DNA methylation protects against cisplatin-induced kidney injury by regulating specific genes, including interferon regulatory factor 8

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DNA methylation is an epigenetic mechanism that regulates gene transcription without changing primary nucleotide sequences. In mammals, DNA methylation involves the covalent addition of a methyl group to the 5-carbon position of cytosine by DNA methyltransferases (DNMTs). The change of DNA methylation and its pathological role in acute kidney injury (AKI) remain largely unknown. Here, we analyzed genome-wide DNA methylation during cisplatin-induced AKI by reduced representation bisulfite sequencing. This technique identified 215 differentially methylated regions between the kidneys of control and cisplatin-treated animals. While most of the differentially methylated regions were in the intergenic, intronic, and coding DNA sequences, some were located in the promoter or promoter-regulatory regions of 15 protein-coding genes. To determine the pathological role of DNA methylation, we initially examined the effects of the DNA methylation inhibitor 5-aza-2′-deoxycytidine and showed it increased cisplatin-induced apoptosis in a rat kidney proximal tubular cell line. We further established a kidney proximal tubule-specific DNMT1 (PT-DNMT1) knockout mouse model, which showed more severe AKI during cisplatin treatment than wild-type mice. Finally, interferon regulatory factor 8 (Irf8), a pro-apoptotic factor, was identified as a hypomethylated gene in cisplatin-induced AKI, and this hypomethylation was associated with a marked induction of Irf8. In the rat kidney proximal tubular cells, the knockdown of Irf8 suppressed cisplatin-induced apoptosis, supporting a pro-death role of Irf8 in renal tubular cells. Thus, DNA methylation plays a protective role in cisplatin-induced AKI by regulating specific genes, such as Irf8.

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Acute kidney injury (AKI), formerly known as acute renal failure, is generally defined as a rapid decrease in kidney function within a few hours to days. AKI is now recognized as a major public health problem with profound consequences, including high mortality rates and increased costs and length of hospital stay.[1] Major clinical causes of AKI include renal ischemia reperfusion, nephrotoxicity, and sepsis. Approximately 20% of AKI cases are associated with exposures to nephrotoxic drugs. Among these, cisplatin (cis-diamminedichloroplatinum II) is one of the most commonly used and highly effective chemotherapy drugs for treating a wide variety of cancer types, such as testicular, ovarian, head and neck, bladder, small and non-small cell lung and cervical cancers, as well as sarcomas and lymphomas.[2,3] However, cisplatin is notorious for its adverse effects in normal tissues and organs, particularly in the kidney, which limits its therapeutic use and efficacy.[4–8] After a single-dose cisplatin treatment, 25% to 35% of patients experience renal function deterioration. In the kidneys, cisplatin accumulates at high concentrations in renal tubular cells (approximately 5 times higher concentration than that in the blood), causing tubular cell injury and death, which is a key determinant of AKI.[9] Recently, the pathogenesis of cisplatin-induced AKI has been intensively studied, and multiple signaling pathways have been implicated, such as apoptosis and necrosis pathways in renal tubular cells, and inflammatory and oxidative stress signaling.[4–8] Despite these studies, the molecular basis of cisplatin nephrotoxicity remains unclear, and no effective therapies are available for renoprotection during chemotherapy.

DNA methylation is an important epigenetic mechanism that involves the covalent addition of a methyl group to the 5-carbon position of the cytosine in the CpG dinucleotide sequences by DNA methyltransferases (DNMTs).[10] DNA methylation profiles are replicated through cell division and heritably regulate gene transcription without changing primary nucleotide sequences. There are 3 major DNMTs (DNMT1, DNMT3a, and DNMT3b). DNMT1 is a maintenance DNMT and is also the most abundant DNMT in various cell types; DNMT3a and DNMT3b are *de novo* methyltransferases that establish initial DNA methylation patterns.[11,12] When DNMT1 is inhibited or absent during cell division, newly synthesized DNA strands cannot be methylated, resulting in passive demethylation in daughter cells and dilution of DNA methylation in the cell population. Conversely, active demethylation can be achieved by the enzymatic replacement of methyl cytosine to cytosine by enzymes such as thymine DNA glycosylase, activation-induced deaminase, and ten-eleven translocation methylcytosine dioxygenase.[13,14] The fundamental role of DNA methylation in cell biology is transcriptional regulation. The hypermethylation of the gene promoter regions generally leads to heterochromatin with highly packed DNA, decreased accessibility of transcription factors, and loss of gene expression.[15,16] In contrast, DNA hypomethylation may correlate with the activation of gene transcription or lead to genomic instability.[17] Hence, DNA methylation plays essential roles in mammalian development, genomic integrity, X chromosome inactivation (females), and genomic imprinting. Moreover, aberrant DNA methylation changes have been implicated in a wide variety of disease conditions such as cardiovascular diseases, neurologic diseases, and cancer.[18–23] For example, global DNA hypomethylation accompanied by hypermethylation of tumor suppressor genes is recognized as an epigenetic hallmark of cancer.[24,25]

Several studies have recently suggested the involvement of DNA methylation changes in kidney diseases, including kidney fibrosis,[26–28] diabetic nephropathy,[29,30] and chronic kidney disease.[31,32] However, little is known regarding the roles and regulatory mechanisms of DNA methylation in AKI.[33–35] In this study, we analyzed global DNA methylation changes during cisplatin-induced AKI or nephrotoxicity. Functionally, the inhibition of DNA methylation by 5-aza-2′-deoxycytidine (5-aza) increased cisplatin-induced apoptosis *in vitro*, and DNMT1 ablation from kidney proximal tubules enhanced cisplatin-induced AKI in mice, suggesting a renoprotective role of DNA methylation. We further identified interferon regulatory factor 8 (Irf8) as a hypomethylated gene during cisplatin treatment and showed that its induction contributes to tubular cell apoptosis.

RESULTS

Genome-wide changes in DNA methylation during cisplatin-induced AKI

To determine genome-wide DNA methylation changes in cisplatin-induced AKI, we used the reduced representation bisulfite sequencing (RRBS) to identify DNA methylation at single-base resolution. Genomic DNAs were isolated from the kidney cortex and outer medulla of control and cisplatin-treated mice and were then subjected to RRBS analysis. A total of 1.5 and 1.9 million of CpG sites were analyzed in the control and cisplatin-treated kidney samples, respectively. Cisplatin treatment induced obvious changes in DNA methylation, as shown in the heat map (Figure 1a). Using 200-base pair (bp) nonoverlapping windows, we identified 215 differentially methylated regions (DMRs) between the control and cisplatin-treated kidneys that showed significant ($>\pm0.25$) differences in methylation (Figure 1b). In DMR genome-wide distribution analysis, 83% of DMRs were found in the intergenic regions, introns, and coding DNA sequences, and only 7% of DMRs were at the 5′-ends and 5′-untranslated regions (UTRs) of the promoters or regulatory regions of protein-coding genes (Figure 1c). This result is consistent with a recent study of genome-wide DNA methylation patterns in chronic kidney disease,[28] in which most DMRs were distributed in the intronic and transcription termination regions and 3′-UTRs and not in the gene promoter regions. DNA methylation in the gene promoter regions is considered to be critical for transcriptional regulation. Our analysis identified 15 genes with DMRs in their 5′-ends and 5′-UTRs of regulatory regions, and subsequent functional analyses indicated that these genes are involved in gene transcription, cell cycle control, and apoptosis (Supplementary Figure S1). Altogether, these results indicate that cisplatin treatment induces changes in DNA methylation patterns in kidney tissues and suggest the roles of DNA methylation in cisplatin-induced AKI by regulating specific genes.

The DNA methylation inhibitor 5-aza increases cisplatin-induced apoptosis in rat kidney proximal tubular cells

To determine whether DNA methylation plays a pathogenic role in cisplatin-induced AKI, we first assessed the effects of 5-aza, a pharmacological DNA methylation inhibitor, on cisplatin-induced apoptosis in rat kidney proximal tubular cells (RPTCs). As a cytidine analog, 5-aza may be incorporated into DNA and irreversibly bind to DNMTs, resulting in DNMT inactivation and degradation.[36–38] Treatment with 1 μM 5-aza could diminish DNMT1 and DNMT3a expressions (Figure 2a) without noticeable toxicity in RPTCs (Figure 2b and c). Importantly, 5-aza significantly increased apoptosis during cisplatin treatment (Figure 2b). Typical apoptotic cells showed cellular shrinkage and formation of apoptotic bodies viewed by phase contrast microscopy (Figure 2b, upper panel) and nuclear condensation and fragmentation viewed by Hoechst 33342 nucleus staining (Figure 2b, bottom panel). Cell counting experiments showed that cisplatin induced 24% apoptosis, which increased to 55% after 5-aza pretreatment (Figure 2c). These morphologic observations were verified by immunoblot analyses that showed enhanced cleavage of caspase 3 and poly (ADP-ribose) polymerase, which are biochemical hallmarks of apoptosis (Figure 2a). Collectively, these results demonstrate a sensitizing