Overexpressed SIRT6 attenuates cisplatin-induced acute kidney injury by inhibiting ERK1/2 signaling

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Sirtuin 6 (SIRT6) is a NAD$^+$-dependent deacetylase associated with numerous aspects of health and physiology. Overexpression of SIRT6 has emerged as a protector in cardiac tissues against pathologic cardiac hypertrophy. However, the mechanism of this protective effect is not fully understood. Here, both *in vivo* and *in vitro* results demonstrated that SIRT6 overexpression can attenuate cisplatin-induced kidney injury in terms of renal dysfunction, inflammation and apoptosis. In addition, SIRT6 knockout aggravated kidney injury caused by cisplatin. We also found that SIRT6 bound to the promoters of ERK1 and ERK2 and deacetylated histone 3 at Lys9 (H3K9) thereby inhibiting ERK1/2 expression. Furthermore, inhibition of ERK1/2 activity eliminated aggravation of kidney injury caused by SIRT6 knock out. Thus, our findings uncover the protective effect of SIRT6 on the kidney and define a new mechanism by which SIRT6 regulates inflammation and apoptosis. This may provide a new therapeutic target for kidney injury under stress.

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Sirtuin 6 (SIRT6) is a NAD$^+$ (nicotinamide adenine nucleotide positive)-dependent enzyme associated with many biological processes, mainly through 2 distinct enzymatic reactions: adenosine diphosphate-ribosylation and deacetylation of both histone and long-chain fatty acyl groups.[1–4] By deacetylating histone 3 at Lys 9 or 56, SIRT6 decreases chromatin accessibility for transcription factors, such as nuclear factor κB, c-Jun, c-Myc, and Foxo3, to their target promoters, which inhibits the expression of their target genes.[5–8] Based on these activities, SIRT6 is involved in DNA damage repair and modulating various inflammation and apoptosis-related signaling.[9]

Cisplatin (CP) is a common and effective chemotherapeutic drug for the treatment of many kinds of solid tumors.[10–12] It is mainly excreted by the kidney and accumulates in renal proximal tubular cells.[13] This results in a positive feedback loop among oxidative stress, inflammation, and apoptosis, which is a major cause of CP-induced renal injury.[14,15] Inhibition of mitogen-activated protein kinase and tumor necrosis factor-α reduces CP-induced kidney injury.[16–18] Exogenous resveratrol and β-lapachone attenuate the CP-induced kidney injury by mediating sirtuins.[19,20] More effective interventions need to be explored.

SIRT6 overexpression is capable of blocking the development of pathologic cardiac hypertrophy and heart failure from hypertrophic stimuli, although no obvious effect is seen without these stressors.[6] Furthermore, SIRT6 protects the heart from ischemia/reperfusion injury through forkhead box O3α activation.[21] SIRT1 and SIRT3 could protect the kidney from different types of injuries.[22] However, the function of SIRT6 on the kidney remains unclear. Our previous study showed that calorie restriction enhances SIRT6 expression, alleviating renal insufficiency caused by aging.[23] To further clarify the function of SIRT6 in the kidney and discover new therapeutic interventions for CP-induced acute kidney injury, SIRT6-mutated mice and primary kidney epithelial cells were used to investigate the effect of SIRT6 on the kidney under CP stress. Our results confirmed that SIRT6 overexpression attenuates CP-induced acute kidney injury in mice; conversely, SIRT6 deficiency exacerbates CP-induced renal injury. Furthermore, we discovered that SIRT6 could inhibit the expression of both extracellular signal–regulated kinase (ERK) 1 and 2, which may define a new mechanism for the SIRT6 protecting effect. Our study provides a new therapeutic target for kidney injury under stress.

RESULTS

SIRT6 protects primary kidney epithelial cells from CP-induced apoptosis

We obtained primary kidney epithelial cells from SIRT6 mutant mice and wild-type (WT) mice to investigate the effect of the protein level of SIRT6 on CP-induced apoptosis. Megalin immunostaining demonstrated that >95% of cells were of proximal tubule origin (Supplementary Figure S1). According to Western blot assay, we found that there were significant changes in SIRT6 expression in the mutant cells (Figure 1a). The cells were treated with CP for 24 hours; we then compared the stress response in different groups, including WT cells treated with dimethylsulfoxide (DMSO) or CP (WT+CP), SIRT6 overexpressed cells treated with DMSO (transgenic [TG]) or CP (TG+CP), and SIRT6-deficient cells treated with DMSO (knockout [KO]) or CP (KO+CP). CP treatment caused a significant decrease in cell viability in the WT and SIRT6 KO cells, but this effect was weaker in the SIRT6 overexpression line (Figure 1b and Supplementary Figure S2A). Under the microscope, we could find massive apoptosis in WT+CP mice, and the percentage of apoptotic cells was markedly reduced in TG+CP mice compared with WT+CP mice, whereas KO+CP mice did not show more apoptosis than WT+CP mice (Figure 1c and Supplementary Figure S2B). Flow cytometry was used to measure cell-cycle status and revealed fewer S-phase cells after treatment with CP. This effect was reduced in TG+CP cells compared with WT+CP cells (Figure 1d and e). As for the

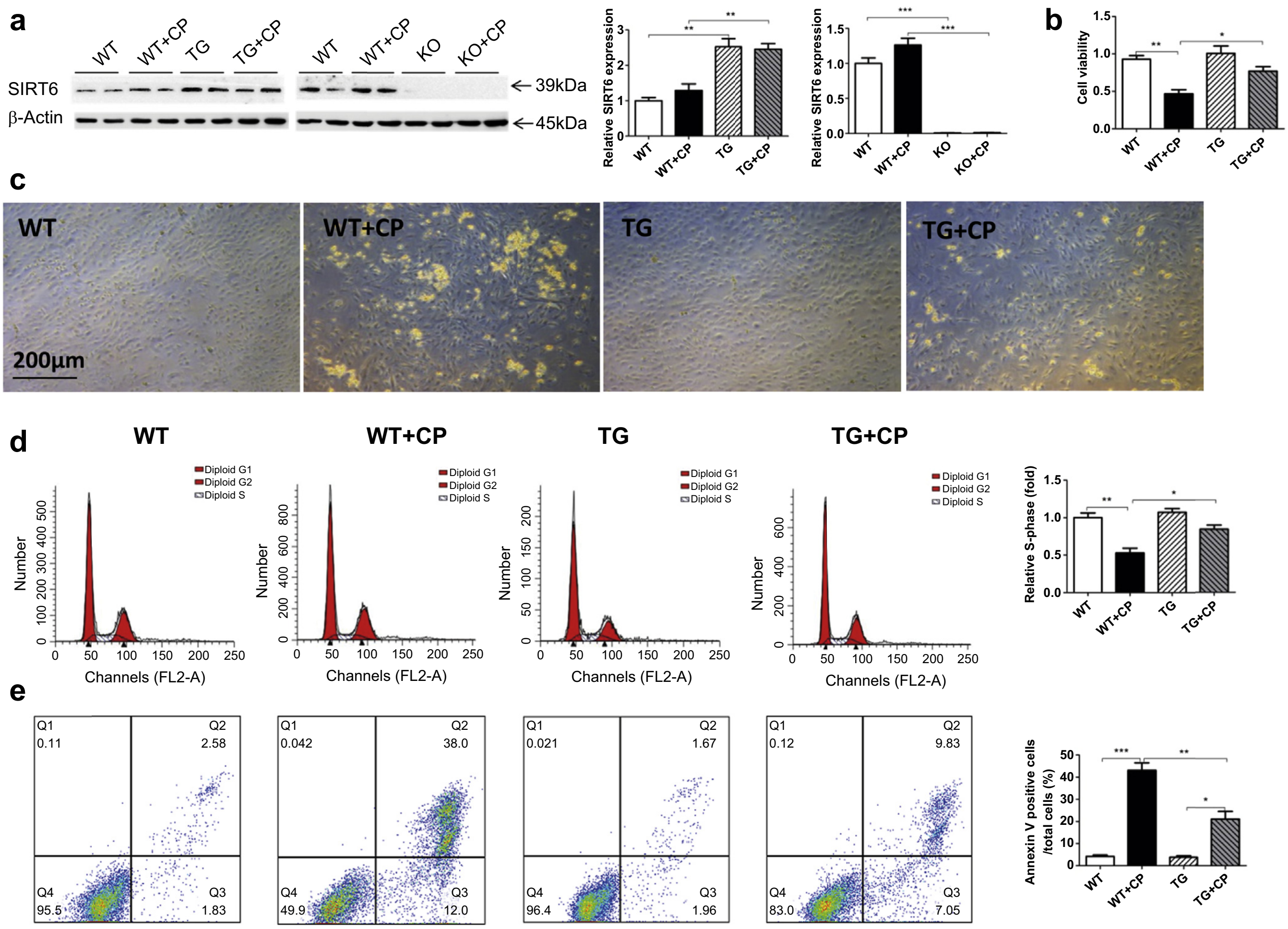

Figure 1 | SIRT6 (sirtuin 6) overexpression protects renal primary cells against cisplatin (CP)-induced apoptosis. (**a**) After treatment with CP for 24 hours, whole-cell lysate was collected for a Western blot assay of SIRT6. Wild-type (WT) cells treated with dimethylsulfoxide (DMSO) (WT) or cisplatin (WT+CP), SIRT6 overexpressed cells treated with DMSO (TG) or cisplatin (TG+CP), and SIRT6 knockout (KO) cells treated with DMSO (KO) or cisplatin (KO+CP) are shown. Representative Western blots and statistical results are shown ($N = 4$). Data are presented as mean $\pm$ SD. **$P < 0.05$, ***$P < 0.001$. (**b**) Cell Counting Kit-8 was used to estimate the cell viability of renal primary cells ($N = 3$). Data are presented as mean $\pm$ SD. *$P < 0.05$, **$P < 0.01$. (**c**) Representative images of renal primary cells processed with or without CP (25 μM). (**d**) Renal primary cells were stained with propidium iodide and subjected to a flow cytometer to determine the fraction of cells in the S phase ($N = 3$). Data are presented as mean $\pm$ SD. *$P < 0.05$, **$P < 0.01$. (**e**) Renal primary cells were stained with annexin V and propium iodine, and the results were tested by flow cytometry ($N = 3$). Data are presented as mean $\pm$ SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Dip, diploid. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.