA demethylation can be passive or active. Passive demethylation occurs in the absence of functional DNMT1 during cell division, as newly synthesized DNA strands are not able to be methylated. Methyl groups can also be actively removed from DNA by an enzymatic replacement mechanism called active demethylation. On one hand, 5-methylcytosine (5mC; a methylated form of cytosine) can be deaminated to thymine by activation-induced cytidine deaminase (AID), which generates a mismatch between thymine and guanine bases. 5mC can also be oxidized by ten-eleven translocation (TET) proteins to 5-hydroxymethylcytosine (5hmC), which can be further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) or deaminated to 5-hydroxymethyl-uracil (5hmU). Finally, these intermediates are replaced by cytosine through thymine DNA glycosylase (TDG) and base excision repair (BER). SAH, S-adenosyl-L-homocysteine; SAM, *S*−adenosyl-L-methionine.

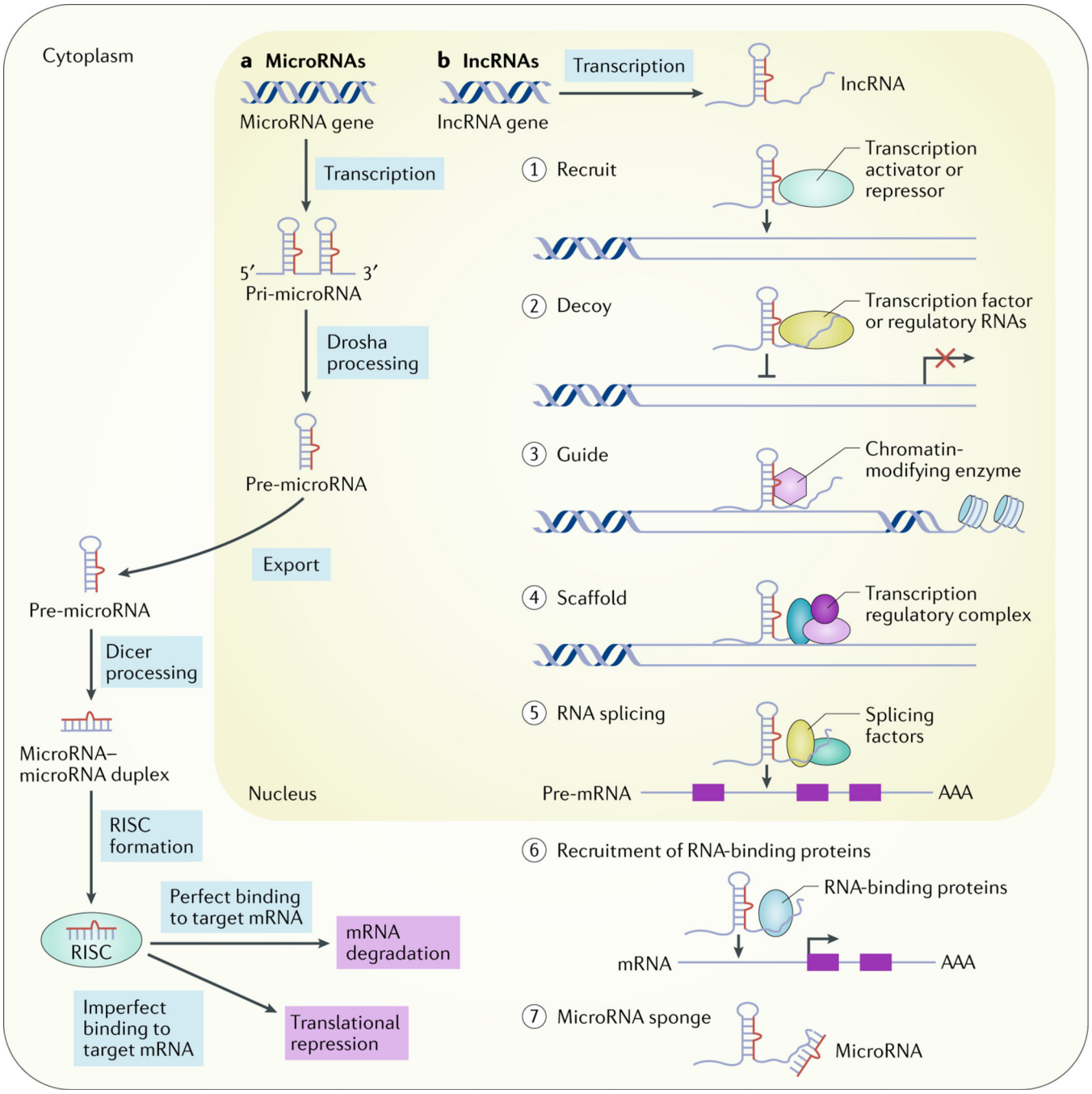

Fig. 4 |. Mechanisms of gene regulation by non-coding RNAs.

a | MicroRNA biogenesis requires initial transcription of a microRNA gene by RNA polymerase II into a large primary transcript that contains multiple hairpin loop structures, known as a primary microRNA (pri-microRNA). This transcript is processed by the ribonuclease, Drosha, exported from the nucleus and processed by another enzyme, called Dicer, to produce a microRNA–microRNA duplex in the cytoplasm consisting of a guide strand and a passenger strand. This complex is unwound and the mature microRNA (the guide strand) is incorporated into the ribonucleoprotein complex, RISC (RNA-induced

silencing complex), which is guided to target genes, leading to post-transcriptional silencing either by mRNA degradation or most commonly by translational repression. **b** | Long non-coding RNAs (lncRNAs) can regulate gene expression at both transcriptional (1–4) and post-transcriptional levels (5–7). At a transcriptional level, lncRNAs can (1) recruit transcription activators or repressors to the target gene, resulting in gene activation or suppression, respectively; (2) act as decoy factors by binding and sequestering transcription factors and other proteins or regulatory RNAs away from chromatin, thereby inhibiting gene transcription; (3) guide chromatin-modifying enzymes to specific gene targets and then modulate chromatin states either in *cis* or *trans*, leading to positive or negative regulation of gene expression; and (4) serve as a structural scaffold to recruit multiple proteins or RNAs to form transcription regulatory complexes, which can positively or negatively regulate gene expression. lncRNAs can also regulate gene expression at a post-transcriptional level by (5) binding to splicing factors to regulate RNA splicing, (6) directing RNA-binding proteins to the target mRNAs to either promote or inhibit protein translation or (7) serving as a molecular sponge that binds and titrates microRNAs away from their targets.

Table 1|

Histone-acetylation-based epigenetic drugs in AKI and repair

Drug	Model	Function	Mechanisms	Refs
HDACis				
TSA	Cisplatin nephrotoxicity in immortalized mouse proximal tubule cells	Protective	Restores CREB-mediated transcription as well as survival	47
	Cisplatin nephrotoxicity in RPTCs	Protective	Delays p53 phosphorylation, acetylation and activation	48
	Mouse cisplatin nephrotoxicity	Protective	Upregulates AMWAP	49
	Mouse cisplatin nephrotoxicity	Protective	Activates autophagy	50
	Mouse UUO	Protective	Enhances H3 and H4 acetylation and inhibits STAT3 activation	53
	Mouse unilateral IRI	Protective	Enhances H3 acetylation and miR-21	54
SAHA	Cisplatin nephrotoxicity in RPTCs	Protective	Inhibits p53 phosphorylation, acetylation and activation and blocks CHK2 phosphorylation	48
	Rat haemorrhagic shock	Protective	Increases H3K9Ac and decreases BAD	55
VPA	Rat haemorrhagic shock	Protective	Increases H3K9Ac and BCL-2 and decreases BAD	55
	Mouse adriamycin-induced nephropathy	Protective	Increases glomerular H3K9Ac	56
	Rat IRI	Protective	NA	57
MS-275	Mouse cisplatin nephrotoxicity	Protective	Upregulates AMWAP	49
	Mouse unilateral IRI	Protective	Enhances H3 acetylation	54
	Mouse folic-acid-induced AKI	Pathogenic	Increases H3 acetylation, suppresses the expression and phosphorylation of EGFR and inhibits STAT3 and AKT phosphorylation	58
	Mouse rhabdomyolysis-induced AKI	Pathogenic	Increases H3 acetylation and suppresses the expression and phosphorylation of EGFR	58
m4PTB	Zebrafish gentamicin-induced AKI	Protective	Increases proliferation of tubular epithelial cells	59
	Mouse IRI	Protective	Increases proliferation and reduces G2/M arrest of renal tubular epithelial cells and prevents interstitial fibrosis	59
	Mouse aristolochic acid nephropathy	Protective	Increases proliferation and reduces G2/M arrest of renal tubular epithelial cells and prevents interstitial fibrosis	60
Tubastatin A	Mouse rhabdomyolysis-induced AKI	Protective	Increases H3 acetylation and inhibits phosphorylation of NF-κB, expression of multiple cytokines and chemokines and oxidative stress	61
	Mouse cisplatin nephrotoxicity	Protective	Increases H3 acetylation, phosphorylation of AKT and autophagy and inhibits phosphorylation of NF-KB, expression of multiple cytokines and chemokines and oxidative stress	62
SIRT1 (HDAC) activators				
Resveratrol	Cultured RMICs treated with H_2O_2	Protective	NA	45

Drug	Model	Function	Mechanisms	Refs
	Rat cisplatin nephrotoxicity	Protective	Inhibits inflammatory cell filtration and reduces lipid peroxidation and glutathione depletion	63
	Rat septic AKI	Protective	Deacetylates SOD2	64
SRT-2183	Cultured RMICs treated with H_2O_2	Protective	Induces COX2 and PGE_2	45
SRT-1720	Mouse UUO	Protective	Induces COX2 and PGE_2	45
	Mouse IRI	Protective	NA	65
HAT inhibitor				
Curcumin	Rat cisplatin nephrotoxicity	Protective	Inhibits inflammatory response and oxidative stress	66
	Rat IRI	Protective	Reduces oxidation products MDA, NO and PC in serum and kidney tissue	67
	Rat LPS-induced AKI	Protective	Inhibits interstitial inflammatory infiltration	68

AKI, acute kidney injury; AKT, protein kinase B; AMWAP, activated microglia/macrophage WAP domain protein; BAD, BCL-2 associated death promoter protein; BCL-2, B cell lymphoma 2; CHK2, checkpoint kinase 2; COX2, cyclooxygenase 2; CREB, cAMP-responsive element binding protein; EGFR, epidermal growth factor receptor; H3K9Ac, histone H3 lysine 9 acetylation; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDACi, HDAC inhibitor; IRI, ischaemia–reperfusion injury; LPS, lipopolysaccharide; m4PTB, 4-methyl-thiobutanate; MDA, malondialdehyde; NA, not available; NO, nitric oxide; PC, protein carbonyl; PGE_2, prostaglandin E_2; RMICs, mouse renal medullary interstitial cells; RPTCs, rat proximal tubular cells; SAHA, suberoylanilide hydroxamic acid; SIRT1, sirtuin 1; SOD2, superoxide dismutase 2; STAT3, signal transducer and activator of transcription 3; TSA, trichostatin A; UUO, unilateral ureteral obstruction; VPA, valproic acid.

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Table 2|

DNA methylation alterations in AKI and repair

Gene	Model	DNA methylation alteration region	DNA methylation level change	Effects on gene expression	Detection method	Refs
C3	Rat IRI, kidney	The IFNγ-responsive element in *C3* promoter	↓	NA	Bisulfite sequencing	121
C3	Rat kidney transplantation, kidney	IL-1-IL-6 response element and NF-κB binding site 2 in *C3* promoter	↓	NA	Pyrosequencing	122
Slc22a12	Mouse contact-freezing-induced AKI, plasma	Promoter	↓	NA	TaqMan-based unmethylated DNA-specific PCR and bisulfite sequencing	123
KLK1	Patients with AKI, blood	Promoter	↑	NA	Pyrosequencing	124
CALCA	Kidney transplant patients, urine	Promoter	↑	NA	Methylation-specific PCR	125
Cxcl10 and *Ifngr2*	Mouse IRI, kidney	Promoter	↓5hmC	↑	hMeDIP-qPCR	126
Irf8	Mouse cisplatin nephrotoxicity, kidney	5′ UTR	↓	↑	RRBS	129
Rasali	Mouse unilateral IRI, kidney	Promoter	↑	↓	DNA MeDIP	133

5hmC, 5-hydroxymethylcytosine; 5′ UTR, 5′ untranslated region; AKI, acute kidney injury; hMeDIP-qPCR, hydroxymethylated DNA immunoprecipitation-quantitative PCR; IRI, ischaemia–reperfusion injury; MeDIP, methylated DNA immunoprecipitation; NA, not available; RRBS, reduced representation bisulfite sequencing.

Table 3|

MictoRNAs in AKI

MictoRNA	Upstream effector	Target	Function	Model	Response	Manipulation	Refs
MicroRNAs in ischaemic AKI							
miR-24	NA	S1P1, H2AX and HO1	Pathogenic	Kidney transplantation patients, mouse IRI, HK-2 cell ATP depletion and endothelial cell hypoxia–reoxygenation	↑	• In vitro: mimic and anti-miR •In vivo: anti-miR	172
miR-150	NA	IGF1R[a]	Pathogenic	Mouse IRI	↓	miR-150-deficient mice	173
miR-494	NA	ATF3	Pathogenic	Mouse IRI and urine from patients with AKI	↑	In vivo: mimic	174
miR-687	HIF1	PTEN	Pathogenic	Mouse IRI, HEK cells and BUMPT cell hypoxia	↑	• In vitro: mimic and anti-miR • In vivo: anti-miR	175
miR-17–5p	p53	DR6	Protective	Mouse IRI and RPTC hypoxia	↑	• In vitro: mimic and anti-miR • In vivo: mimic	176
miR-21	HIF1α	PDCD4, TSP1 and Rab11a	Protective and/or pathogenic	Mouse IPC, mouse IRI, rat IRI, urine and plasma from patients with AKI, NRK-52E cell hypoxia–reoxygenation, cultured human renal epithelial cell $CoCl_2$ or hypoxia	↑	• In vitro: mimic and anti-miR • In vivo: anti-miR	169,184–188
miR-26a	NA	IL-6	Protective	Mouse IRI	↓	In vivo: mimic	177
miR-126	NA	PI3KR2	Protective	Mouse IRI	NA	miR-126 conditional overexpressing mice	178
miR-127	HIF1α	KIF3B	Protective	Rat IRI, and NRK-52E cell and HK-2 cell hypoxia–reoxygenation	↑	In vitro: mimic and anti-miR	179
miR-146a	NF-KB	IRAK1	Protective	Kidney transplantation patients, mouse unilateral IRI and LPS-challenged kidney following IRI	↑	• In vitro: mimic and anti-miR • In vivo: anti-miR and miR146a-deficient mice	169,180,181
miR-205	NA	EGLN2	Protective	HK-2 cell hypoxia–reoxygenation	↓	In vitro: mimic and anti-miR	182
miR-489	HIF1α	PARP1	Protective	Mouse IRI, RPTC hypoxia and ATP depletion	↑	• In vitro: mimic and anti-miR • In vivo: anti-miR	183
MicroRNAs in cisplatin nephrotoxicity							
miR-34a	p53	SIRT1[a]	Protective	Mouse, BUMPT cells, NRK-52E cells	↑	In vitro: mimic and anti-miR	191,192
miR-125b	NRF2	AhRR	Protective	Mouse, NRK-52E cells	↑	In vitro: mimic	193
miR-155	NA	c-Fos	Protective	Mouse	NA	miR-155-deficient mice	195
miR-122	NA	FOXO3	Pathogenic	Mouse, NRK-52E cells	↓	In vitro: mimic and anti-miR	192
miR-146b	NA	ErbB4	Pathogenic	Serum from patients with AKI, rat, NRK-52E cells	↑	In vitro: anti-miR	194

MictoRNA	Upstream effector	Target	Function	Model	Response	Manipulation	Refs
miR-181a	NA	BCL-2	Pathogenic	HK-2 cells	↑	In vitro: anti-miR	196
miR-375	p53 and NF-KB	HNF1β	Pathogenic	Mouse and RPTCs	↑	In vitro: anti-miR	198
miR-449	NA	SIRT1[a]	Pathogenic	NRK-52E cells	↑	In vitro: anti-miR	197
miR-709	NA	mtTFA	Pathogenic	Kidney biopsy samples from patients with AKI, mouse and mPTCs	↑	• In vitro: mimic and anti-miR • In vivo: anti-miR	199
MicroRNAs in other types of AKI							
miR-150	NA	IGF1R[a]	Pathogenic	Myocardial-infarction-induced AKI	NA	miR-150-deficient mice	173
miR-192	NA	MDM2	Pathogenic	Aristolochic acid nephropathy	↑	NA	200

AhRR, aryl hydrocarbon receptor repressor; AKI, acute kidney injury; anti-miR, anti-microRNA oligonucleotides; ATF3, activating transcription factor 3; BCL-2, B cell lymphoma 2; BUMPT cells, a mouse renal proximal tubular cell line; DR6, death receptor 6; EGLN2, Egl nine homologue 2; ErbB4, erb-B2 receptor tyrosine kinase 4; HEK cells, human embryonic kidney cells; HIF1, hypoxia-inducible factor 1; HK-2 cells, an immortalized human proximal tubular epithelial cell line; HNF1β, hepatocyte nuclear factor 1 homeobox B; HO1, haem oxygenase 1; IGF1R, insulin-like growth factor 1 receptor; IPC, ischaemic preconditioning; IRAK1, IL-1 receptor-associated kinase 1; IRI, ischaemia-reperfusion injury; KIF3B, kinesin family member 3B; LPS, lipopolysaccharide; MDM2, murine double-minute 2; mPTCs, mouse proximal tubular epithelial cells; mtTFA, mitochondrial transcriptional factor A; NA, not available; NRF2, nuclear factor erythroid-2-related factor 2; NRK-52E cells, a rat renal proximal tubular cell line; PARP1, poly(ADP-ribose) polymerase 1; PDCD4, programmed cell death protein 4; PI3KR2, phosphoinositide 3-kinase regulatory subunit 2; PTEN, phosphatase and tensin homologue; RPTC, rat proximal tubular cell; S1P1, sphingosine-1-phosphate receptor 1; SIRT1, sirtuin 1; TSP1, thrombospondin 1.

[a]An indirect target.

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Table 4|

potential microRNA biomarkers for the diagnosis of AKI

MictoRNA	Model	Source	Species	Response	Refs
miR-210, miR-16 and miR-320	Patients with AKI	Plasma	Human	↑ (miR-16 and miR-320↓)	202
miR-21 and miR-155	Patients with AKI	Urine	Human	↑ (miR-155↓)	204
miR-21	Patients with AKI after cardiac surgery	Plasma and urine	Human	↑	205
miR-494	Patients with AKI	Urine	Human	↑	174
miR-21, miR-200c, miR-423 and miR-4640	Patients with AKI	Urine	Human	↑ (miR-4640↓)	206
miR-101–1-3p, miR-127–3p, miR-210–3p, miR-126–3p, miR-26b-5p, miR-29a-3p, miR-146a-5p, miR-27a-3p, miR-93–3p and miR-10a-5p	Patients with AKI	Serum	Human	↓	211
miR-10a and miR-30d	Ischaemic AKI	Urine	Mouse	↑	203
miR-10a, miR-192 and miR-194	Ischaemic AKI	Plasma	Rat	↑	208
miR-714, miR-1188, miR-1897–3p, miR-877 and miR-1224	Ischaemic AKI	Plasma and kidney	Mouse	↑	210
let-7g-5p, miR-93–5p, miR-191a-5p and miR-192–5p	Cisplatin nephrotoxicity	Urine	Rat	↑	207
miR-200a–c, miR-192 and miR-194	Contact-freezing-induced AKI	Plasma	Mouse	↑	212

AKI, acute kidney injury