Chicken ovalbumin upstream promoter-transcription factor II protects against cisplatin-induced acute kidney injury

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Abstract. The chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) plays essential roles in organogenesis of embryos. Recently COUP-TFII is also implicated in several diseases in adults. Here we focus on the role of COUP-TFII in cisplatin-induced acute kidney injury (AKI). COUP-TFII was the most abundantly expressed in the kidney among organs. Male tamoxifen-inducible COUP-TFII-knockout mice or control mice were intraperitoneally treated with 30 mg/kg body weight of cisplatin at 12 weeks old to induce AKI. The kidney samples were subject to morphological studies, terminal deoxynucleotidyl transferase-mediated deoxyuridine nick-end labeling (TUNEL) assay, immunohistochemistry and RT-qPCR. Serum levels of creatinine, blood urea nitrogen (BUN) and tumor necrosis factor alpha (TNF-α) were measured. Administration of cisplatin induced a more severe AKI in adult COUP-TFII-knockout mice. An increase in dead cells in both the proximal tubules and thick ascending limb of Henle’s loop (TAL) was observed in the knockout mouse kidney. The expression levels of COUP-TFII decreased in the TAL by cisplatin administration. There was no difference in the expression levels of transporter mRNAs responsible for cellular cisplatin uptake between control and knockout mouse kidney. COUP-TFII-knockout mice and COUP-TFII-depleted cells exhibited an elevation in TNF-α levels, suggesting the involvement of the TNF-α pathway. Chromatin immunoprecipitation showed that COUP-TFII was enriched in the potential binding site, suggesting that COUP-TFII might directly suppress the TNF-α gene at transcriptional level. These results indicate the involvement of COUP-TFII in the pathophysiology of AKI and COUP-TFII may be a potential therapeutic target for AKI.

Key words: Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), Acute kidney injury (AKI), Cisplatin, Tumor necrosis factor alfa (TNF-α), Nuclear receptor

CHICKEN OV ALBUMIN UPSTREAM PROMOTER-TRANSCRIPTION FACTOR II (COUP-TFII), also known as NR2F2, is an orphan nuclear hormone receptor whose ligand is yet to be identified [1]. COUP-TFII plays essential roles in organogenesis and cell-type commitment during development in murine models [2, 3]. Knockout mice die around embryonic day 10 due to defects in angiogenesis and cardiogenesis [4]. Notably, COUP-TFII plays an essential role in cell viability and organogenesis in the embryonic kidney [5]. In the adult mouse kidney, COUP-TFII is expressed in the distal tubules, podocytes, and epithelial cells of the Bowman’s capsule, but not in the proximal tubules or collecting ducts. In addition, COUP-TFII is highly expressed in interstitial cells [6]. Recently COUP-TFII has been implicated in the pathogenesis of several diseases, including obesity, cancer, and muscular dystrophy [7-9]. Considering the abundant expression of COUP-TFII in the kidney, and its essential role in the organogenesis and cell viability, particularly during several diseases, we hypothesized that it may be involved in renal diseases. Therefore, we set out to determine if COUP-TFII is involved in adult renal diseases using mouse model.

Acute kidney injury (AKI) is characterized by a sudden decrease in renal functions and leads to high mortality and morbidity. A systematic review by the American Society of Nephrology reported that approximately 20% of hospitalized patients suffering from AKI have an overall mortality rate of 23% [10]. Risk factors for AKI include critical illness, sepsis, burns, surgery, iodinated radiocontrast agents, and nephrotoxic agents. Therefore, AKI is a heterogeneous group of conditions with various
etologies. The treatment regimens vary depending on the cause of AKI, but effective therapies supported by high quality evidence are not yet established [11].

Cisplatin is a platinum-based chemotherapeutic agent that is widely used for the treatment of several cancers in various organs. Cisplatin binds to DNA and induces mitochondrial damage and apoptosis through multiple mechanisms [12]. One serious side effect of cisplatin treatment is AKI, as cisplatin accumulates in tubular cells of the kidney after excretion into primitive urine [13]. The S3 segment of the proximal tubules and the thick ascending limb of the Henle’s loop (TAL) are primarily affected in the nephrotoxic type of AKI [14]. Multiple pathways are reported to be involved in cisplatin-induced AKI [15]. Particularly, tumor necrosis factor alpha (TNF-α) produced in renal parenchymal cells plays an important role in cisplatin-induced AKI [16, 17]. In addition, therapeutic approaches for this side effect are still under development.

Here we deleted the COUP-TFII gene in adult mice and induced AKI by cisplatin treatment, to study whether COUP-TFII is involved in the pathogenesis of AKI.

Materials and methods

Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine and follow the National Institutes of Health guide for the care and use of Laboratory animals. The COUP-TFII-floxed [18] and ROSA26CRE-ER<sup>12</sup> strains [19] were crossed to generate a tamoxifen-inducible COUP-TFII-knockout system [8]. The mice were backcrossed to the 129 Sv background for more than 10 generations. The 8-week-old male control or knockout mice were intraperitoneally injected with 0.5 mg of tamoxifen for 5 consecutive days to delete the COUP-TFII gene, as previously described [8]. Intraperitoneal administration of cisplatin (cis-diammineplatinum(II) dichloride, Sigma) (30 mg/kg body weight) was performed on 12-week-old mice. Mice were deeply anesthetized with isoflurane and then sacrificed by cervical dislocation 24 or 72 hours after treatment. Trunk blood was collected to measure the serum levels of creatinine and blood urea nitrogen (BUN) which was performed by the Comparative Pathology Laboratory at Baylor College of Medicine.

Cell culture

MDCK cells and VA-13 cells were cultured in D-MEM supplemented with 10% FBS. For TUNEL assay in MDCK cells, cells were cultured on the poly-L-lysine-coated coverslips and incubated with 100 μM of cisplatin either in the presence or absence of pentoxifylline (0.5 mM, Tokyo Chemical Industry) for 24 h. Transfection in VA-13 cells with siRNAs was done using TransIT-X2 Dynamic Delivery System (Mirus) according to the manufacturer’s instructions. The target sequence of siRNA against COUP-TFII is 5'-ccaacccagacgagauu-3'.

Antibodies and lectins

The primary antibodies used in this study include COUP-TFII (Perseus Proteomics), Tamm-Horsfall protein (THP, Santa Cruz), and CD10 (Novus). Lotus tetragonolobus lectin (LTL) and peanut agglutinin (PNA) were obtained from Vector Laboratories.

Histological studies

Periodic acid-Schiff staining was done by incubating renal sections in 1% periodic acid solution for 10 minutes and in Schiff’s reagent for 15 minutes, followed by hematoxylin counterstaining. Experimental procedures for immunofluorescence and immunohistochemistry were previously reported [5]. The terminal deoxynucleotidyl transferase-mediated deoxyuridine nick-end labeling (TUNEL) assay on renal tissues or MDCK cells was done according to a previous report [20].

Quantitative RT-PCR

Total RNA was extracted from control and COUP-TFII-knockout mice kidney and siRNA-treated VA-13 cells with QIAzol (Qiagen) according to the manufacturer’s instructions. Premium total RNA (Clonetech) was used for tissue distribution studies. Approximately 0.5 μg of total RNA was used for cDNA synthesis. Quantitative RT-PCR was performed using either TaqMan Assays (Applied Biosystems) or THUNDERBIRD SYBR qPCR mix (TOYOBO) according to the manufacturer’s instructions. The mRNA levels were normalized to those of 18S rRNA and were expressed as the relative amount compared to the control group. The primers used for quantitative RT-PCR are: 5'-tggtctgtcacaccttctc-3' (forward) and 5'-cccagcagagaagccacacc-3' (reverse) for OCT2, 5'-cgacacattcctacac-3' (forward) and 5'-tcacccattttcgcgtt-3' (reverse) for Ctr1, 5'-cccagagggagaagtttc-3' (forward) and 5'-tacaggtgtgtctcggg-3' (reverse) for TNF-α, 5'-ctctgtggtgatgggtgg-3' (forward) and 5'-acgggcgggtgtagcaggg-3' (reverse) for 18S rRNA. TaqMan assays were used for COUP-TFII.

Measurements of serum TNF-α levels

TNF-α levels in mouse serum were measured using the mouse TNF-α ELISA kit (Invitrogen) according to the manufacturer’s instructions.
**Chromatin immunoprecipitation**

Chromatin immunoprecipitation was done by using VA-13 cells and EZ-ChIP (Upstate) Kit according to the manufacturer’s instructions. Immunoprecipitation was done using either COUP-TFII antibody or normal IgG and immunoprecipitated chromatin was analyzed by quantitative PCR. The primers used for PCR are: 5'-ctctctcccctggaaagga-3' (forward) and 5'-gccacgatcaggagagaa-3' (reverse) for negative control in the coding region at and around TNF-α locus. To identify evolutionally conserved potential COUP-TFII binding site, rVista 2.0 (https://rvista.dcode.org) was used.

**Statistical analyses**

The Student’s t-test, one-way analysis of variance (ANOVA) and Tukey’s HSD test were done using JMP (SAS Institute).

**Results**

**COUP-TFII is substantially expressed in human kidney**

To study the roles of COUP-TFII in adults, we first analyzed the tissue distribution of COUP-TFII in human. Among multiple organs examined, COUP-TFII was the most highly expressed in kidney (Fig. 1A), indicating that COUP-TFII may be involved in renal function even in adult.

COUP-TFII is essential for the development of mouse embryos. Knockout mouse die around embryonic day 10 due to defects in angiogenesis and cardiogenesis [4]. Therefore, conventional knockout mice cannot be utilized to study the functions of COUP-TFII in postnatal or adult animals. To overcome this problem, we developed a tamoxifen-inducible systemic COUP-TFII-knockout system by crossing COUP-TFII-floxed mice with the ROSA26CRE-ER<sup>T2</sup> strain [8]. In this model, the COUP-TFII gene remains intact until tamoxifen administration. We deleted the COUP-TFII gene in 8-week-old adult mice (Fig. 1B). This approach resulted in more than 90% of reduction in the COUP-TFII mRNA levels in the knockout mouse kidney (Fig. 1C). In addition, efficient systemic decrease in COUP-TFII protein levels was previously reported in these mice [21]. Consistent with previous report [6], COUP-TFII was expressed in the distal tubules, podocytes, epithelial cells of the Bowman’s capsule and interstitial cells (Fig. 1D). Immunohistochemistry of the knockout mouse kidney revealed that COUP-TFII signal was almost undetectable in most of the cells. The signal remained only in some of the interstitial cells of the knockout mouse kidney (Fig. 1D).

**COUP-TFII-knockout mice are more susceptible to cisplatin-induced acute kidney injury**

Serum levels of creatinine and BUN usually increase when the filtration function of the kidney does not work properly. We did not detect any differences in the levels of these molecules in serum between COUP-TFII-knockout mice and control mice, indicating that the knockout mice are not suffering from overt renal failure. However, it has been reported that COUP-TFII plays important roles in some pathological conditions in adults [7-9]. Moreover, COUP-TFII plays an essential role in cell viability in the embryonic kidney [5]. Therefore, we studied the functions of COUP-TFII in one of the serious renal diseases, AKI, in which tubular cell death plays central roles [11]. AKI was induced at 12 weeks of age by cisplatin administration, an established animal model for AKI [16]. Analyses of mice were done 24 or 72 hours after cisplatin exposure (Fig. 1B). Although more specific markers have been proposed for AKI [22], serum creatinine and BUN are the most traditionally established markers of renal failure. The levels of creatinine were significantly higher in the COUP-TFII-knockout mice, as compared to the controls, at both 24 and 72 hours after the induction of AKI (Fig. 2A). These results indicate that the mutant mice suffer from a more severe AKI. Similar findings were obtained for the levels of serum BUN, a less specific marker when compared to creatinine, but the difference was significant only at 24 hours (Fig. 2B).

Consistent with the findings of serum creatinine and BUN levels, no obvious morphological abnormalities were identified in COUP-TFII-null kidneys without cisplatin administration (Fig. 2C). The major lesions in cisplatin-induced AKI are found in the renal tubules [15]. In accordance with previous reports [14], dilation of renal tubules and formation of casts were observed in control kidneys exposed to cisplatin. The COUP-TFII-knockout mouse kidneys showed more severe structural changes, including a thyroid-like appearance, which is induced by the dilation of renal tubules and deposition of excessive THP in the lumen of the tubules (Fig. 2C). These morphological changes in the mutant animals indicate the occurrence of a more severe AKI.

**COUP-TFII-knockout mouse kidney contains more TUNEL-positive cells in both proximal tubules and TALs in the murine model of cisplatin-induced AKI**

To visualize tubular cell death in cisplatin-induced AKI, we utilized the TUNEL assay, which is widely used to detect apoptotic cells, although it also detects non-apoptotic cells such as necroptotic cells [23]. We did not find any TUNEL-positive cells in the mouse kidneys without cisplatin treatment (Fig. 3A). In control mouse
kidneys treated with cisplatin, only a few TUNEL-positive cells were found 24 hours after cisplatin exposure, and the number of positive cells increased substantially 72 hours after treatment. As compared to the control subjects, a greater number of TUNEL-positive cells were identified in COUP-TFII-null mutants at both 24 and 72 hours after treatment (Fig. 3A and B). These results indicate that the more severe AKI phenotype in COUP-TFII-null mutants is associated with increased tubular cell death.

Fig. 1  COUP-TFII is substantially expressed in human kidney. (A) COUP-TFII mRNA levels among multiple human organs. The mRNA levels were expressed as relative values to the level in the pancreas. (B) Schematic representation of the experimental schedule. Adult male tamoxifen-inducible COUP-TFII-knockout mice and control mice were treated with tamoxifen at 8 weeks of age to delete COUP-TFII. Cisplatin was administered at 12 weeks of age, and the mice were subjected to the analyses 24 or 72 hours after cisplatin treatment. (C) Efficiency of COUP-TFII deletion in the knockout mouse kidney as determined by mRNA levels. (D) Representative images of COUP-TFII immunohistochemistry. COUP-TFII was expressed in the distal tubules (circle), podocytes (arrowhead), epithelial cells of the Bowman’s capsule (thin arrows) and interstitial cells (thick arrows) in the control mice. Nuclear COUP-TFII signals remain in some of the interstitial cells of the knockout mouse kidney. *: \( p < 0.05, n = 4 \) for each of the control group and knockout group. Scale bars: 20 μm.
Next, we tried to determine the identities of the TUNEL-positive cells in the mutant mice. We stained renal specimens using tubule-type specific lectins after the TUNEL assays (Fig. 3C). While many dead cells were co-stained by a proximal tubule-specific LTL, some TUNEL-positive cells were positive for PNA, a marker for distal tubules and TALs [24]. These results are consistent with findings in wild-type C57BL/6 mice treated with cisplatin [25]. The S3 segment of the proximal tubules and TALs are primarily affected in the nephrotoxic type of AKI [14]. Since COUP-TFII is not expressed in the proximal tubules [6], we further examined the damage to TALs using a specific marker THP. At least some TUNEL-positive cells in the COUP-TFII-
Fig. 3  
*COUP-TFII*-knockout mouse kidney has more TUNEL-positive cells in both the proximal tubules and TAL in the murine model of cisplatin-induced AKI.

(A) TUNEL assays using kidney sections of mice 24 or 72 hours after exposure to cisplatin show more TUNEL-positive cells (green, nuclear signal) in the knockout subjects. (B) Numbers of TUNEL positive cells were counted and represented as relative values to total cell numbers. (C) TUNEL-positive cells were observed in the proximal tubule and distal tubule/TAL. Renal sections from *COUP-TFII*-knockout mice, 72 hours after cisplatin treatment, were stained with LTL (proximal tubule marker; left, red, membrane signal on the lumen side) or PNA (distal tubule marker and TAL; right, red, membrane signal on the lumen side) after the TUNEL assay (green, nuclear signal). (D) Some TUNEL-positive cells are observed in the TAL. TUNEL assays were performed on *COUP-TFII*-knockout mouse kidney, 72 hours after cisplatin treatment. The adjacent section was stained for the TAL marker THP. Some of the TAL cells are positive for TUNEL (red circles). CTRL: control mice, KO: knockout mice, w/o: without cisplatin, DAPI: 4′,6-diamidino-2-phenylindole. *: p < 0.05, n = 4 for each group. Scale bars: 50 μm (A), 25 μm (B, C).
knockout animals appeared to be positive for the TAL marker, as determined by staining of the adjacent section (Fig. 3D). These results indicate that proximal tubules and TALs are more severely affected in cisplatin-treated COUP-TFII-knockout mice as compared to the controls.

**The expression level of COUP-TFII decreases in the TALS in the murine model of cisplatin-induced acute kidney injury**

In the adult mouse kidney, COUP-TFII is expressed in the distal tubules, but not in the proximal tubules. In addition, COUP-TFII is highly expressed in interstitial cells [6]. Since the primary affected sites in AKI include the S3 segment of the proximal tubules and the TAL, we tested whether COUP-TFII is expressed in these cells (Fig. 4A). Immunofluorescent staining for COUP-TFII in control kidneys revealed that COUP-TFII immunoreactive cells were negative for CD10, a marker for the S3 segment. These results are consistent with previous reports [5, 6]. Based on morphology, at least some of the COUP-TFII-positive cells were indicative of interstitial cells. In contrast, TAL epithelial cells expressed COUP-TFII, suggesting potential cell-autonomous roles for COUP-TFII in the TAL in AKI. After exposure to cisplatin, the expression levels of COUP-TFII gradually decreased (Fig. 4A and B). In addition, immunohistochemistry of COUP-TFII showed that its signal diminished in all types of cells, including the distal tubules, podocytes, epithelial cells of the Bowman’s capsule and interstitial cells, in the cisplatin-treated kidneys (Fig. 4C). These results suggest that decreased expression of COUP-TFII might be involved in the defects of AKI induced by cisplatin.

**COUP-TFII-knockout mice exhibit elevated levels of TNF-α**

Cellular uptake of cisplatin in the kidney is mediated by transporters, including organic cation transporter 2 (OCT2) and copper transport protein (Ctr1) [26, 27]. The up-regulation of these transporters would increase the intracellular concentration of cisplatin, resulting in severe nephrotoxicity. To test whether the more severe AKI phenotype in COUP-TFII-null mice is due to increased cisplatin uptake, we studied the expression levels of these transporters in the kidney. We did not observe any differences between control and knockout mice, indicating that the knockout phenotype was not due to the different cellular uptake levels of cisplatin (Fig. 5A and B).

Immune responses are involved in several aspects of AKI, including multiple types of cellular death in the renal tubules. TNF-α produced in renal parenchymal cells plays an important role in cisplatin-induced AKI [16, 17]. On the other hand, neutralizing TNF-α antibody ameliorates cisplatin nephrotoxicity [28]. These previous studies indicate the involvement of TNF-α on pathogenesis of AKI. To examine the involvement of TNF-α on cisplatin-induced AKI, we used cultured MDCK cells, which are derived from tubular epithelial cells of dog kidney, and treated with cisplatin. As shown in Fig. 5C, cisplatin treatment markedly increased the number of TUNEL-positive cells, whereas inhibition of TNF-α by pentoxifylline decreased the number of TUNEL-positive cells, indicating the involvement of TNF-α on the pathogenesis of cisplatin-induced AKI. Then, we measured the serum levels of TNF-α in AKI-induced mice. The levels of TNF-α in the serum increased after cisplatin treatment in control animals. On the other hand, the increases in TNF-α levels were much more dramatic in the COUP-TFII-null mice, 24 hours after cisplatin administration (Fig. 5D). This is consistent with the more severe phenotype of cisplatin-treated knockout animals. Interestingly, the serum levels of TNF-α in the COUP-TFII-knockout mice were similar to the levels in the control mice, 72 hours after cisplatin treatment. Consistent with these facts, kidneys in COUP-TFII-knockout mice showed elevated levels of TNF-α mRNA (Fig. 5E).

To further dissect the potential mechanisms of TNF-α overproduction in COUP-TFII-knockout mice, we utilized RNA interference and ChIP assay. Since knockdown of COUP-TFII did not work in dog-derived MDCK cells (data not shown), we used VA-13 cells, which are derived from human fibroblast and known to express COUP-TFII [29]. Depletion of COUP-TFII by siRNA significantly increased the mRNA levels of TNF-α (Fig. 5F), recapitulating in vivo phenomena. Since COUP-TFII directly binds DNA and works as a transcriptional repressor [1], we searched for evolutionally conserved COUP-TFII binding sites around TNF-α gene locus. Sequence analyses revealed a potential COUP-TFII binding site that is conserved between human and mouse, at 3.5kb downstream of TNF-α gene body. Chromatin immunoprecipitation showed that COUP-TFII was enriched in the potential binding site but not in the coding region (Fig. 5G). These results suggest that COUP-TFII might directly suppress the TNF-α gene at transcriptional level.

**Discussion**

In this study, we found that adult COUP-TFII-knockout mice are more susceptible to cisplatin-induced AKI compared to control mice, as determined by serum markers for renal failure, renal morphology, and tubular cell death. COUP-TFII is highly expressed in mouse embryos and plays pivotal roles during development. In
contrast, the expression levels of COUP-TFII are dramatically decreased in adults, including the kidneys [1, 6]. Thus, little is known about the roles of COUP-TFII in adults as compared to those in embryos. In addition, no obvious changes were observed when the COUP-TFII gene was deleted in adult mice using the ROSA26CRE-ERT2 system. These findings suggest that the function of COUP-TFII in healthy adult subjects is limited; however, roles for COUP-TFII in adult animal models of several diseases have been reported [7-9]. Our findings now provide additional information on the involvement of COUP-TFII in adult disease models.

The increase in cell death was observed in the COUP-TFII-null mutants, as determined by the TUNEL assay. While this method has traditionally been used to detect apoptotic cells, it was reported recently that the TUNEL assay also labels cells undergoing programmed necrosis (necroptosis) [30, 31]. Although tubular cell apoptosis has been regarded as the main etiology of AKI, necroptosis is also involved in its pathophysiology [32, 33]. In addition, it is accepted that autophagy is observed in AKI [34]. Although the targeted pathway(s) of COUP-TFII in AKI is not clear, it was reported that COUP-TFII-knockout embryos exhibit apoptosis in renal precursor

Fig. 4  Expression levels of COUP-TFII decrease in the TAL in the murine model of cisplatin-induced AKI.
(A) Renal sections from wild type mice, without cisplatin treatment or 24 or 72 hours after treatment, were stained for COUP-TFII (green, nuclear signal) with the marker for the S3 segment of proximal tubules CD10 (upper, red, membrane signal on the lumen side) or the TAL marker THP (bottom, red, membrane signal on the lumen side), shown in red. COUP-TFII is expressed in the TAL as well as other cell types, but not in the S3 segment. The expression levels of COUP-TFII decreased after cisplatin administration. (B) Decrease in COUP-TFII mRNA levels in the kidneys 72 hours after cisplatin treatment. (C) Decrease in COUP-TFII levels in the cisplatin-treated kidneys were determined by immunohistochemistry. Nuclear COUP-TFII signals diminished in all types of cells including the distal tubules (circle), podocytes (arrowhead), epithelial cells of the Bowman’s capsule (thin arrows) and interstitial cells (thick arrows), in the cisplatin-treated kidneys. w/o: without cisplatin. Scale bars: 50 μm.
metanephric mesenchyme cells [5]. Therefore, it seems likely that COUP-TFII has a protective role against apoptosis of tubular cells in the AKI model. In addition, we cannot exclude the possibility that COUP-TFII might regulate necroptosis and autophagy as well.

In drug-induced AKI, the S3 segment of proximal tubules and the TAL are mainly affected [14]. Although the S3 segment is considered as the most sensitive site, the TAL is also highly affected in AKI as it is a metabolically active site [35]. In addition, Srichai et al. reported that apoptosis of TAL cells alone results in AKI [36]. While the S3 segment is negative for COUP-TFII, TAL cells express COUP-TFII. Thus, loss of COUP-TFII might contribute to severe apoptosis of TAL cells and AKI. In addition, a previous study has shown that proinflammatory cytokines including TNF-α suppress COUP-TFII [37]. Therefore, a decrease in COUP-TFII expression in TAL might be a result of cisplatin-stimulated TNF-α production. A TAL-specific deletion of the COUP-TFII gene in mice would be needed to

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**Fig. 5** COUP-TFII-knockout mice exhibit elevated levels of TNF-α.

(A, B) Relative mRNA levels for Oct2 (A) and Ctr1 (B) in control and COUP-TFII-knockout mouse kidney without cisplatin treatment. (C) Inhibition of TNF-α by pentoxifylline decreased the number of TUNEL positive cells in cisplatin-treated MDCK cells. (D) Serum levels of TNF-α were evaluated 24 or 72 hours after treatment with cisplatin in control and COUP-TFII-knockout mice. (E) Relative mRNA levels for TNF-α were elevated in COUP-TFII-knockout mouse kidney. (F) Knockdown of COUP-TFII stimulated the expression of TNF-α in VA-13 cells (G) Chromatin immunoprecipitation showed that COUP-TFII was enriched in the potential binding site but not in the coding region of TNF-α gene. All data are expressed as the mean ± SEM. PTX: pentoxifylline, CTRL: control mice, KO: knockout mice, w/o: without, n.s.: not significant, *: p < 0.05, n = 4 for the w/o cisplatin groups and n = 6 for the cisplatin 24 h groups and cisplatin 72 h groups. n = 3 for each group in cell-based experiments.
study cell-autonomous functions of COUP-TFII in the AKI model.

On the other hand, we observed increased cell death in COUP-TFII-null mice, not only in the TAL, but also in the S3 segment, where COUP-TFII is not expressed. These findings cannot be explained by a cell-autonomous anti-apoptotic effect of COUP-TFII. One potential explanation could be that some diffusing molecules mediate the effects of the COUP-TFII deletion in a non-cell-autonomous manner. One such candidate is TNF-α. The TNF-α pathway plays an important role in AKI, including cisplatin-induced toxicity [16, 38]. The serum levels of TNF-α increased after cisplatin treatment in control animals, consistent with a previous report [28]. On the other hand, COUP-TFII-knockout mice showed significantly higher levels of serum TNF-α, 24 hours after exposure to cisplatin but not after 72 hours. This transient elevation of TNF-α might be responsible for the more severe phenotype in the knockout mice. However, several questions remain to be addressed. First, the cell type(s) of origin of TNF-α production is not specified in this system. Zhang et al. reported that renal parenchymal cells are responsible for the production of TNF-α, rather than bone marrow-derived immune cells [17]. However, several other types of cells may also be involved in our system, because we utilized systemic knockout mice. A significant increase in TNF-α mRNA level was observed in the knockout mouse kidney tissue, but there exist many types of cells in the kidneys. In addition, our experiments revealed that COUP-TFII negatively regulates TNF-α gene in VA-13 cells, which are not derived from kidney. Cell-type specific knockout systems would be necessary to solve this question. A remaining question would include the role of transient TNF-α overproduction in the serum of COUP-TFII-null mice. It is possible that the transient increase in serum TNF-α in the knock-out animals is harmful enough to induce long-term toxicity.

In summary, our data indicate that COUP-TFII plays a renoprotective role in the cisplatin-induced AKI mouse model and suggests that potentiating the function of COUP-TFII might be a therapeutic strategy to avoid or treat cisplatin-induced nephrotoxicity.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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